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SOME ASPECTS OF
THE ANALYTICAL CHEMISTRY
OF
MALATHION

a thesis submitted by

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for the degree of

DOCTOR OF PHILOSOPHY

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Birmingham

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SOME ASPECTS OF THE ANALYTICAL CHEMISTRY OF MALATHION

by

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submitted for the degree of Ph.D.

SUMMARY

A thorough investigation of the recommended colorimetric method for the determination of malathion (an organophosphorus pesticide) has led to the identification of the major cause of all the problems with which the method suffers. The method, which involves the extraction of the copper(II) complex of the hydrolysis product of malathion from aqueous solution into immiscible organic solvents, has many drawbacks. For example, the colour of the organic extract fades very quickly and a slight increase in the contact time of the hydrolysis product and the copper reagent within the aqueous solution, results in a decrease in the absolute absorbance. Also, the presence of any reducing agents can be a significant source of error. In the present work, it has been shown that the basic cause of all these problems is the ability of copper(II) ion to be reduced to copper(I) ion. It has further been shown that these problems can be resolved by replacing copper(II) by bismuth(III). This has led to the development of a modified colorimetric method for the determination of malathion, which has distinct advantages over all other existing methods in terms of reagents required, ease in application, avoidance of interferences and stability of colour for extended periods of time.

The modified colorimetric method described above has been further improved by making use of a ligand exchange reaction involving dithizone. The resulting final organic extract in this case is bright orange in colour, the absorbance of which can be measured even with simple photometers.

The usefulness of the modified colorimetric method has been demonstrated by determining malathion in technical products, and in aqueous solution containing the compound down to sub ppm levels.

The scope and applicability of atomic absorption spectrophotometry has been extended by demonstrating for the first time that the technique can be used for the indirect determination of malathion.

Almost all of the work described above has been accepted for publication by international journals and considerable interest in the work has been shown by chemists working in the field of pesticide analysis and research.

Key Words: Malathion hydrolysis; Colorimetric determination;

Water analysis; Atomic absorption spectrophotometry.

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The help and advice received from
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CHAPTER I

GENERAL INTRODUCTION

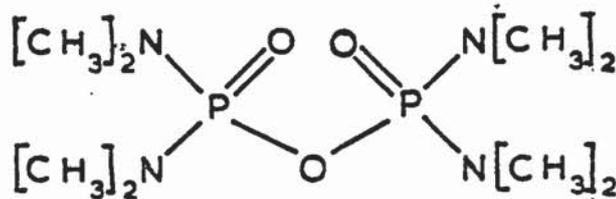
I.1. BRIEF HISTORY OF ORGANOPHOSPHORUS PESTICIDES

This subject has been discussed in detail in books by Eto (1), O'Brien (2) and Green (3) etc. Very briefly, the history of the development of organophosphorus pesticides may be recounted as follows.

Research in the chemistry of organophosphorus compounds was first undertaken in early 19th century by Lassaigne who was interested in preparing phosphate esters. The chemistry of the organic compounds of phosphorus was further developed by Michaelis in Germany during the late 19th century and the beginning of the present century. The Russian chemist Arbuzov and his son made further contributions to this subject.

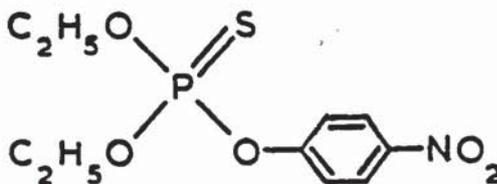
Physiologically abnormal effects of organophosphorus compounds were first observed in dialkyl phosphorofluoridates in 1932 by Lange and Krueger who were trying to develop new types of organic pesticides.

During the Second World War, Saunders in England and Schrader in Germany worked on toxic phosphorus compounds. Saunders synthesised nerve poisons, including diisopropyl phosphorofluoridate (DFP). In 1941, Schrader et al. identified a systemic insecticide (OMPA) named 'schradan' after its discoverer.



schradan

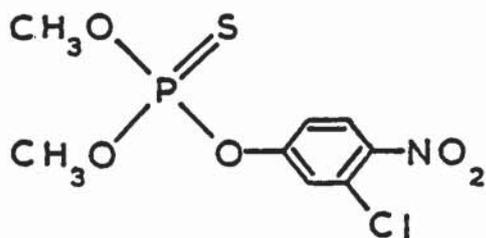
The field of organophosphorus pesticide research and development was much widened by the discovery of parathion, (diethyl p-nitrophenyl phosphorothionate) by Schrader in 1944.



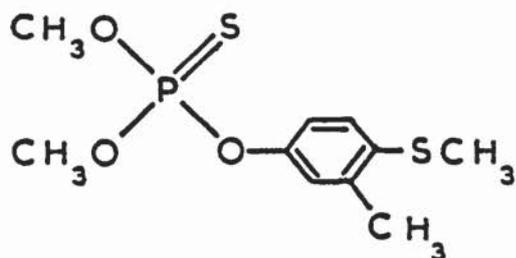
Parathion

Although parathion itself is extremely toxic to mammals as well as to insects, many less toxic insecticides have been developed by slight modifications of the molecular structure,

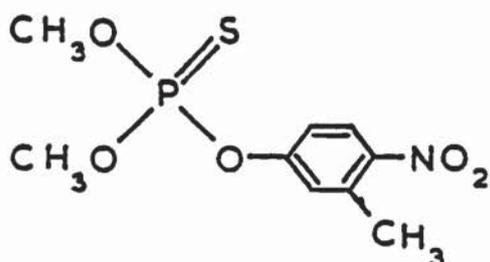
for instance, chlorthion, fenthion and fenitrothion were discovered in 1952, 1958 and 1959 respectively. Another important compound with low mammalian toxicity, malathion had earlier been discovered in 1950 and Bayer AG in Germany discovered demeton in 1951.



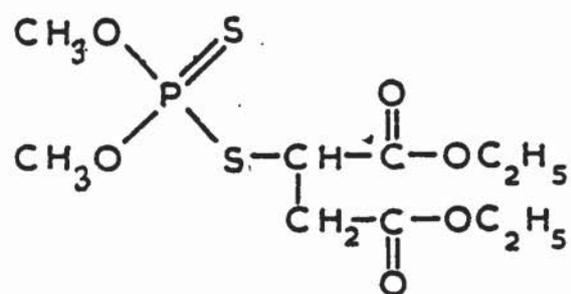
chlorthion



fenthion



fenitrothion

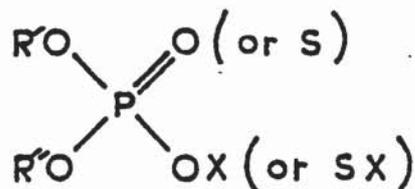


malathion

In recent years hundreds of patents are granted annually by the British government for use of new compounds as pesticides or for the formulations of existing chemicals. The same is true of other European countries, Canada, and the U.S.A.

I.2. GENERAL STRUCTURE OF ORGANOPHOSPHORUS PESTICIDES (3)

The structures of most active compounds in this class can be described by the general formula:

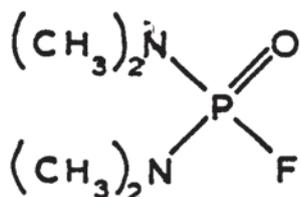


A few compounds have been made in which a higher group VI element, selenium or tellurium, replaces oxygen or sulphur, but these have been reported to be uneconomic. Amide groups have also been tried in place of oxygen or sulphur but such compounds tend to polymerize during synthesis.

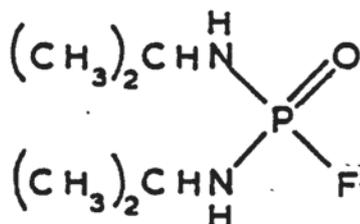
The stable alkoxy groups R'O and R''O are usually the same and most frequently C₂H₅O- or CH₃O-. Propyl esters occasionally appear but esters of higher alcohols usually show reduced activity. The group 'X' which is linked to the phosphorus atom through oxygen or sulphur can be a wide range of alkyl, aralkyl, aryl and heterocyclic groups with various substituents.

A few earlier prepared compounds had (CH₃)₂N- groups in place of R'O and R''O, and F in place of OX or SX.

Two commercially used compounds were dimefox and mipafox, but these are little used nowadays because of their high mammalian toxicities (oral LD50 5 mg/kg).

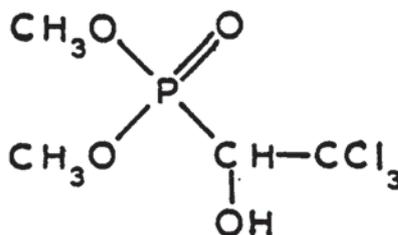


dimefox



mipafox

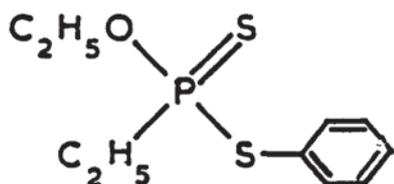
The only other compound with $(\text{CH}_3)_2\text{N}$ - groups instead of $\text{R}'\text{O}$ and $\text{R}''\text{O}$ which had any commercial application was schradan, but this too is not used now because of its high mammalian toxicity (oral LD50 8 mg/kg). There are one or two commercially useful compounds in which the group 'X' is directly linked to phosphorus but not through oxygen or sulphur, i.e. they are phosphonates and not phosphates. The most important compound of this type is trichlorophon.



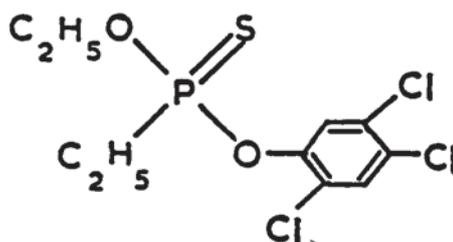
trichlorophon

There are some useful phosphonates in which one of the alkoxy groups $R'O$ and $R''O$ is replaced by an alkyl or aryl group.

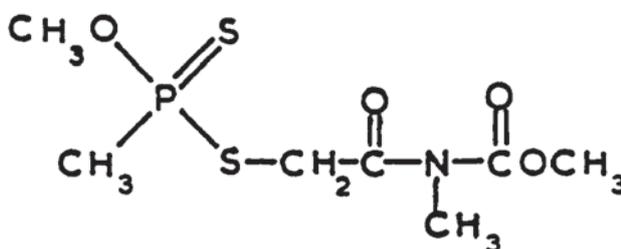
The most important compounds of this type are fonofos, mercarphon, trichloronate, leptophos and cyanofenphos.



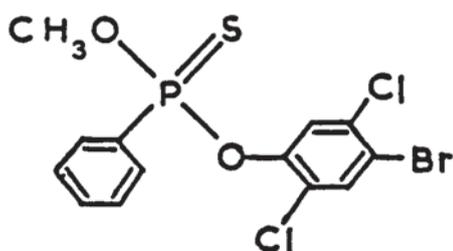
fonofos



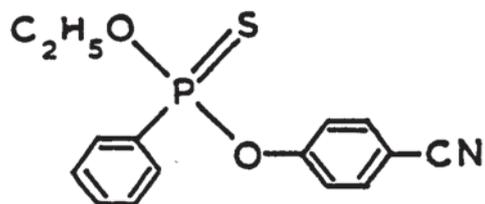
trichloronate



mercarphon

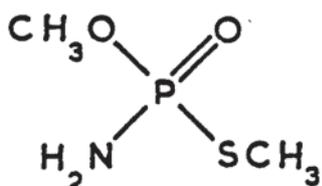


leptophos

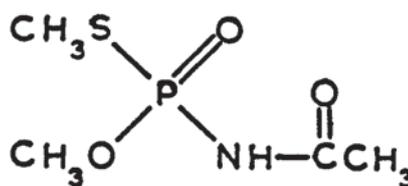


cyanofenphos

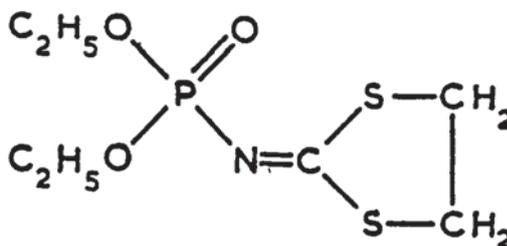
Recently there has been considerable interest in compounds in which the OX or SX group is replaced by NXY so that the compounds are phosphoramidothioates. 'Y' in this case is similar to 'X', and representatives of this class of compounds are methamidophos, acephate, phosfolan and mephosfolan.



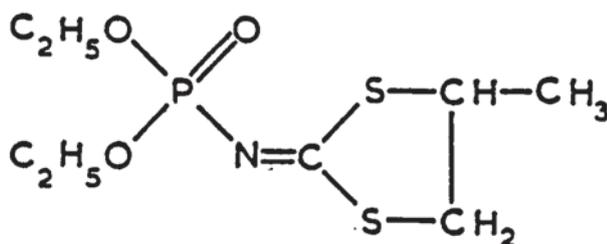
methamidophos



acephate

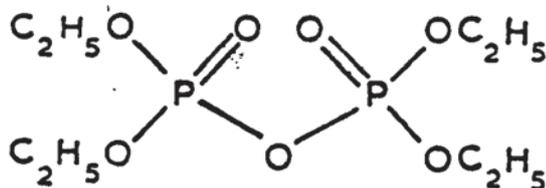


phosfolan

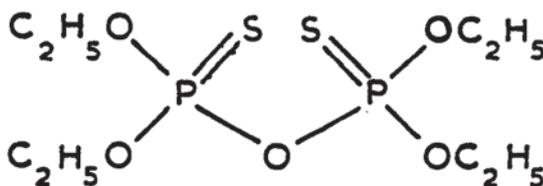


mephosfolan

Most of the common important organophosphorus insecticides are, however, derivatives of phosphoric or thiophosphoric acid. The earliest useful compounds of this type were phosphoric anhydrides such as TEPP (tetraethyl pyrophosphate) and sulfotep, but these are little used now, again because of their high mammalian toxicities (oral LD50 5 mg/kg).



TEPP



sulfotep

I.3. MODE OF ACTION OF ORGANOPHOSPHORUS PESTICIDES

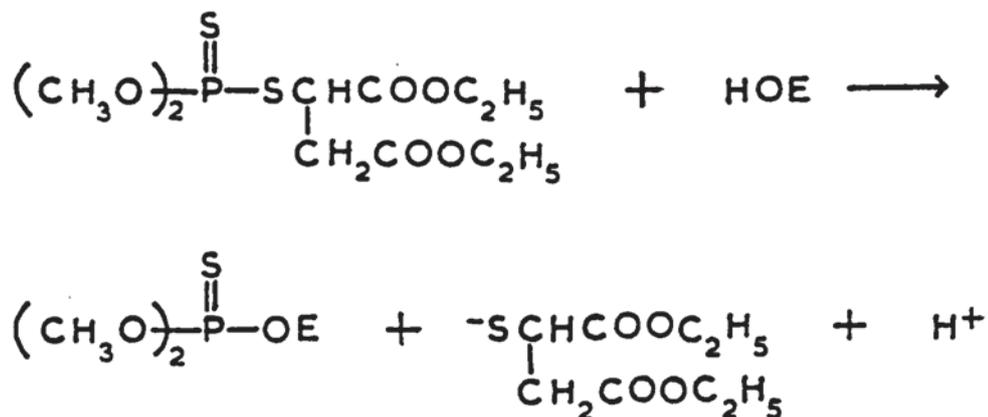
For a detailed discussion about this subject chapter IV of the book by Eto (1) and references cited therein may be consulted. In the paragraph below, only a very simple view of the actual process is given.

It is an accepted view nowadays that organophosphorus pesticides kill animals, both vertebrate and invertebrate, by inhibiting the enzyme acetylcholinesterase. This results in the disruption of nervous activity caused by accumulation of acetylcholine at the nerve endings. The enzyme acetylcholinesterase plays a very important part in the transmission of nerve impulses and the contraction of muscles. When sensory cells associated with the senses of sight, touch or taste receive a signal from the external or internal environment of an organism, an electrical signal or impulse is transmitted along a fibre-like portion of the nerve, the axon, to the central nervous system. From there, another electric impulse is transmitted along another axon to another cell. The junction between the end of the axon and the muscle cell is called a neuromuscular junction or synapse. At the junction, the electric signal is converted to a chemical transmission to the muscle cell. The signal transmitted chemically to the muscle cell causes contraction of the muscle. Figure I-1 below illustrates the path of the impulse.

Acetylcholinesterase has a serine amino acid residue $-\text{CH}_2-\text{OH}$, at its active site. Inhibitors act by forming a stable, covalently bonded ester with the hydroxyl group of the serine residue. This reaction and the resulting inhibition of the enzyme is therefore not reversible. The reaction of organophosphorus pesticides with the enzyme involves the transfer of a group from the pesticide to the enzyme. If the enzyme is represented simply by 'E-OH' and the pesticide by 'A-X', the inhibition reaction may be represented as follows:



Thus, the reaction with malathion would be:



The inhibition of acetylcholinesterase prevents the degradation of acetylcholine generated by the first nerve impulse. Additional impulses arriving at the junction cause a build-up of acetylcholine, leading first to overstimulation of the nerves and muscles, then to paralysis and death by asphyxiation due to respiratory failure.

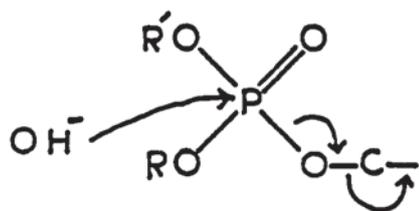
I.4. HYDROLYSIS OF ORGANOPHOSPHORUS PESTICIDES

With only a few exceptions, all organophosphorus pesticides are neutral phosphoryl or thiophosphoryl compounds. A great majority of them are ester derivatives. The polarized phosphoryl group creates a positive charge on the phosphorus atom, which consequently becomes highly electrophilic and reactive with nucleophiles. This is the basic principle of the various reactions of organophosphoryl compounds.

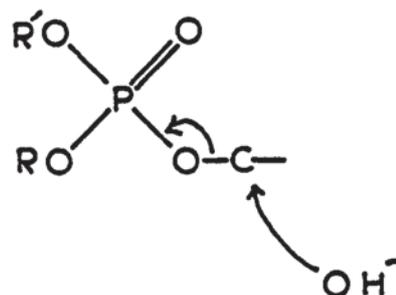
Fully esterified phosphorus acids, like carboxylic esters, are susceptible to hydrolysis. The hydrolysis rates of organophosphorus pesticides are of great importance, because the hydrolysis results in detoxication of the pesticides and, also, their susceptibility to alkaline hydrolysis is significantly related to their biological activity.

Alkaline Hydrolysis of Phosphorus Triesters

In the hydrolysis of the phosphate ester linkage P-O-C in the triesters of phosphoric acid, there exist two possibilities: a nucleophile attacks either the phosphorus atom accompanied by the P-O bond fission (see 1 below), or the attack is at the carbon atom resulting in the O-C bond fission (see 2 below).



1



2

Of the four P-O linkages in the phosphate molecule, the most acidic group AO is the most susceptible to alkaline hydrolysis. Thus, the P-O-A bond is cleaved and the acidic group displaced with the hydroxide ion to form a diester and an anion AO^- which is the most stable of the three possible ions (AO^- , RO^- , $\text{R}'\text{O}^-$) which could be produced by hydrolysis of the triester.



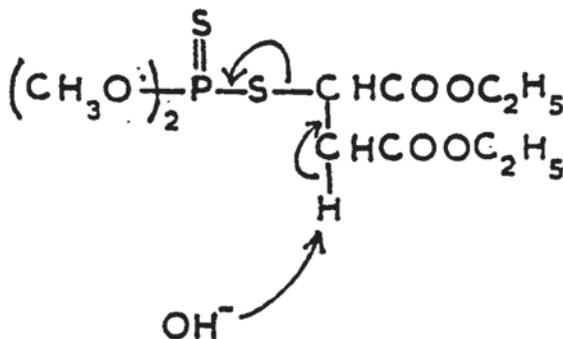
Since the alkaline hydrolysis of the triesters is initiated by the nucleophilic attack of the hydroxide ion at phosphorus, the reaction depends upon the electron deficiency of the phosphorus atom, which in turn depends on the electronic properties of substituents on phosphorus. Thus, the hydrolysis potential of the esters is increased by the presence of electron-withdrawing groups and is decreased by the presence of electron-releasing groups. Therefore, methyl esters are generally less stable than the corresponding ethyl and higher alkyl esters.

The substituents on the phosphorus atom are not the only factors which controls the hydrolyzability of an organophosphorus pesticide. Another aspect of the chemistry of these compounds must also be considered in this context. Many of the organophosphorus pesticides have bonds connecting hetero atoms such as oxygen, nitrogen, sulphur and halogens which all possess a lone pair

of electrons. This lone pair can be donated into the vacant 3d orbitals of the phosphorus atom and due to this contribution, the electron density of phosphorus increases and the phosphorus compounds become less susceptible to the attack of nucleophiles.

It has been observed that the phosphorothionate esters (containing a P=S group) are much more stable than the corresponding phosphate esters. This is so because of the fact that sulphur is less electronegative than oxygen and consequently the phosphorus atom is less electrophilic and less reactive with the hydroxide ion. In contrast to this, phosphorothiolate esters are much more reactive than the corresponding phosphate or phosphorothionate esters.

An unusual alkaline hydrolysis reaction, accompanying C-S bond fission takes place in certain phosphorus esters having an electron attractive group such as carbonyl, cyano or sulfonyl group in the β position. As the β -hydrogen is activated by the electron attractive group, β -elimination occurs by the action of the alkali. An example of such an hydrolysis reaction is the hydrolysis of dioxydemeton-S-methyl (dimethyl S-2-ethylsulfonylethyl phosphorothiolate). The reaction is of special importance in the present work because the compound under investigation (malathion) is also quickly and quantitatively hydrolysed by alkali by this process:



I.5. GENERAL METHODS OF ANALYSIS OF ORGANOPHOSPHORUS

PESTICIDES

(i) Ultraviolet and Visible Spectrophotometry

Ultraviolet and visible spectrophotometric methods are widely used for the analysis of pesticides in general and organophosphorus pesticides in particular because of the easy availability of the instruments (for visible spectrophotometry), ease of application and reasonable sensitivity and accuracy. Also, ultraviolet or colorimetric determinations are generally specific for a single compound or a group of compounds, although care must be taken of any interfering substances present that may lead to false results.

(ii) Infrared Spectrophotometry

Most applications of infrared spectrophotometry are in the analyses of formulations and technical materials. This method is not used for residue analysis due to the non-availability of instruments and comparative lack of sensitivity. Also, the technique of infrared spectrophotometry is useful for materials which are comparatively more pure. It is possible that this technique may gain increasing importance in future because both quantitative and qualitative information can be obtained only by physical measurements.

(ii) Fluorescence Spectrophotometry

Fluorescence can be used for compounds which are themselves fluorescent, which can be converted to fluorescent derivatives or which decrease the fluorescence of another compound by

reacting with it. Although fluorescence has been of limited use in the analysis of organophosphorus pesticides, it is an important technique, in being 100-1,000 times more sensitive than ultraviolet or visible spectrophotometry and being highly specific.

(iv) Gas Chromatography

This is the most important technique for pesticide analysis and every laboratory engaged in this type of work is usually equipped with elaborate glc instruments. The flame-ionisation and phosphorus-specific detectors provide a sensitivity (and precision) for the organophosphorus pesticides which no other technique can match. On the other hand the cost of the instrument and accessories, the extreme conditions of cleanliness required and the skill and experience of the analyst put a limit to its use.

(v) Thin-layer Chromatography

Tlc will always occupy a unique position in organophosphorus pesticide analysis because,

- a) it is a useful separation technique,
- b) is a quick method of identifying the compounds present in the sample, and
- c) in conjunction with fluorescence or densitometry is an important analytical method in itself.

(vi) Cholinesterase Inhibition Techniques

The cholinesterase inhibition method for organophosphorus pesticides is not specific and thus of limited use, but

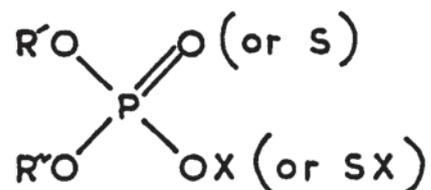
this technique is highly sensitive and is useful for the analysis of compounds for which no specific chemical residue method could be found.

(vii) Insect Bioassay

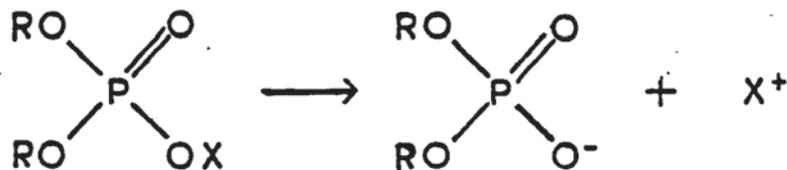
In the early development of a pesticide, when no specific chemical analysis methods are available, bioassay methods are very useful. These are highly sensitive, simple in operation, easily adapted to the assay of newer compounds and capable of detecting the toxic metabolites as well as the parent compound.

I.6. THE PRESENT WORK

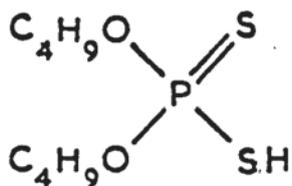
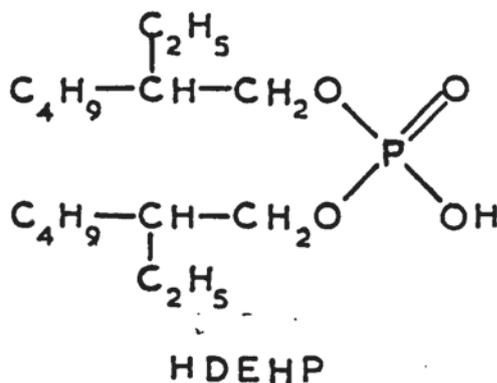
As has been shown earlier, due to their functional nature, organophosphorus pesticides in general have a structure as follows:



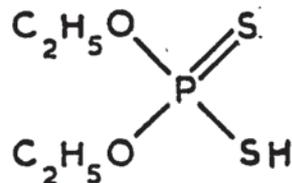
Another characteristic feature of all of these compounds is their ability to undergo hydrolysis and in many cases to release the dialkylphosphates.



These dialkylphosphates in turn are potential metal complexing agents since similar compounds have been in use in analytical chemistry for a long time. For example, di-2-ethyl hexylphosphoric acid (HDEHP), di-n-butylidithiophosphoric acid (HDBDTP), and diethyldithiophosphoric acid (HDEDTP) are excellent metal extractants. Of all these compounds, the dithiophosphates are of special significance because they form coloured complexes with metal ions such as bismuth(III), copper(II) and iron(III), (4).



HDBDTP



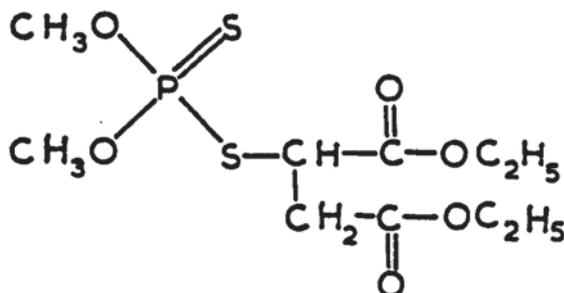
HDEDTP

The above mentioned facts have previously been utilised in the development of colorimetric procedures of analysis for those pesticides which give dialkyl dithiophosphates as their hydrolysis products, the most important in this context being malathion. Working along the same lines, the objectives of the work described in this thesis can be summarised as follows:

- a) to examine in detail, the present colorimetric method for the determination of malathion, and if possible to overcome the serious drawbacks with which this method suffers,
- b) to apply the above improved method to the analysis of malathion technical products and spiked water samples,
- c) to attempt to develop an indirect atomic absorption method for the determination of malathion.

CHAPTER IIMODIFIED COLORIMETRIC METHOD
FOR THE DETERMINATION OF
MALATHIONII.1. INTRODUCTION

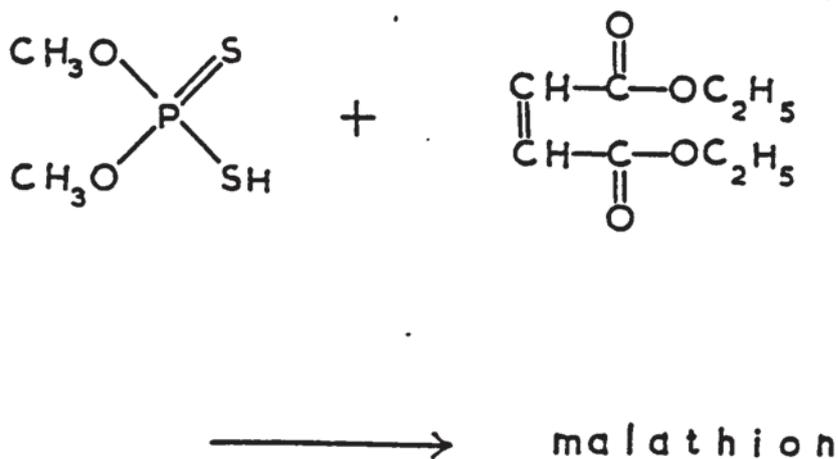
Malathion is the common name approved by the British Standards Institution and by International Standards Organisation for S-1,2-di(ethoxy carbonyl)ethyl O,O-dimethyl phosphorodithioate, which has a structure as given below.



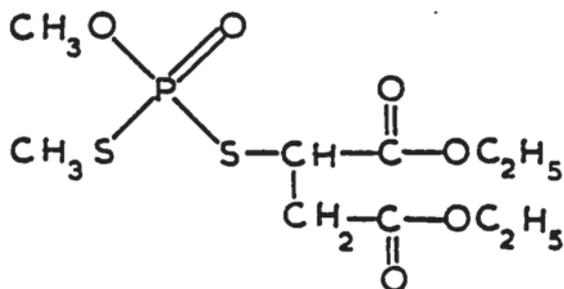
Malathion was first synthesised in the laboratories of the American Cyanamid Company and is covered under U.S. patents 2,578,652 and 2,962,521.

(1) Commercial Synthesis of Malathion (5)

Malathion is synthesised by addition of O,O-dimethyl dithio-phosphoric acid to diethyl maleate. Commercially, the preparation is carried out as a one stage process in which the dithioic acid (obtained from the reaction of methanol and phosphorus pentasulfide) is added to diethyl maleate in presence of catalytic quantities of a base and hydroquinone to prevent polymerization of the maleate.



There are numerous impurities in the technical material but these are generally in trace amount and are measured by GLC methods. One of the most important of these impurities is isomalthion which is an isomer of malathion where the doubly bonded sulphur atom is exchanged with one of the oxygen atoms of the methoxy groups attached to the phosphorus atom..



isomalthion

(ii) Physical Properties of Malathion (6)

Malathion has the following important physical properties:

Melting point: 3.7 °C

Boiling point: 156-157 °C

Solubility in water, approximately 145 mg/l, at 25 °C

Completely soluble in most alcohols, esters, ketones etc.

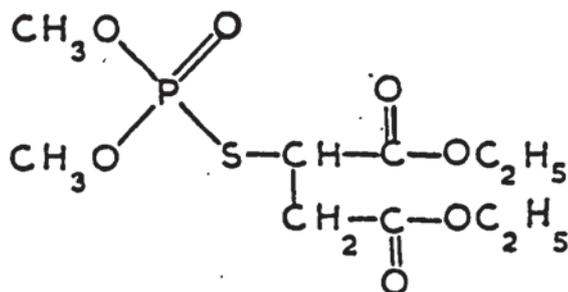
Vapour pressure: 0.00004 mm Hg/30 °C

Viscosity: 36.78 centipoises (25 °C)

Specific gravity: 1.2315 (25 °C)

(iii) Toxicity of Malathion (5)

Malathion is a pesticide with a good spectrum of insecticidal activity combined with a remarkably low mammalian toxicity LD₅₀ (rats) 2,500 mg/kg. This selectivity of insecticidal activity arises from metabolic activation of malathion to its phosphoryl analogue, malaoxon, which is more toxic to both insects and mammals LD₅₀ (rats) 88 mg/kg. Both malathion and its toxic metabolite malaoxon are detoxified by carboxyesterase enzymes which hydrolyse the carbethoxy moiety leading to polar water soluble compounds which are excreted out of the organism. Vertebrates show a greater carboxyesterase activity as compared with insects, so that the toxic agent malaoxon builds up more in insects than in mammals accounting for the selective toxicity of malathion towards insects.



m a l a o x o n

(iv) Uses of Malathion (7)

Malathion is one of the most widely used pesticides in the world, the importance of which can be realised from the vast number of plants which can be protected from insects by the use of pesticide. Some of the plants which have been protected by malathion are alfalfa, almonds, anise, apples, apricots, asparagus, avocados, barley, beans, beets, blackeye beans, blue berries, cranberries, brocolli, cabbage, carrots, cauliflower, citrus, cowpeas, celery, cherries, chestnuts, clover, collards, corn, cucumbers, currants, dandelion, dates, ~~de~~berries, egg plant, endive, figs, filberts, flax, garlic, gooseberries, grapes, grasses, guavas, hops, horse radish, kale, kohlrabi, kumquats, leeks, lentils, lespedza, lettuce, macadamia, mangoes, melons, mint, mushrooms, mustard, nectarines, nuts, oats, okra, onions, parsley, papayas, parsnips, passion fruit, peaches, peanuts, pears, peas, pecans, peppers, pineapples, potatoes, plums, prunes, pumpkins, quince, radishes, sugar beets, Swiss chard, sweet potatoes, tobacco, tomatoes, turnips, vetch, walnuts, watercress and wheat.

In addition to its use as a plant protector, malathion has been used to protect cattle, poultry, sheep, goats, and swine from body lice etc. Malathion is also extensively used in greenhouses, grain bins and for domestic purposes. It has also been found to be extremely useful as a means of controlling forest insects and mosquitoes.

Malathion has been found to be effective in controlling the following pests: aphids, mites, scale, flies, leafhoppers,

leaf mowers, thrips, loopers, pear prylla, mealy bugs, Japanese beetles, lygus bugs, spittlebugs, corn earworms, China bugs, grasshoppers, army worms, boll weevils, bollworms, lice, ticks, ants, spiders and many others.

(v) Analytical Determination of Malathion - GLC vs Spectrometry

With ever increasing use of malathion all over the world, it has become of major importance to have an analytical method for malathion which is of reasonable accuracy, precision and general reliability. For the determination of organophosphorus pesticides in general, the gas-liquid chromatographic (GLC) method is found to be the most accurate. However, good accuracy and precision is obtained only when a high quality instrument coupled to a digital integrator is used. This type of equipment is quite expensive and is not commonly available in most laboratories, in particular in the developing countries.

In contrast to the above, the instrumentation required for a spectrophotometric method is generally available in laboratories throughout the world. However, the standard copper colorimetric method for malathion, which has been in use for over two decades, is difficult to carry out. Several of its steps require exact timing and extreme care to make sure that the same procedure is used for each sample and standard assayed. The result of these problems frequently shows up in poor accuracy and precision, particularly when the assay is carried out by inexperienced operators. Keeping these factors in mind, the present work presents efforts to modify the standard method in such a way that it becomes easy to apply and free from its drawbacks.

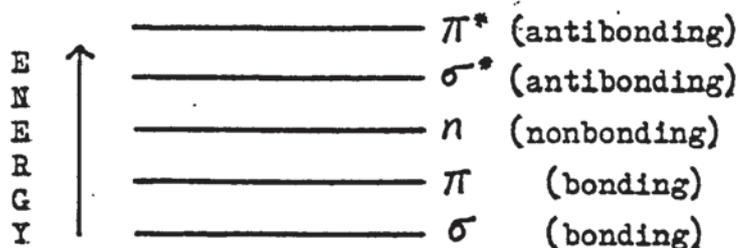
II.2. THEORETICAL ASPECTS OF ULTRAVIOLET AND VISIBLE

ABSORPTION SPECTROPHOTOMETRY (8,9)

Absorption of radiation in the ultraviolet and visible regions of the electromagnetic spectrum results in electronic transitions between molecular orbitals. The energy changes for such transitions occur mostly in the wavelength range 800 to 200 nm although in some cases higher energy absorption below the wavelength of 200 may also be encountered. The energy changes involved in the above transitions are so large that they are almost always accompanied by simultaneous changes in rotational and vibrational energy changes. Such vibrational and especially rotational lines are not usually observed for samples run in solution because of physical interactions between solute and solvent molecules which cause collisional broadening of the lines. The resulting overlapping bands coalesce to give one or more broad-band envelopes. For polyatomic molecules and metal complexes, the spectrum may contain several bands arising from more than one electronic transitions.

(1) Absorption of Light by Polyatomic Organic Molecules

According to the molecular orbital theory of bonding, the interaction of atomic orbitals leads to the formation of bonding and antibonding molecular orbitals. Depending on the nature of the overlapping orbitals, molecular orbitals may be of the σ type or the π type. The corresponding antibonding orbitals are then denoted as σ^* and π^* . In addition to these, there can be nonbonding orbitals denoted by n . The energies associated with these orbitals are in general in the following order:



(ii) Absorption of Light by Metal Complexes

Complexes of metals with organic and inorganic ligands which absorb in the visible or ultraviolet region of the spectrum are of great importance in quantitative analysis. Transitions giving rise to coloured complexes are of the following types.

(a) d-d Transitions

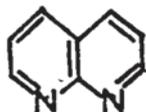
These are associated with the transition metal ions whose unfilled degenerate orbitals have split into different energy levels due to ligand field effects. Bands due to d-d transitions are responsible for the colour of transition metal ions in aqueous solutions. Various colours are produced in the aqueous solutions depending on the nature of the metal and the coordinating ligand; the absorption band shifts towards the ultraviolet with increasing strength of the ligand field. In most cases of d-d transitions, the bands are of low intensity because the electronic transitions are spectroscopically forbidden by the rules of symmetry. The fact that these transitions occur at all to a limited extent is because of vibrational distortions which relax these rules.

(b) Charge Transfer Transitions

A charge transfer band is associated with the transition of an

electron from an orbital belonging to a ligand to an orbital situated essentially on the central atom or conversely from a central metal atom orbital to a ligand orbital. Charge-transfer of the first type may occur with an oxidizable ligand and a high oxidation state central atom. The absorbance coefficients of charge transfer bands are about hundred times those of d-d transition absorption bands.

Charge-transfer bands caused by the excitation of the central metal electron to a ligand orbital appear in the spectrum of complexes formed by low oxidation state metals with unsaturated ligands. Electron transitions of this type are significant primarily for those complexes in which there is a transition even without excitation radiation. Such a charge-transfer band is for example responsible for the red colour of the iron(II)-2,2'-dipyridyl complex.



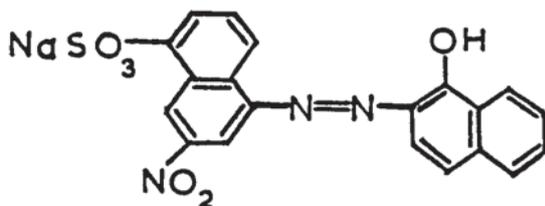
2,2'-dipyridyl

(c) Excitation Within an Organic Ligand

A large number of metal complexes involve organic ligands in which the absorption bands of the ligand are modified to a varying degree by coordination to the metal. Where the central atom is linked primarily by electrostatic forces, the metal ion modifies the spectrum of the ligand in much the same way as does protonation of the ligand. In such cases, complex

formation is accompanied by only a small displacement of the ligand bands towards shorter or, more rarely, towards longer wavelengths. The shape of the absorption curve and the values of the absorption coefficients at the peak maxima are only slightly, if at all, modified. Moreover, the displacement of the absorption bands is quite uncharacteristic of the nature of the cation in these compounds.

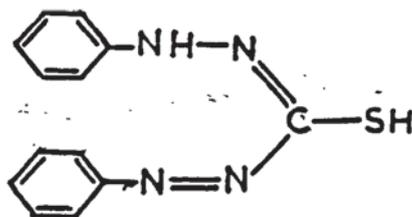
In contrast to the above, with more stable complexes, where the central atom is bound by a more or less covalent bond to the ligand, the extent of the modification in the spectrum of the ligand also depends on the degree of covalency of this bond. The absorption maxima of complexes of Eriochrome Black T appear at shorter wavelengths than that of the free ligand, and with an increase in complex stability the absorption maxima are shifted towards the ultraviolet region.



E r i o c h r o m e B l a c k - T

In those complexes where the nature of the bond between the central atom and the ligand is very covalent, the characters and energies of the molecular orbitals of the ligand and also the probabilities of electronic transitions are greatly altered. In the spectra of these complexes, charge-transfer bands occur

in addition to the absorption of the ligands, particularly if complex stability is enhanced by back coordination from the metal atom. In many cases, differentiation between these two types of absorptions is difficult, in particular with more complicated molecules; an example of such a molecule is afforded by dithizone.

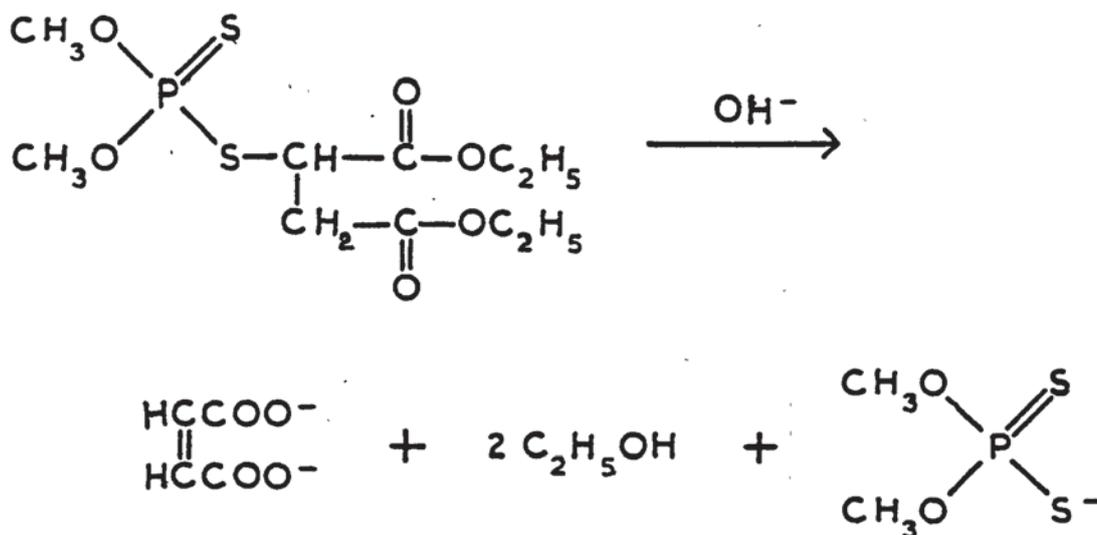


d i t h i z o n e

II.3. REVIEW OF COLORIMETRIC METHODS FOR THE DETERMINATION OF MALATHION

A recommended method for the determination of malathion has been in use for about 25 years but suffers from some serious drawbacks. Experiments described in the present work were designed to overcome these problems, and the first step in this direction was to review critically the existing methods. The purpose of this review was to find out the nature of the deficiencies and drawbacks of the standard method, and to suggest ways of overcoming these problems.

The first colorimetric method for the determination of malathion was developed by Norris et al. (10) of the American Cyanamid Company. The method was based on the fact that malathion is decomposed by alkali (e.g. NaOH) into maleate, ethanol and dimethyl dithiophosphate, as shown below.



The dimethyldithiophosphate is converted to the copper(II) complex which is extracted into carbon tetrachloride (or n-hexane) with the formation of intense yellow colour, with an absorption peak at about 420 nm.

The method outlined above has been successfully used for some years for the determination of malathion residues in various plant materials, e.g. apples, spinach, potatoes, canned peaches and strawberries, (10). Later, the method was adapted for estimation of the pesticide in fat, meat, egg-white, egg-yolk and milk(11), in cottonseed(12), in wheat and maize(13), in barley and rice bran(14,15), in a range of stored products(16), in pimento(17) and corn and cereal products (18).

In fact, this method has become well established and was recommended by the Malathion Panel set up jointly by the

Scientific Sub-committee of the Inter-departmental Advisory Committee on Poisonous Substances used in Agriculture and Food Storage; the Analytical Methods Committee of the Society for Analytical Chemistry; the Association of British Manufacturers of Agricultural Chemicals (19). In addition to this, it is also the recommended method of the Association of the Official Analytical Chemists of the United States (20) and is listed in the CIPAC handbook (21) as well as in the World Health Organisation's publication entitled 'Specification for Pesticides (22).

In spite of such wide-spread use, the method suffers from certain inherent drawbacks, some of which are mentioned here:

- (a) It has been found that the yellow colour which forms the basis of the colorimetric measurements, fades very quickly. Measurement of the absorbance after various periods of time reveals that there is significant change in the intensity of the colour even one minute after extraction of the copper(II)-dimethyldithiophosphate complex (23).
- (b) The various steps in the process leading to the final measurement of absorbance have to be carried out precisely according to the conditions stated, otherwise there are bound to be significant errors in the results. For example, it has been found that a delay of 15 seconds between the addition of the copper reagent and subsequent shaking with the organic

solvent will result in an absorbance reading which is as much as 4% lower than when shaking is started immediately. Longer delays result in much lower readings, with losses up to 40% after a 2 minute delay (6).

- (c) Determination and removal of any impurities which would otherwise reduce copper(II) to copper(I) is also important, since copper(I) forms a colourless and more stable complex with DMDTP than does copper(II). In the modified procedure of Norris et al. (11), this is achieved by the addition of 1 ml of a 5% solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and repeated extraction with carbon tetrachloride. It is possible that certain indirect problems might arise from this step and which may have previously been overlooked: even 'Analar' grade iron(III) chloride can contain sufficient copper which might combine with DMDTP, resulting in the loss of the latter by repeated extraction, thus lowering the sensitivity and introducing errors. Also, it is possible that DMDTP like DEDTP (diethyl dithiophosphate) might also partially reduce iron(III) to iron(II) and be destroyed in the process (24).
- (d) Whereas it has been mentioned that removal of impurities like mercaptans etc. which might reduce copper(II) to copper(I) is very important, cognisance has not been taken of the fact that DMDTP, again like DEDTP, may itself have the ability to reduce copper(II) to copper(I) (24).

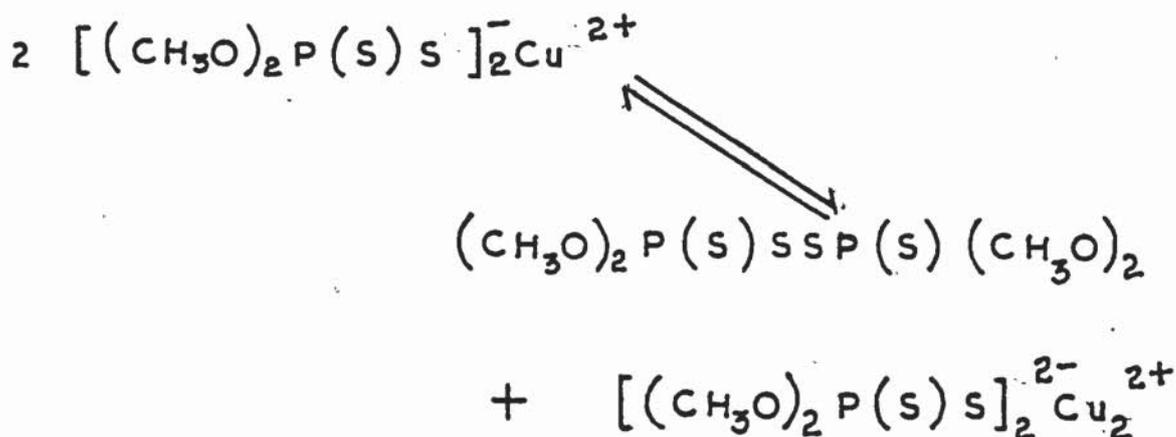
Many attempts have been made to overcome some of these difficulties by modifying the original method or by developing newer colorimetric techniques. For example, Getz (25) suggested a method for the quantitative determination of organophosphorus pesticides including malathion by conversion into orthophosphate ion. Getz and Watts (26) have also described a method for the estimation of organophosphates based on the reaction of 4-(p-nitrobenzyl) pyridine and the phosphate pesticide in the presence of cyclohexyl-amine. These methods are obviously not selective for malathion and also are tedious and lengthy to use.

Rossouw (13) put forward a slight modification of the method of Norris et al. (10). In this case, colour development is carried out at 18 °C, and the absorbance is measured after 30 minutes. It is claimed that the colour intensity changes only very slightly between 15-45 minutes after development, if the temperature is maintained between 16-20 °C.

Mora (27) has determined malathion in technical formulations by reacting a solution of the pesticide in methanol with a pH 7.2 buffer, hydroxyl ammonium chloride and iron(III) chloride.

Long (28) developed a method for malathion determination based on the formation of a phosphorofluoridate by the reaction of malathion with hydroxytrifluoro boric acid, followed by reaction of the fluoridate with hydrogen peroxide and fluorene-2,7-diamine.

Hill (29) has presented evidence to show that in the method of Norris et al. (10), the copper(II) complex of DMDTP exists in reversible equilibrium with its two dissociation products, copper(I)-dimethyl dithiophosphate and bis(dimethoxy phosphorothiono) disulphide and that this dissociation accounts in part for the instability of the yellow complex.



Hill also demonstrated that incorporation of the disulphide could limit the dissociation of the coloured complex.

Wayne et. al. (30) developed a non-aqueous copper colorimetric method for malathion based on the reaction of Norris et. al. (10). This method has not been favoured by some workers on the basis that the method is tedious and requires a large amount of glassware. Also, the availability of truly anhydrous solvents, or their preparation in the laboratory, can be a difficult problem (31). It has also been pointed out that strict cleanliness of glassware must be maintained in order to get reproducible results and that the method is time-consuming as compared to the original method. At least one worker has also reported difficulties with colour stability, (32).

More recently, Wisweswriah and Jayaram (33) suggested that the use of palladium(II) chloride reacting with the acid hydrolysis product of malathion would give a yellow coloured complex suitable for the determination of malathion. Their observations are confusing, however, because they state that the acid hydrolysis of malathion gives primarily dimethyl thionophosphoric acid but that the yellow colour is due to the reaction of palladium(II) with dimethyl dithiophosphoric acid, a product not formed under their recommended acid hydrolysis conditions.

The use of palladium(II) chloride has also been reported by some Japanese workers (34, 35). These methods involve a series of reactions with the pesticide and the development of colour takes between 20 minutes to two hours. These factors make the methods unsuitable for routine use.

In addition to the above, the work of Upham (143), Ware (144, 145) and Orloski (146) is also important.

II.4. THE PRESENT WORK

(i) Introduction

The critical survey of the literature dealing with the colorimetric methods for malathion which have been developed up to now and which have been discussed in the previous section, led to the following important conclusions:

(a) The original method of Norris et al. (10), although suffering from some drawbacks, is simple and the analysis can be carried out in a reasonable period of time.

(b) All the other methods developed to date are either more tedious and time consuming and/or involve the use of expensive metals like palladium.

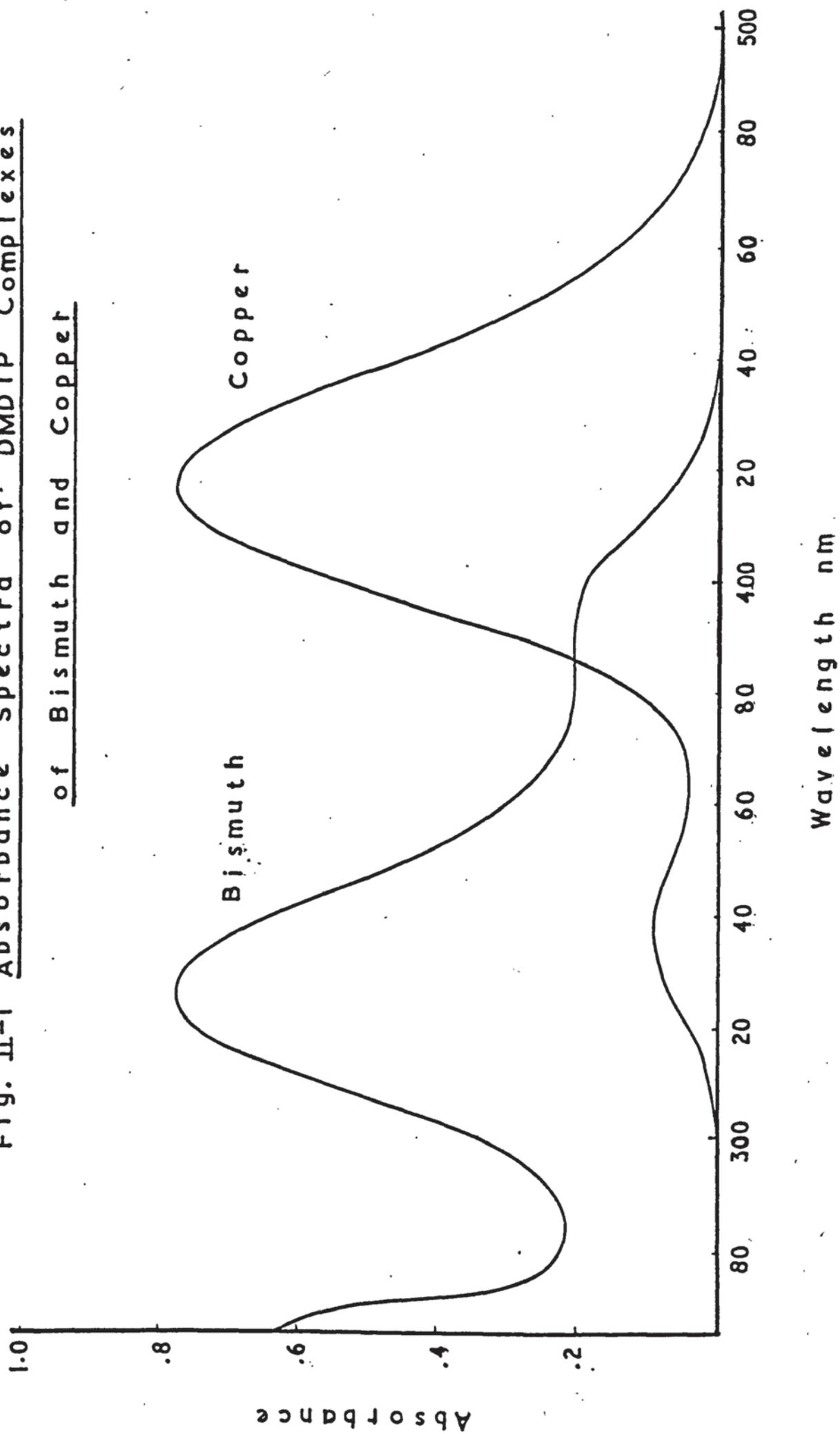
(c) The problems associated with the original method must be related to the complex forming behaviour of DMDTP (dimethyl dithiophosphate) and copper(II) ions.

These considerations led to the belief that copper(II) could be advantageously replaced by another metal ion. Now a compound very similar to DMDTP i.e. diethyl dithiophosphate (DEDTP) has long been used for the solvent extraction of many metals (as mentioned in the introductory chapter), some of the extracted complexes being coloured. Several metal ions form complexes with DEDTP (4), but of these bismuth(III) seemed the most suitable for further study.

Preliminary experiments indicated that bismuth(III) like copper(II) also forms a yellow coloured complex with DMDTP, the intensity of the colour for the same amount of DMDTP being less than the copper complex. On the other hand it was also noticed that the colour of the bismuth complex was stable for as long as 24 hours. The absorption spectra of the DMDTP complexes of copper(II) and bismuth(III) extracted into carbon tetrachloride are shown in figure II-1 page 37.

It would seem to be desirable to understand the reaction of bismuth(III) ions with DMDTP thoroughly, i.e. to investigate

Fig. II-1 Absorbance Spectra of DMDTP Complexes
of Bismuth and Copper



the stoichiometry involved and to compare the stability constant of the bismuth(III)-DMDTP complex with that for the copper(II) complex. To give the author some experience in the determination of formation constants, an experiment from a standard inorganic text was selected, (36), i.e. the well known nickel-glycine complex formation was chosen as a model system.

(ii) Formation Constants of Nickel-glycine Complexes.

As mentioned above, this experiment was carried out to gain experience and to assess the applicability of the method to our particular problem. The complete experimental details are presented in Appendix I at the conclusion of the thesis. The values obtained for the formation constants for the Ni(gly) and Ni(gly)₂ complexes were:

$$\begin{array}{l} \log K'_1 = 6.65 \\ \text{and } \log K'_2 = 5.42 \end{array} \quad (22 \text{ }^\circ\text{C})$$

These values are in reasonable agreement to those found by Li et al. (37), (5.97 and 4.95 respectively). The slight discrepancy between the two set of values could be due to errors in the present experiment, most probably arising from the incorrect calibration of the pH-meter and other minor factors. No attempts were made to further improve the present results by repeating the experiment. It was quickly realised that it would not be feasible to apply this technique to the determination of formation constants of DMDTP because in aqueous solutions copper(II) and

bismuth(III) form thick precipitates with DMDTP, thus creating a two phase system. Also, the precipitate could form a thin film over the electrodes used for pH measurements thus leading to incorrect results and hence to wrong conclusions.

It was therefore decided to use an alternative approach for the assessment of the complex forming ability of DMDTP with copper and bismuth, and for this purpose, a method proposed by Likussar and Boltz (38) was selected.

(iii) Formation Constant of the Copper-DMDTP Complex

Using the method of Likussar and Boltz (38), complete details of which together with the experimental data are given in Appendix II, the value for the formation constant for the copper(II)-DMDTP complex in ethanol-water (19:1) was found to be

$$\log K_f' = 10.5 \quad (22 \text{ }^\circ\text{C})$$

It was not possible to determine the formation constant of the bismuth complex because even very small quantities of the metal and the ligand give precipitate in the solvent, thereby making the spectrophotometric measurements impossible (the method of Likussar and Boltz is essentially based on absorbance measurements).

(iv) Extraction Constants of the Copper(II) and Bismuth(III) Complexes of DMDTP from Water into Carbon tetrachloride

Once again, using the method of Likussar and Boltz (38), the values obtained for the two complexes were:

Cu-DMDTP	$\log K'_e = 11.9$	(22 °C)
Bi-DMDTP	$\log K'_e = 13.9$	

The above experiments indicated that, as expected, copper(II) forms a 1:2 complex with DMDTP while bismuth(III) forms a 1:3 complex with the ligand.

The large values obtained for the extraction constants of the copper and bismuth complexes indicated that no special problems would be encountered if copper(II) is replaced by bismuth(III) for complexation with DMDTP in the method for the determination of malathion. It could be foreseen that the large value of the extraction constant for the bismuth complex could be useful in the following contexts:

- (a) the complex $\text{Bi}(\text{DMDTP})_3$ could be quantitatively transferred from water into carbon tetrachloride, and
- (b) for a certain amount of DMDTP, even three to four times the theoretical amount of bismuth would be sufficient for complete extraction.

(v) Infrared Study of the Copper and Bismuth Complexes of DMDTP

To further understand the true nature of the bismuth and the copper complexes of DMDTP, the complexes were precipitated from aqueous solutions, filtered through^asintered glass funnel and recrystallised from diethyl ether. Infrared spectrum of the complexes was recorded by the Analytical Services Division of the Chemistry Department on^aPerkin-Elmer 599 B IR spectrophotometer, using the KBr disc method. The energy range studied was from 200 to 4000 cm^{-1} . The absorption bands observed in the pure ligand (ammonium salt of DMDTP) and in the two metallic complexes

are listed in Table II-1, together with the probable assignments of these bands based on the findings of other workers about DMDTP or related ligands and their metallic complexes.

TABLE II-1

Infrared Absorption Bands of DMDTP (Ammonium Salt) and its Complexes with Copper(II) and Bismuth(III) Metal ions

NH_4DMDTP	Cu(DMDTP)_2	Bi(DMDTP)_3	Assignment	Reference
-	-	270 (w)	ν (M-S)	39, 40
-	290 (w)	290 (s)	δ (P-O-CH ₃)	40
330 (s)	-	-		
360 (w)	365 (s)	365 (s)		
-	-	380 (w)		
395 (w)	395 (s)	395 (m)		
420 (w)	420 (w)	440 (w)		
-	490 (s)	-		
-	-	505 (m)		
540 (m)	530 (m)	520 (m)	ν (P-S)	40, 41
580 (s)	-	-		
-	-	625 (s)		
635 (m)	635 (s)	640 (m)		
-	-	660 (s)		
760 (br)	830 (s)	780 (s)		
-	-	795 (s)		
-	-	800 (s)		
1030 (br)	1030 (br)	1020 (s)		
-	-	1040 (s)		
-	-	1165 (w)		
1180 (s)	1180 (s)	1180 (s)	ν (P-O-CH ₃)	42, 43
1400 (br)	1450 (s)	1450 (s)	δ (C-H)	" "
-	2820 etc.	2820 etc.	ν (C-H)	" "
3010 (v br)	-	-	ν (N-H)	" "

(v) Persistence of Colour Studies for the Copper(II) and the Bismuth(III) Complexes of DMDTP

The list of reagents and apparatus used for experiments reported in the following sections is as follows:

Reagents

Dimethyl dithiophosphate, (DMDTP) Solution, 5×10^{-3} M

Dissolve 0.1752 g purified ammonium salt (see below) in distilled water and dilute to 200 ml.

Bismuth Solution

Dissolve 0.1 g bismuth oxide (Bi_2O_3) (BDH Laboratory Reagent) in 3 ml of conc. nitric acid and dilute to 100 ml with distilled water.

Copper Solution

Dissolve 2 g copper sulphate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ('Analar' Hopkins and Williams Ltd.) in a few ml of distilled water and dilute to 100 ml with distilled water.

Carbon tetrachloride

Redistil commercial grade material and store in a dry glass bottle.

Ethanol

Medical grade (99.5%) used for all work reported here.

Apparatus

Spectrophotometers

UNICAM SP6-100 and UNICAM SP8-100 ultraviolet-visible spectrophotometers equipped with 1 cm quartz cells were used for all experimental work.

(v-a) Purification of ammonium dimethyldithiophosphate

10 g of the commercial product (Aldrich Chemical Co. 95% purity) was dissolved in 50 ml of ethanol. The solution was filtered and to the filtrate 30 ml of carbon tetrachloride were added. The solution was kept overnight at room temperature after which time the purified compound had been recrystallised as well-defined crystals. These were washed several times with diethyl ether, dried and the purity of the compound was confirmed by determining the melting point (143 °C).

(v-b) Persistence of colour studies

In a 100 ml separating funnel containing about 9 ml of distilled water, the following solutions were added: 0.3 ml of 5×10^{-3} M DMDTP, 1 ml 4M HNO_3 , 10.0 ml carbon tetrachloride and 1 ml of the copper (or bismuth) solution. The funnel was stoppered and vigorously shaken for exactly 1 min. The organic layer was allowed to separate and then was transferred to a 1 cm quartz cell, via a cotton wool plug placed in the stem of the funnel. The change in absorbance of the copper and bismuth complexes with time was studied on the single beam spectrophotometer connected to a chart recorder, (Fig.II-2).

(vi) Beer's Law Studies

The absorbance for the two complexes using various amounts of DMDTP were measured as described above. For the copper complex the wavelength used was 418 nm whereas for the bismuth

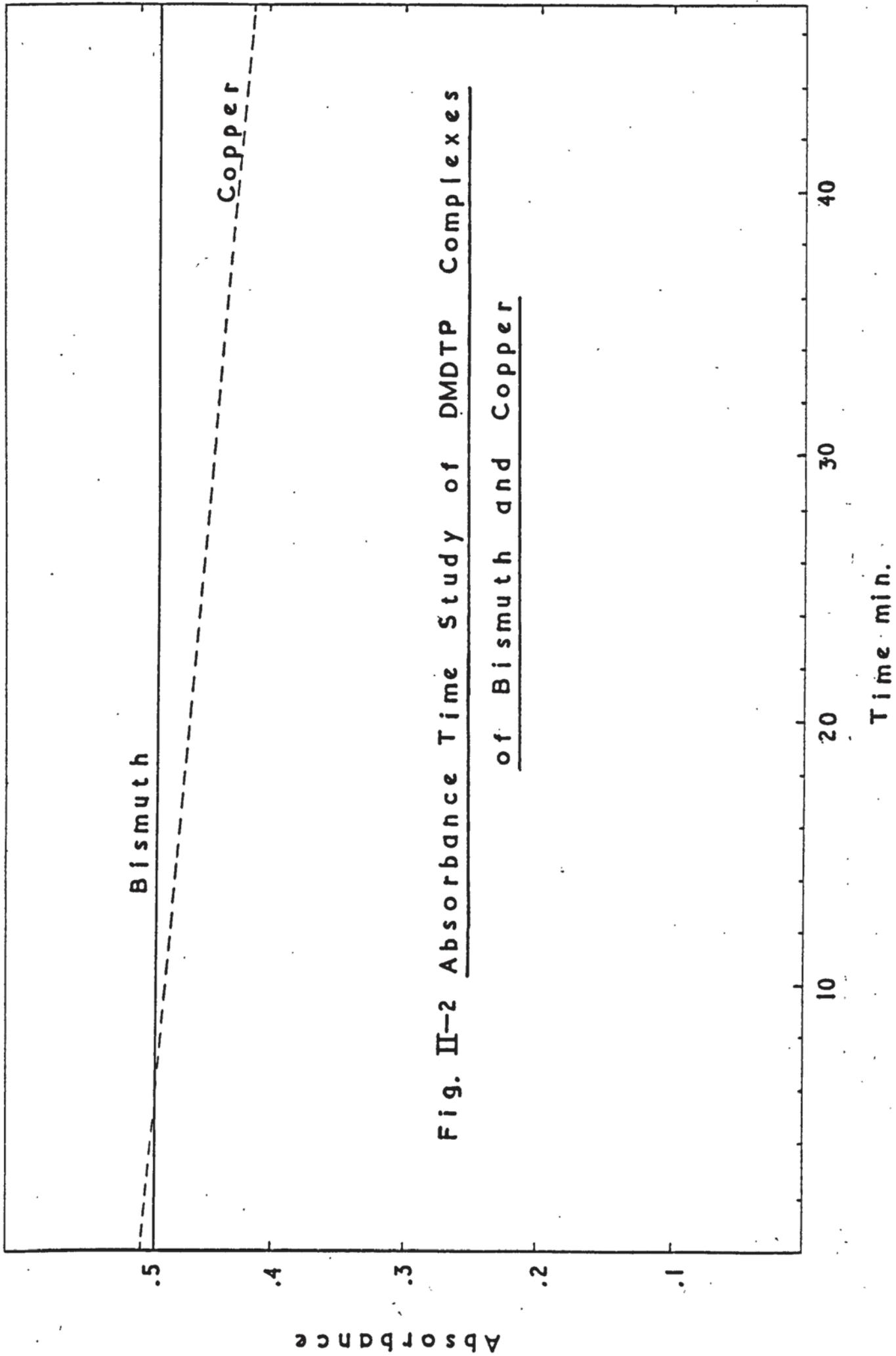


Fig. II-2 Absorbance Time Study of DMDTP Complexes
of Bismuth and Copper

complex, both the 325 nm and 390 nm absorption measurements were recorded. The resulting absorbances were then plotted against the amount of DMDTP used, (see Fig. II-3).

(vii) Effect of Reducing Agents

In the original method of Norris et al. (10), it has been reported that the presence of any reducing agents with the hydrolysis product of malathion is a major source of error. It was, therefore, decided to investigate the effect of a typical reducing agent. Ascorbic acid was chosen to study such an effect on the absorbance characteristics of the copper and bismuth complexes of DMDTP. For this purpose, the absorbances of these complexes for a fixed amount of solution, 0.3 ml, were measured after extraction from aqueous layers containing varying amounts of ascorbic acid. The results of this experiment are presented in Table II-2 below.

TABLE II-2

Effect of Ascorbic Acid on the Absorbance of Copper and Bismuth Complexes of Dimethyldithiophosphate*

ml of 10^{-2} M ascorbic acid	0	2	4	6	8	10
Absorbance Cu-DMDTP	0.82	0.62	0.51	0.44	0.38	0.37
Absorbance Bi-DMDTP	0.74	0.76	0.75	0.74	0.74	0.73

* 0.3 ml DMDTP extracted with excess of copper and bismuth in the presence of varying amounts of ascorbic acid.

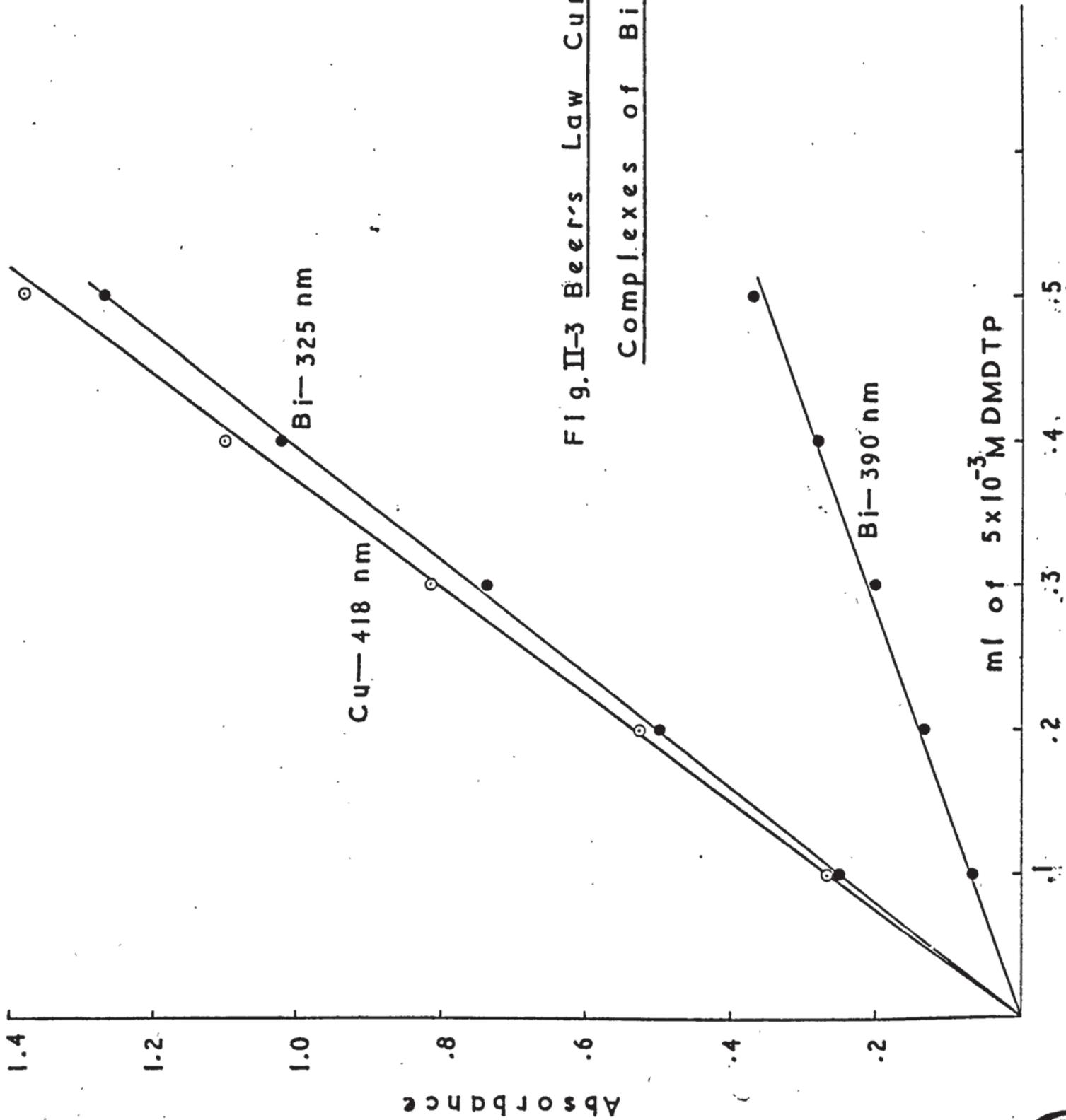


Fig. II-3 Beer's Law Curves for DMDTP
Complexes of Bismuth and Copper

(viii) Effect of Lapse of Time Between the Addition of
Metal Reagent and Subsequent Extraction into the
Organic Solvent

In the standard method of malathion determination it has been recommended that there should be a minimum lapse of time between the addition of the copper(II) reagent and subsequent shaking with the organic solvent. A delay of only a few seconds causes a decrease in the absolute absorbance and thus introduces errors. To confirm these findings and to investigate the effect of this lapse of time on the bismuth complex of DMDTP, a fixed amount of DMDTP solution was used, (0.8ml of 5×10^{-3} M DMDTP solution). It was found that if the complexes were extracted immediately after addition of metal ion, the absorbances obtained for the copper and bismuth complexes were 1.92 and 1.79 respectively. On the other hand, when the extraction was started one minute after the addition of the metal reagents, the absorbance for the bismuth complex remained virtually the same whereas the average absorbance for the copper complex fell to 1.31 (average of 10 readings).

(ix) Analysis of Commercial Samples

The standard copper method and a modified method using bismuth instead of copper were compared by applying them to the analysis of two commercial samples as described below.

Reagents

Malathion Analytical Standard Solution. Dissolve 0.1219 g of 99% malathion analytical standard supplied by the American Cyanamid Company, in 100.0 ml of ethanol.

Malathion Emulsifiable Concentrate Solution. Dissolve 0.1633 g of 60% malathion emulsifiable concentrate (Murphy Chemical Ltd.) in 100.0 ml of ethanol.

Technical Malathion Solution. Dissolve 0.1115 g of technical malathion (Cyanamid of Great Britain Ltd.) in 100.0 ml ethanol.

Sodium Hydroxide, 6 M. Dissolve 24 g NaOH (BDH Laboratory Reagent) in 100 ml distilled water.

Hydrochloric Acid, 7 M. Dilute 4.4 ml concentrated acid (BDH 'Analar') to 100 ml with distilled water.

Carbon tetrachloride. Redistil commercial grade material and store in a dry glass bottle.

Copper Solution. Dissolve 2 g $\text{CuSO}_4 \cdot 6\text{H}_2\text{O}$ in 100 ml distilled water.

Bismuth Solution. Dissolve 0.1 g Bi_2O_3 in a minimum amount of conc. nitric acid and dilute to 100 ml with distilled water.

Ferric Chloride Solution. Dissolve 5 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 100 ml of 1 M HCl.

Sodium Chloride Solution. Dissolve 20 g NaCl in 1 l distilled water and place in refrigerator until cool to about 15 °C.

Procedure

(a) Preparation of the Calibration Graph

Using 0.1 -1.0 ml of the malathion analytical standard solution, the following procedure was carried out:

Copper Method: An aliquot of malathion was transferred to a dry 250 ml separating funnel. 1 ml of 6 M NaOH was added and the contents of the funnel swirled gently for 1 minute. 75 ml of the cooled NaCl solution were added followed by 1 ml of the 7 M HCl. Then 10.0 ml of carbon tetrachloride were added to the

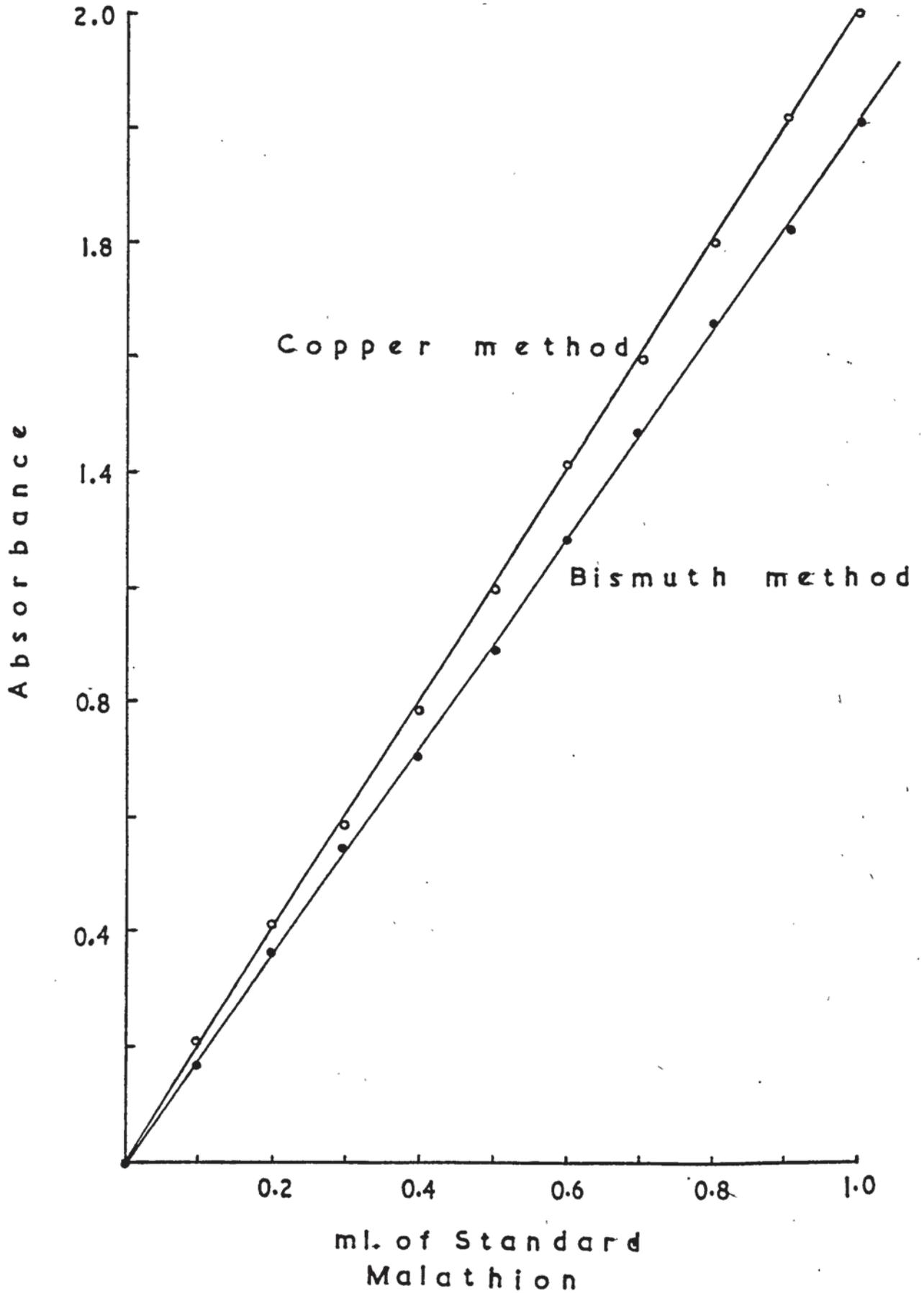
funnel, followed by 1 ml of the copper solution and 1 ml of the ferric chloride solution. The funnel was immediately stoppered and vigorously shaken for exactly one minute. The organic layer was allowed to separate from the aqueous layer, the former being transferred to a 1 cm quartz cell via a cotton-wool plug placed in the stem of the funnel. The absorbance of the carbon tetrachloride extract was measured without delay at 418 nm against a carbon tetrachloride blank.

Bismuth Method: An aliquot of malathion was transferred to a dry 250 ml separating funnel. 1 ml of 6 M NaOH was added and the contents of the funnel swirled gently for 1 minute. 50 ml of distilled water were added followed by 2 ml of the 7 M HCl. This was followed by the addition of 10.0 ml of carbon tetrachloride and 1 ml of the bismuth solution. The funnel was stoppered and vigorously shaken for about one minute. The organic layer was transferred to the spectrophotometric cell as described above and the absorbance of the extract measured at 325 nm.

(b) Analysis of 60% Malathion Emulsifiable Concentrate

1.0 ml of the emulsifiable concentrate solution was taken through the procedure described for preparation of the calibration graph. Using the absorbance readings of the organic layer and from the calibration graph, the volume of the standard malathion solution equivalent to 1.0 ml of the emulsifiable concentrate was found. Using the volumes of the concentrate and the standard solutions and their concentrations, the malathion content of the emulsifiable concentrate was determined. The procedure was repeated ten times for both the copper and the bismuth methods using exactly

Fig.II-4 Calibration Graphs for
Malathion



1 ml of the emulsifiable concentrate solution. The results of this experiment are presented in Table II-3.

(c) Analysis of the 93.9% Technical Malathion Sample

1.0 ml of the technical malathion solution was taken through the procedure described above for the emulsifiable concentrate. The results are again presented in Table II-3.

TABLE II-3

Analysis of 60% Emulsifiable Concentrate and 93.9% Technical Malathion by the Copper and Bismuth Methods of Malathion

Determination

<u>No.</u>	<u>60% Emulsifiable Concentrate</u>		<u>93.9% Technical Malathion</u>	
	<u>Copper Method</u>	<u>Bismuth Method</u>	<u>Copper Method</u>	<u>Bismuth Method</u>
1.	60.4	58.8	93.2	94.7
2.	58.4	58.9	93.8	95.2
3.	59.9	60.2	93.8	91.0
4.	60.3	59.7	94.4	95.3
5.	60.2	60.0	92.1	94.7
6.	58.1	58.1	94.4	95.3
7.	59.2	58.2	94.9	94.1
8.	60.2	59.6	94.4	94.1
9.	60.1	59.8	94.9	92.8
10.	58.4	59.7	94.9	95.2
Average	59.48	59.30	94.08	94.24
Std. Dev.	0.83	0.79	1.18	1.43
Coeff. of Variation	1.40	1.33	1.26	1.52

II.5. DISCUSSION OF RESULTS

(i) Nature of the DMDTP Complexes of Copper(II) & Bismuth(III)

The infrared study of the copper and bismuth complexes of DMDTP indicated that as expected, the complexation of the metal and the ligand occurs through the sulphur atom. This is borne out from the negative shifts in the P-S frequency bands, (see Table II-1, page 41).

During the experiments for the formation constants of the DMDTP complexes (see page 39) it was observed that a solution of the complexes in 95% ethanol absorbed strongly in the visible and near ultraviolet regions whereas a similar solution of the free ligand did not show any absorbance except in the far ultraviolet region. This indicates that the complexation between DMDTP and the metal ions involves a fair degree of covalency with some charge-transfer character.

The structure of the copper complex, $\text{Cu}(\text{DMDTP})_2$, like the copper dithiocarbamates, would be a square planar one. On the other hand, the bismuth complex $\text{Bi}(\text{DMDTP})_3$ would, in similarity to the vanadium(III) and chromium(III) complexes of diethyldithiophosphate (39, 43), have a distorted octahedral shape with D_3 symmetry.

(ii) Comparison of the Copper and Bismuth Methods of Malathion

Analysis

From the absorption spectra of the copper and bismuth complexes of DMDTP (Fig. II-1, page 37) and the Beer's Law curves for DMDTP using both copper(II) and bismuth(III), (Fig. II-3, page 46), it is clear that if bismuth is used for the determination

of malathion, in the visible region (390 nm) it is 4 times less sensitive than the standard copper method. On the other hand, in the ultraviolet region (325 nm), the sensitivity of the modified method is almost the same as that of the standard method.

From Fig. II-2, page 44, it can be seen that the yellow colour due to the bismuth complex of DMPTP remains unchanged even after a period of 40 minutes, whereas the absorbance of the copper complex falls appreciably during this period of time. In fact, the bismuth complex does not show any change in absorbance even 24 hours after extraction. This makes the bismuth method of malathion determination much more attractive than the copper one because one of the major drawbacks in the standard method is removed without taking the extra trouble of developing the colour at a lower temperature as suggested by Rossouw (13), using a precious metal like palladium (33) or incorporating the use of the disulphide (29).

Fig. II-3, page 46, shows that the bismuth complex obeys Beer's Law at least within the concentration range for which the copper method is applicable. This is found to be true for both the wavelengths of interest, i.e. 325 nm and 390 nm.

Results of experiments with ascorbic acid (Table II-2, page 45), show that whereas even a small amount of the reducing agent has a drastic effect on the results obtained by the copper method of malathion determination, no such detrimental effect is observed when bismuth is used in place of copper. This is a distinct advantage in that the analyst has no longer to worry about the

impurities which in the standard method would have to be oxidised by the addition of iron(III) reagent. In fact, the addition of iron(III) is no longer required.

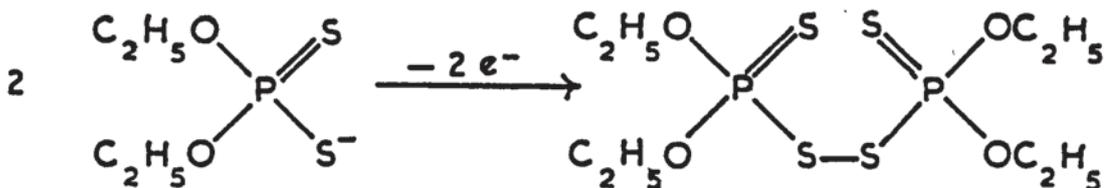
A further advantage of the bismuth method over the standard copper method is demonstrated by the lapse of time between the addition of metal and subsequent extraction studies. Whereas a delay of one minute has a noticeable effect on the absorbance of the copper complex of DMDTP, no such problem is encountered when bismuth is used in place of copper. This makes the bismuth method much simpler to use. Whereas the method of Norris et al. (10) requires strict adherence to time factors, the new method does not, and it is suggested that it gives equally good results.

The application of the bismuth method of malathion determination to the analysis of 60% emulsifiable concentrate and the 93.9% technical malathion samples confirms the usefulness of the new method. As observed from the results of Table II-3, page 51, within experimental error, both the copper and the bismuth methods yield similar results, proving that the standard method could well be replaced by the modified bismuth method with distinct advantages.

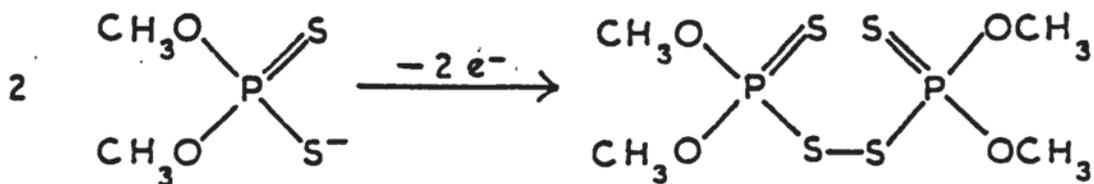
(iii) Basic Cause of the Drawbacks of the Copper Method

The results of the above experiments not only prove the superiority of using bismuth in place of copper in the method of malathion determination, but also give a good indication about the root cause of all the problems associated with the standard method. All the evidence supports the view that the basic cause of all the problems is reduction of copper(II) to

copper(I) not only by the impurities present in the hydrolysis products of malathion, but also by the major hydrolysis product DMDTP itself. It is well known that aqueous solutions of iron(III) and copper(II) oxidise diethyldithiophosphate to its disulphide as follows (23, 24),



and it is expected that DMDTP (dimethyldithiophosphate) would behave similarly. :



This would account for the reduced absorbance of the copper(II)-DMDTP complex when there is a delay between the addition of the copper reagent and subsequent extraction of the complex into the organic solvent, i.e. with longer time intervals, more and more copper(II) is reduced to copper(I) forming a colourless complex with DMDTP resulting in lower absorbance readings. Also, this could account for the instability of the yellow coloured

complex when extracted into the organic solvent.

Mention must be made here that due to the possibility of the oxidation of DMDTP, addition of large amounts of iron(III), as recommended in the standard method (6) may be a source of error in itself. The dangers are twofold in residue analysis where some authors recommend repeated shaking of the aqueous solution of DMDTP and iron(III) with the organic solvents and discarding of the extract. In this case, it is likely that even 'Analar' grade iron(III) chloride can contain sufficient copper(II) to make serious losses of DMDTP possible.

(iv) Extension of the Bismuth Method to other Pesticides

An attempt was made to extend the use of the bismuth method for the determination of some other organophosphorus pesticides. The pesticides tested were isomalathion, malaaxon, dimethoate, phorate, formothion, ethion and menazon. Alcoholic solutions of these compounds were taken through the hydrolysis and complex formation/extraction procedures but it was found that none of these compounds yielded the $\text{Bi}(\text{DMDTP})_3$ complex and thus the bismuth method could not be used for these pesticides. It is clear from the structures of isomalathion and malaaxon that neither would yield DMDTP on hydrolysis because both lack a doubly bonded sulphur atom to the phosphorus atom. The other compounds listed above do contain such a sulphur atom but lack a labile β -hydrogen atom and would not be hydrolysed by the mechanism specific for malathion, again failing to yield DMDTP and not participating in the reaction.

CHAPTER IIIFURTHER IMPROVEMENT OF THE
MODIFIED COLORIMETRIC METHOD
FOR THE DETERMINATION OF
MALATHIONIII.1. INTRODUCTION

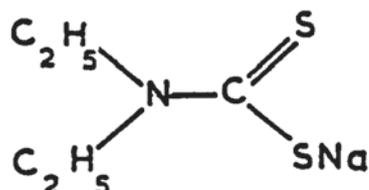
The modified colorimetric method for the determination of malathion described in the previous chapter has distinct advantages over all the existing methods in terms of the number of reagents required, the ease in application, avoidance of interferences and the stability of the colour for extended periods of time. The only disadvantage of the method is that the absorbance peak of the bismuth-DMDTP complex lies in the ultraviolet region (325 nm), and the full potential of the method can be exploited only when an expensive spectrophotometer is used. As this limits the use of the method for routine applications, it was thought desirable either to develop a new method for malathion having all the advantages of the modified method using bismuth, or to improve the method so that the final measurement of the absorbance can be made in the visible region. In the work described in this chapter, the latter approach was adopted.

The fact that the Bi-DMDTP complex extracts into carbon tetrachloride only from acidic solutions led to some preliminary studies with the yellow coloured organic extract. The following points were observed during these studies.

It was found that when the carbon tetrachloride solution of the yellow bismuth-DMDTP complex was shaken with pH 10 buffer solution, the yellow colour disappeared completely and the absorbance at 325 nm fell to zero. Also, when the buffer solution of this experiment was acidified (with conc. nitric acid) and shaken with another portion of the organic solvent (equal in volume to the original portion) the yellow colour was imparted to the latter. Most importantly, the absorbance of the second extract was the same as that of the first one.

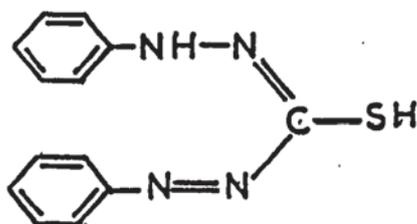
From these observations, it was concluded that when an organic solvent containing the bismuth-DMDTP complex is brought into contact with pH 10 buffer, the complex is completely broken up and the bismuth(III) and the DMDTP ions are quantitatively transferred to the aqueous layer. Thus, an alternative method for the colorimetric determination of bismuth could bring about the desired improvement. The only limitation required of such a method was that it should be applicable under the strongly alkaline conditions described above.

Fortunately, two well known reagents for the determination of bismuth fulfil these requirements (4). The first of these, sodium diethyldithiocarbamate (NaDEDTC) has a structure as shown below and occurs as a trihydrate while crystalline. The compound is soluble in water and less soluble in organic solvents.



The reagent was originally introduced for solvent extraction of copper but forms coloured complexes with several metal ions including bismuth, cobalt, copper, and iron, and these can be determined spectrophotometrically after extraction into an organic solvent. Besides direct and indirect spectrophotometry, flame photometry has also been applied to the extracts. The bismuth complex can be extracted into carbon tetrachloride at pH 4-11. The complex has a strong absorbance at 370 nm but the absorbance is usually measured at 400 nm.(4).

The other reagent of interest is called 'dithizone' which is more appropriately known as diphenylthiocarbazone. The compound is a purplish black solid soluble in chloroform and carbon tetrachloride but is insoluble in water and aromatic hydrocarbons. Dithizone exists in two tautomeric forms, the keto (or thione) and the enol (or thiol) forms, and it is the enol form which forms complexes with many metals most of which are strongly coloured and can be easily determined by spectrophotometry.



The bismuth complex of dithizone can be extracted into carbon tetrachloride at pH 3-10 and shows a maximum absorbance at 490 nm.

(NOTE: Although a comprehensive review of the reagents available for the colorimetric determination of bismuth was undertaken and is presented in Appendix IV, none of these could be used in the present work because of the low pH conditions at which they have to be used).

III.2. THE PRESENT WORK

Since the experiments for the determination of the extraction constants had previously indicated that bismuth forms a 1:3 complex with DMDTP (see Chapter II), both DEDTC and dithizone were first studied with 1.66×10^{-3} M bismuth(III) solution. Later, the same reagents were employed for the indirect determination of 5×10^{-3} M DMDTP (NOTE: 1:3 = $1.66 \times 10^{-3} : 5 \times 10^{-3}$).

The list of reagents for the experimental work reported in this chapter is as follows:

DMDTP Solution, 5×10^{-3} M: See page 42.

Bismuth reagent solution: See page 42.

Bismuth solution, 1.66×10^{-3} M: Dissolve 0.3880 g Bi_2O_3 (BDH Laboratory Reagent) in a few ml of conc. nitric acid and dilute to 100.0 ml with distilled water. Dilute 10.0 ml of this solution to 100.0 ml with distilled water.

Diethyl dithiocarbamate solution (DEDTC): Dissolve 1.0 g of the reagent (Hopkins & Williams 'Analar') in 100 ml of distilled water.

Dithizone solution: Dissolve 0.5 g of the reagent (Hopkins & Williams 'Analar') in 500 ml of purified carbon tetrachloride. Dilute 10 ml of this solution to 100 ml with carbon tetrachloride when required.

Carbon tetrachloride: Redistil commercial grade material and store in a glass bottle.

pH-10 Buffer solution: Dissolve 26.2 g ammonium nitrate in a minimum amount of deionised water, transfer to a 250 ml volumetric flask and add 142.5 ml A.R. ammonia (S.G. 0.88) solution. Make up to mark with water.

Malathion Analytical Standard solution: Dissolve 0.1046 g of the analytical standard (99.9 % purity, American Cyanamid Co.) in a few ml of ethanol and dilute to 100.0 ml with ethanol.

Malathion Emulsifiable Concentrate solution: Dissolve 0.1633 g of the 60% emulsifiable concentrate (Murphy Chemical Ltd.) in a few ml of ethanol and dilute to 100.0 ml.

The UNICAM SP6-100 spectrophotometer with 1.0 cm quartz cells was used for all experimental work.

(i) Extraction of Bi-DEDTC complex.

To a 50 ml separating funnel were added, 2 ml of the buffer solution, about 8 ml of distilled water, and 10 ml of carbon tetrachloride. After adding measured amounts (0.30---1.90 ml) of the 1.66×10^{-3} M bismuth(III) solution 1 ml of DEDTC was added and the funnel stoppered and shaken vigorously. The organic layer was allowed to separate and was then transferred to the 1 cm quartz cell, via a cotton wool plug placed in the stem of the funnel. The absorption of the complex was measured at 400 nm using carbon tetrachloride as a reference solution. The results are presented as solid circles in Fig. III-1, page 64.

(ii) Extraction of bismuth-dithizone complex

To the 50 ml separating funnel were added 2 ml of the buffer, 8 ml deionised water and 10 ml of the dithizone working solution. The funnel was stoppered and shaken in order to transfer the dithizone to the aqueous alkaline layer. The organic layer was discarded and the buffer solution shaken with another portion of pure carbon tetrachloride (10 ml). The two layers were allowed to separate, and before discarding the second organic layer, its absorbance at 495 nm was measured and this value was subtracted from all further readings. A third aliquot of carbon tetrachloride (10.0 ml) was added to the funnel followed by 0.01 and 0.02 ml of 1.66×10^{-3} M bismuth(III) solution. The contents of the funnel were shaken and the absorbance of the organic layer measured at 495 nm, after transferring it to the 1 cm

cell as described above. The results are presented as solid squares in Fig. III-1.

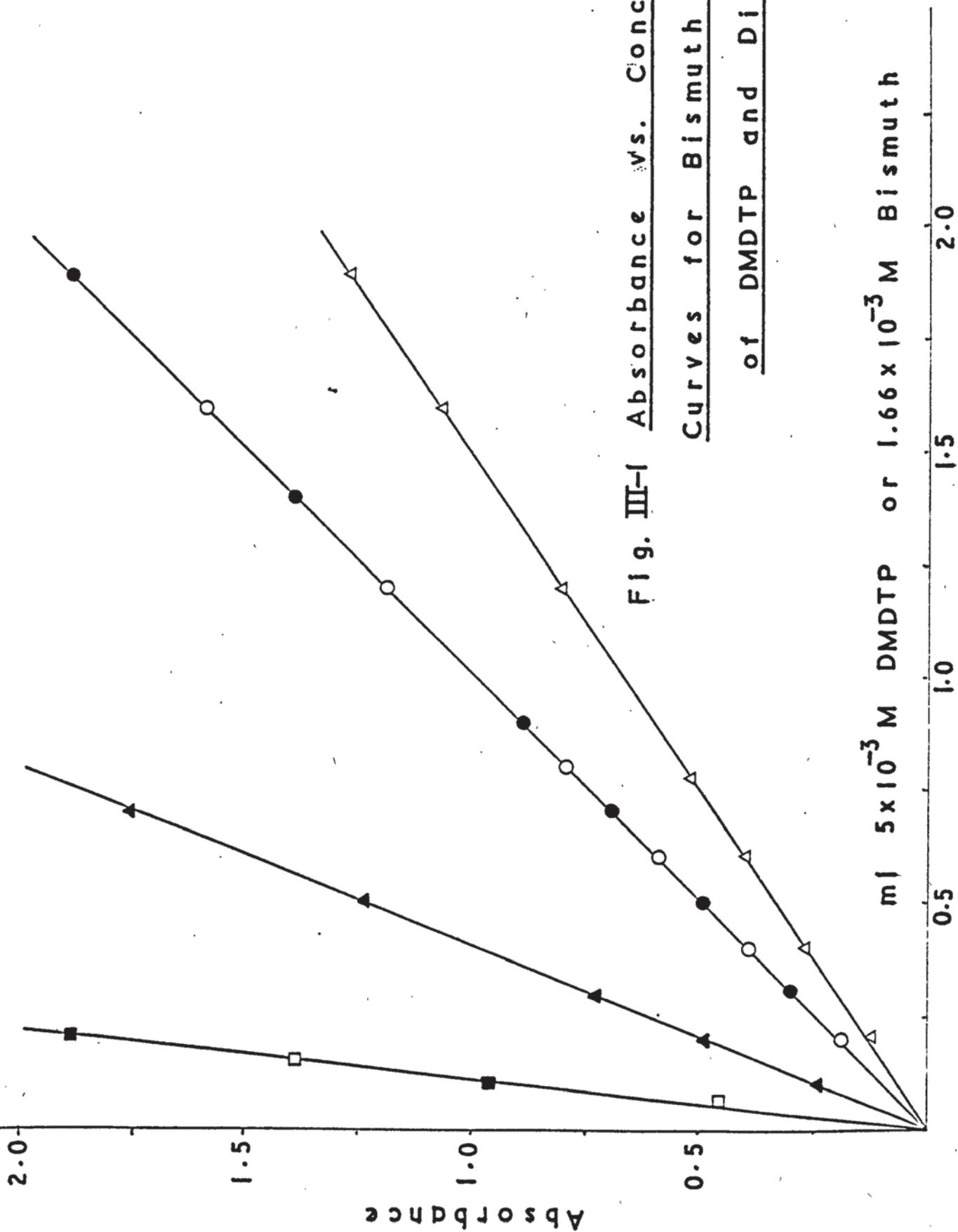
(iii) Indirect determination of DMDTP using DEDTC

The bismuth-DMDTP complex, using measured amounts (0.2 -1.6 ml) of 5×10^{-3} M DMDTP was extracted into 10 ml portions of carbon tetrachloride as described in Chapter II, page 43. The absorbance of the solution was measured at 325 nm and a portion of this solution was transferred to another 50 ml funnel containing the DEDTC reagent and the buffer solution (pH 10). (It is easy to see at this point that it is not important to transfer the carbon tetrachloride extract quantitatively to the second funnel). The contents of the second funnel were shaken and the absorbance of the organic layer measured at 400 nm as described in (i) above. The results are presented as open circles in Fig. III-1. :

(iv) Indirect determination of DMDTP using dithizone

Again, the bismuth-DMDTP complex (using 0.005 and 0.015 ml of 5×10^{-3} M DMDTP) was extracted into 10.0 ml carbon tetrachloride, as described in Chapter II, page 43, followed by the transference of part of the extract to a second funnel containing the dithizone in the buffer solution which had been purified by repeated shaking with the solvent (see (ii) above). The contents of the second funnel were shaken and the absorbance of the organic layer was measured at 495 nm. The results are presented as open squares in Fig. III-1.

Fig. III-1 Absorbance vs. Concentration
Curves for Bismuth Complexes
of DMDTP and Dithizone



ml 5×10^{-3} M DMDTP or 1.66×10^{-3} M Bismuth

(v) Analysis of the 60% Emulsifiable Concentrate

0.1 ml portions of the malathion standard solution (or the 60% emulsifiable concentrate solution) were hydrolyzed by mixing with 1 ml of 4 M sodium hydroxide solution in a 50 ml separating funnel. The solution was then made strongly acidic with 2 ml of 4 M nitric acid. The bismuth complex of the DMPTP produced by hydrolysis was extracted into 10.0 ml carbon tetrachloride. A portion of the organic extract was then shaken with the buffer solution containing the purified dithizone in a second funnel. The absorbance of the resulting orange-yellow organic solution being measured at 495 nm. The results of these experiments are presented in Table III-1. In order to compare the precision of the dithizone method and the bismuth method (Chapter II), 0.4 ml samples of the 60% emulsifiable concentrate were hydrolyzed and the bismuth complex extracted into carbon tetrachloride. The absorbance of the extract was measured at 325 nm.

The concentration of the emulsifiable concentrate was calculated as follows:

$$\begin{aligned} \text{malathion concentration} &= \frac{\text{weight of std.}}{\text{weight of conc.}} \times \frac{\text{av. abs. of conc.}}{\text{av. abs. of std.}} \\ \text{of the concentrate} &= \frac{0.1046}{0.1633} \times \frac{0.57}{0.60} = 60.9\% \end{aligned}$$

TABLE III-1Indirect Determination of Malathion using Dithizone

Absorbance readings of bismuth complexes			
Sample No.	Malathion Analytical	Malathion Emulsifiable	
	<u>Standard</u>	<u>Concentrate</u>	
	Bi-dithizone (495 nm)	Bi-dithizone (495 nm)	Bi-DMDTP (325 nm)
1.	0.58	0.57	0.60
2.	0.56	0.64	0.62
3.	0.54	0.58	0.61
4.	0.60	0.63	0.60
5.	0.58	0.59	0.61
Average	0.57	0.60	0.608
Standard Deviation	0.02	0.03	0.0075
Coefficient of Variation	3.5	5.0	1.2

III.3. DISCUSSION OF RESULTS

In addition to the bismuth complexes of DEDTC and dithizone, Fig. III-1 also represents the absorbance readings due to the bismuth-DMDTP complex both at 325 nm (solid triangles) and at 390 nm (open triangles).

The results shown in Fig. III-1 demonstrate the fact that the bismuth-DMDTP complex can be quantitatively converted to either the bismuth-dithizone complex or the bismuth-DMDTP complex, since the plots of the absorbance vs. concentration i.e. the open and closed squares and the open and closed circles fall respectively on two independent straight lines.

The other important information that can be obtained from Fig. III-1 is about the sensitivity of the two reagents. The use of DEDTC gives a slight improvement to the intensity of the colour but it is not sensitive enough. On the other hand, when dithizone is used, not only does an almost colourless carbon tetrachloride solution containing bismuth-DMDTP complex change to a bright orange coloured one, but the sensitivity is increased as well.

Thus, it is clear that by the inclusion of one more step in the modified colorimetric method, in which the final extract is shaken with dithizone solution, malathion could be determined even with simple filter photometers with much improved sensitivity.

Experiments with the emulsifiable concentrate gave a result which is in good agreement to that obtained by the copper and the bismuth methods (see Chapter II). The only drawback of the



dithizone method is the larger variation of the absorbances as compared to the direct bismuth-DMDTP measurements. As seen from the results in Table III-1, the dithizone method is three to four times less precise than the direct bismuth method. Thus, it is recommended that when using the dithizone method, at least five sample measurements should be made and an average value of the absorbance readings should be used.

CHAPTER IVEVALUATION OF THE MODIFIED
COLORIMETRIC METHOD FOR ITS
APPLICATION TO THE ANALYSIS
OF MALATHION RESIDUES IN WATERIV.1. INTRODUCTION

Since the introduction of the original colorimetric method for the determination of malathion by Norris et al. (10) it has been successfully used for the analysis of malathion residues in a variety of fruits, vegetables, cereals and animal products etc, (10—18).

Malathion residues in water have been determined mostly by gas chromatography. For example, Askew et al. (44) described a general and comprehensive scheme for the extraction of organophosphorus pesticides (including malathion), from river water and sewage effluents. The pesticides, after extraction with chloroform are determined by gas and thin-layer chromatography.

Similarly, Ripley et al. (45), developed a method for the determination of the pesticides in natural waters at less than p.p.b. concentrations. They determined the concentrated compounds by isothermal gas chromatography using a flame photometric detector that was quantitatively sensitive to phosphorus.

There seems to be no reference in the literature regarding the determination of malathion residues in water by any colorimetric method. Because of the ease in application and lower cost of the equipment involved, it was thought to be particularly desirable to evaluate the modified colorimetric method for the determination of malathion (see Chapter II) as regards its application to water analysis. A series of experiments were carried out in this context and these are described below.

IV.2. SEPARATION AND CONCENTRATION OF MALATHION FROM DILUTE AQUEOUS SOLUTIONS

Two of the most important techniques used for separating (and concentrating) pesticides and other trace organic compounds from water are solvent extraction and adsorption on solids. Methods employing solvent extraction are time-consuming and troublesome in their execution. On the other hand, rapid and efficient adsorption techniques for pre-concentration of trace organic contaminants have been reported using inert polymeric resins. A few examples are mentioned in the following text.

Riley and Taylor (46) found that when sea water was passed through a column of Amberlite XAD-1 resin, several classes of organic compounds including vitamins, surfactants, pesticides, dyes and humic acids were taken up quantitatively by the resin. These compounds could be recovered completely by elution with suitable solvents and determined by gas chromatography.

Burnham et al. (47) developed a method for extracting trace organic contaminants from potable water using Amberlite XAD-2 and XAD-7 macroreticular resins. They found that the resins extracted weak organic acids and bases and neutral organic compounds quantitatively from water solutions at p.p.b. to p.p.m. levels.

Glaze et al. (48) examined sewage wastewater effluent from a municipal treatment plant before and after laboratory chlorination. They concentrated neutral organic materials by adsorption on Amberlite XAD-2 resin followed by elution with diethyl ether.

Kennedy (49) described a process for treating chlorinated pesticide waste effluents. The pesticides were adsorbed on Amberlite XAD-4 polymeric resin, which could be regenerated with an organic solvent, and the adsorbed pesticides recovered in a concentrated form.

Junk et al. (50) detected organic contamination in the water which had flowed through tubes of polyethylene, polypropylene, black latex etc., by isolating the contaminants by sorption on Amberlite XAD-2 resin beads. The sorbed organic compounds were then concentrated by evaporation, and the organic contaminants were separated and measured quantitatively by gas chromatography.

Osterroht (51) described a method for extracting several chlorinated pesticides and other non-polar substances from sea water by sorption on Amberlite XAD-2. A similar procedure

has been developed by McNeil et al. (52) for monitoring organochlorine pesticides in potable water. Material adsorbed from water on to Amberlite XAD-2 resin was eluted with n-hexane and the concentrated eluate was analysed by gas chromatography.

Kim et al. (53) reviewed the published studies dealing with the use of synthetic resins for the removal of organic substances from water and presented results of experimental studies that were conducted to show in detail what factors affect the removal of weak aromatic acids by weak-base anion exchange resins.

In addition to the organochlorine pesticides, Amberlite XAD-2 resin has also been evaluated for multiresidue extraction of ng/l levels of several polychlorinated biphenyls (PCB's) from fortified distilled water and fortified and unfortified natural waters (54).

Recent work by Berkane et al. (55) with fenitrothion adsorption from river water is of special interest because they found that the pesticide remains stable on the resin column for extended periods of time, making the procedure suitable as a sampling and preservation technique.

With these considerations in mind, it was decided to investigate the use of Amberlite XAD-2 polymeric resin in conjunction with the modified colorimetric method for malathion as a suitable technique for the analysis of synthetic aqueous solution of malathion.

IV.3. THE PRESENT WORK

The present work concerns the development of a complete analytical scheme for the analysis of aqueous solutions of malathion at p.p.m. level. The approach that was adopted involved the adsorption of the pesticide on a column of Amberlite XAD-2 polymeric resin followed by its elution by an immiscible solvent. Malathion thus concentrated would then be hydrolysed to give dimethyl dithiophosphate (DMDTP), which in turn would be complexed with bismuth(III), the absorbance of the resulting complex being measured at 325 nm after extraction into carbon tetrachloride.

The reagents and apparatus used in the experimental work are listed below.

Reagents:

Malathion Standard Solution, 1.046 gm/l (3.17×10^{-3} M).

See page 61.

Dimethyl dithiophosphate (DMDTP) (3.17×10^{-3} M).

Dissolve 0.1752 g purified ammonium salt (Aldrich Chemical Company Ltd.) in distilled water and make up the volume to 100.0 ml. Dilute 31.70 ml of this solution to 100.0 ml with distilled water.

Bismuth Solution. See page 42.

Sodium hydroxide 6 M.

Dissolve 24 g material (BDH 'Analar') in 100 ml distilled water.

Sodium in Ethanol, 1% solution.

Dissolve 0.5 g freshly cut sodium in 50 ml ethanol.

Prepare daily and use fresh.

Diethyl ether. Technical grade without any further purification used.

Di-isopropyl ether. BDH Technical Material with 0.01% hydroquinone used without any further purification.

Carbon Tetrachloride. See page 42.

Amberlite XAD-2 Resin. Supplied by Rohm and Haas through BDH Ltd.

Apparatus.

Glass Columns.

2.5 cm x 50 cm fitted with teflon stop-cocks.

Spectrophotometer.

UNICAM SP-8 100 UV-visible spectrophotometer equipped with 1.0 cm quartz cells.

(ii) Hydrolysis of Malathion in Organic Solvents

Preliminary studies had indicated that if malathion is dissolved in carbon tetrachloride and then shaken with 6M-NaOH, as suggested by Norris et al. (10), the conversion of malathion to DMDTP is not quantitative. The hydrolysis of malathion obviously involves the pesticide's transfer from the organic layer to the aqueous layer where it is attacked by the hydroxide ion. The greater solubility of malathion in organic solvents as compared to water, is

obviously the controlling factor in this reaction. It was thought that if the hydrolysis could be carried out within the organic solvent, better results may be achieved. The DMDTP produced within the solvent could then easily be transferred to the aqueous layer by later shaking the organic solvent with water.

To find a suitable means of carrying out the hydrolysis of malathion within the organic solvent, recourse was made to the methods of titration in non-aqueous media. It is well known that alkoxide ions (RO^-) serve as powerful bases in non-aqueous titrations. These are produced by dissolving sodium or potassium metal in appropriate alcohols, (56). It was therefore decided to use a 1% solution of sodium in ethanol for the present work. To demonstrate the difference in the potential of the hydroxide and the ethoxide ions as hydrolysis agents for malathion, 1.0 ml of malathion standard solution was allowed to react with the two reagents in ethanol and also in carbon tetrachloride. The degree of hydrolysis was then estimated by measuring the absorbance of the bismuth-DMDTP complex. The actual procedure followed is described below.

(a) Hydrolysis of malathion in ethanol

1.0 ml of malathion standard solution ($3.17 \times 10^{-3} M$) was transferred to a 50 ml separating funnel followed by 1 ml of the hydrolysing reagents (6 M sodium hydroxide or 1% sodium in ethanol) and the mixture swirled gently for one minute. About 10 ml of distilled water

was then added and this solution made acidic with 2-4 ml of 6 M nitric acid. 10.0 ml of carbon tetrachloride was added followed by 1 ml of bismuth solution and the resulting complex extracted into the organic solvent by shaking for one minute. The absorbance of the carbon tetrachloride layer was then measured at 325 nm in the manner described previously (see Chapter II). Each experiment was repeated five times and the absorbance readings are presented in Table IV-1.

(b) Hydrolysis of malathion in carbon tetrachloride

To the 50 ml separating funnel, about 10 ml of carbon tetrachloride was added followed by 1.0 ml of malathion standard solution, and the contents mixed with gentle swirling. For the actual hydrolysis of malathion, slightly different procedures were adopted for the two reagents. For sodium hydroxide, 1 ml of the reagent was added followed by 10 ml of water and the contents of the funnel vigorously shaken for 1 minute. For sodium in ethanol, 1 ml of the reagent was added and the solution swirled gently for a minute, followed by the addition of 10 ml of distilled water and vigorous shaking. In both cases, after allowing the organic layers to separate, the carbon tetrachloride layer was disposed of. A fresh portion of carbon tetrachloride (10.0 ml) was added and the bismuth-DMDTP complex formed and extracted as previously described. The results of these experiments are also presented in Table IV-1, which also shows the absorbances due to the

TABLE IV-1

Hydrolysis of Malathion by the Hydroxide and Ethoxide
ions in Ethanol and Carbon Tetrachloride*

No.	1.0 ml malathion soln. in		1.0 ml DMDTP soln.		
	Ethanol		Carbon Tetrachloride		
	OH ⁻	OC ₂ H ₅ ⁻	OH ⁻	OC ₂ H ₅ ⁻	
1.	1.41	1.45	1.22	1.42	1.42
2.	1.42	1.45	1.20	1.41	1.42
3.	1.39	1.41	1.19	1.42	1.41
4.	1.41	1.42	1.15	1.44	1.39
5.	1.38	1.42	1.21	1.43	1.40
Average	1.40	1.43	1.19	1.42	1.41

* readings in absorbance units

bismuth-DMDTP complex formed from 1.0 ml of 3.17×10^{-3} M DMDTP solution.

(iii) Screening of Solvent for the Elution of Malathion from the Resin Column

It can be seen from Table IV-1 that a 1% solution of sodium in ethanol is the most suitable reagent for the hydrolysis of malathion, but it was important to test this reagent with the organic solvent to be used for the desorption of the pesticide from the resin column. Since diethyl ether has frequently been used for this purpose (38, 40, 44, 45), experiments were carried out to study the hydrolysis of malathion in this solvent as follows:-

1.0 ml of malathion standard solution (3.17×10^{-3} M) was dissolved in 10 ml diethyl ether, the resulting solution being treated in a way similar to the carbon tetrachloride solution in ii-b above. The absorbances of the final bismuth-DMDTP extracts are presented in Table IV-2 below,

Table IV-2

Hydrolysis of 1.0 ml Malathion Solution in Diethyl Ether and Diisopropyl Ether*

Solvent	1	2	3	4	5	average
Diethyl ether	1.26	1.27	1.28	1.32	1.32	1.29
Diisopropyl ether	1.41	1.42	1.39	1.40	1.40	1.40

* readings in absorbance units

From these results it is indicated that the amount of the bismuth-DMDTP complex obtained is less than the theoretical amount corresponding to an absorbance of 1.41. The most probable causes of this discrepancy (all arising due to the slight miscibility of the ether with water) are discussed later. It was concluded that this problem may not be encountered if diisopropyl ether is used because of its negligible miscibility with water. Hydrolysis experiments with malathion dissolved in the solvent were therefore carried out in a manner exactly as that described for diethyl ether. The results of these experiments are also presented in Table IV-2.

(iv) Recovery of Malathion from Aqueous Solutions

In this experiment three glass columns were filled with the XAD-2 resin up to a height of 10 cm. Each of the columns was washed successively with 50 ml ethanol, diethyl ether, ethanol and distilled water. 1.0 ml of malathion standard solution was dissolved in 2 l of distilled water and allowed to percolate through the column at an average rate of 20 ml per minute. When all of the water had passed through, the columns were allowed to drain for 10 minutes. The taps were closed and about 90 ml of diisopropyl ether was added to the columns and left to condition for a further period of 10 minutes, after which time the columns were drained into 250 ml separating funnels. The water was disposed of and the ether was transferred to 100.0 ml measuring flasks.

50 ml more ether was added to the columns and the ether allowed to pass through the columns directly into the flasks, until the latter reached the 100.0 ml mark. 10.0 ml portions of these well mixed solutions were then analysed for malathion by hydrolysing the pesticide with 1% solution of sodium in ethanol and extracting the bismuth-DMDTP complex into 10.0 ml carbon tetrachloride as previously described (Chapter II). The results of this experiment are presented in Table IV-3.

The percentage recovery for each column was calculated by using the following relationship:

$$\text{percentage recovery} = \frac{\text{average absorbance} \times 10}{1.40*} \times 100$$

* The value of 1.40 was taken from Table IV-2

(v) Storage of Malathion on the Resin Column

The experiment described above (iv) was repeated by eluting the malathion from the columns after a time period of four weeks after the aqueous solution had been passed through the columns. The results of this experiment are presented in Table IV-4.

Table IV-3

Elution of Malathion from the Resin Column with Diisopropyl
Ether, 10 ml Portions Analysed.*

Portion No.	Column I	Column II	Column III
1.	0.14	0.13	0.13
2.	0.14	0.15	0.12
3.	0.13	0.14	0.12
4.	0.13	0.13	0.13
5.	0.13	0.12	0.14
Average	0.134	0.134	0.128
Percentage Recovery	96	96	93

* readings in absorbance units

TABLE IV-4

Storage of Malathion on the Resin Column. Elution after
Four Weeks With Diisopropyl Ether. 10 ml Portions Analysed.*

Portion No.	Column I	Column II	Column III
1.	0.13	0.14	0.14
2.	0.13	0.13	0.14
3.	0.12	0.12	0.13
4.	0.15	0.12	0.13
5.	0.14	0.13	0.14
Average	0.134	0.128	0.136
Percentage Recovery	96	92	97

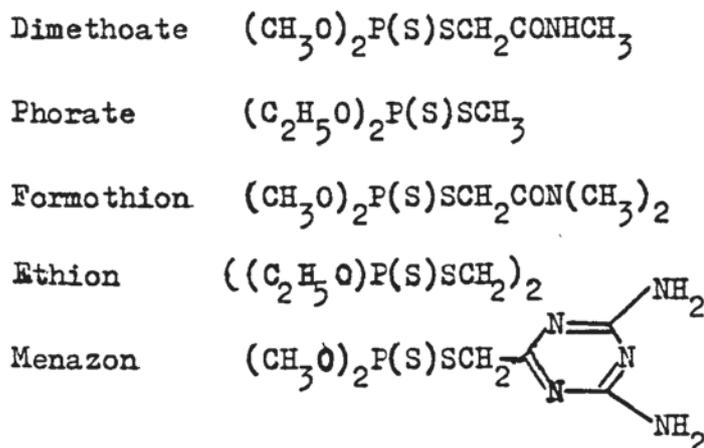
* readings in absorbance units

(vi) Determination of Malathion in Stream Water

The method developed above was applied to the analysis of agricultural run-off water sample collected from a stream in Newtown, North Wales. The water sample was brought to the laboratory in 3 litre plastic containers. The stream water (pH 7.5) was spiked with malathion at 50 p.p.b. level (0.1 ml of standard malathion solution was dissolved in 2 l water sample). This water sample was then analysed for malathion in the manner described in the previous pages, the only difference being that the whole of the 100 ml ethereal sample was hydrolysed instead of 10 ml samples. The average recovery of the pesticide from three experiments was 95 per cent. No malathion was detected in unspiked sample.

(vii) Interferences

Since the following organophosphorus pesticides do not participate in the hydrolysis/complexation reaction responsible for the Bi-DMDTP complex, which is the basis of the method for the determination of malathion, they are not expected to interfere in the present method. The pesticides are



A 10 p.p.m. solution (2 l) of NH_4DMDTP when passed through the resin column did not show any DMDTP in the ether extract, thus demonstrating the fact that free dimethyldithiophosphate is

not likely to interfere as well. This is to be expected since dimethyldithiophosphoric acid like the diethyl and the dibutyl acids (pK_a -1.10 and 0.22 respectively (4)) would behave as a reasonably strong acid and as such would not be adsorbed on the inert resin from aqueous solutions of pH values normally encountered in environmental samples.

IV.4. DISCUSSION OF RESULTS

The results in Table IV-1 indicate that for malathion in ethanol, both the 6 M sodium hydroxide and 1% sodium in ethanol convert the pesticide quantitatively into its hydrolysis products. On the other hand, if malathion is dissolved in a solvent which is immiscible with water, the hydrolysis reaction with sodium hydroxide is not quantitative, confirming the preliminary doubts about the usefulness of sodium hydroxide and indicating the replacement of the hydroxide by a 1% solution of sodium in ethanol in the standard procedure for the analysis of malathion is advantageous. Results of Table IV-2 show that the absorbance due to the Bi-DMDTP complex from malathion in diethyl ether is less than is theoretically possible. This may be due to the slight miscibility of diethyl ether with water, so that when the ether is shaken with water following the hydrolysis of malathion, and the organic solvent is disposed of, it may be possible that some DMDTP is also drawn off. Also, the slight miscibility of ether with water means that the ether in the aqueous layer ultimately gets transferred to the carbon tetrachloride layer, thus diluting the final extract and lowering the absorbance reading.

Results for malathion dissolved in diisopropyl ether on the other hand are very near to the theoretical absorbances. Therefore, the use of diisopropyl ether as a potential eluent for malathion from the resin column is advisable. These findings are confirmed by the next experiment (Table IV-3, page 81). These results show that the average recovery of malathion from dilute aqueous solutions is 95 per cent when the pesticide is adsorbed on the resin and later eluted with diisopropyl ether.

Results of experiments involving the storage of malathion adsorbed on the resin for four weeks showed no detrimental effects. This is of particular significance because it could make the collection of samples from polluted waters much easier in that a large volume of water can be passed through the resin, draining the column and transporting it to the analytical laboratory for later elution and analysis. This method of sample collection could possibly overcome many of the difficulties faced at present in this field.

Some of the most obvious advantages are the ease in transportation in terms of glass-ware; chemicals and space; and extension of time periods for which the sample collector can stay away from the laboratory and possibly collect a larger number of samples in one tour, thus saving time and money.

Although the method described above (which uses a 2 l water sample) is suitable for the determination of malathion :

at 0.5 p.p.m. level, malathion at lower levels may be estimated by using a larger water sample (20 -30 l) and by using all of the 100 ml eluted ethereal sample (as was done for the stream water in the present study) instead of analysing 10 ml portions. Sensitivity could further be improved by the use of a 4 cm cell instead of a 1 cm cell for the absorbance measurements.

CHAPTER V

DETERMINATION OF MALATHION BY
INDIRECT ATOMIC ABSORPTION
SPECTROPHOTOMETRY

V.1. ATOMIC ABSORPTION SPECTROPHOTOMETRY - AN INTRODUCTION

The phenomenon of atomic absorption was first noticed by Wollaston in 1802, when he observed a few dark lines in the solar spectrum. In 1814, Fraunhofer observed about 700 lines in the spectrum of the sun, and, although he could not explain the origin of these, he made a complete map of the lines and assigned the letters A to H to the most dark ones (including the so called sodium D lines).

It was Kirchoff who in 1860 established the basic principles underlying atomic absorption. Kirchoff showed that a flame containing sodium chloride would not only emit the yellow sodium D lines, but also absorb the same yellow light from a continuous source placed behind the flame. Thus, the Fraunhofer lines were attributed to the absorption by certain elements present in the cooler outer atmosphere of the sun, of the continuous spectrum emitted by the hot interior. Kirchoff emphasised the great significance of the characteristic spectrum of the different elements and the foundation of analytical spectroscopy was laid.

Although emission methods of analysis (flame, arc & spark) became

well established, it was not until 1953 that Walsh realized the analytical potential of atomic absorption and demonstrated the advantages of the new technique. The first commercial instrument appeared in the early sixties, and since then no other technique has been found to be better for the determination of a large number of metallic elements. It is only very recently that atomic absorption is facing a strong rival in inductively coupled plasma emission technique which, if linked to a suitable computer can determine upto 70 elements in a single sample in less than five minutes.

(i) Theoretical Aspects of Atomic Absorption Spectrophotometry

When electromagnetic radiation characteristic of electronic transitions in the outer orbitals of atoms of a particular element is passed through an atomic vapour of that element, the intensity of radiation at certain frequencies is decreased. The absorbed radiation excites electrons from the ground state to various higher energy levels and the degree of absorption is a quantitative measure of the concentration of ground-state atoms in the vapour. The energy changes involved correspond to radiation in the ultraviolet and visible regions of the spectrum. As only atoms in the ground state will absorb the radiation, the conditions used for volatilising and decomposing the sample to produce an atomic vapour must induce a minimum of ionization. This can be achieved by flame excitation where temperatures in general do not exceed about 3000°K . At this temperature, for most of the elements, practically 100% of atoms will be in the ground state.

The extent to which the radiation of a particular frequency is absorbed by an atomic vapour is related to the length of the path traversed and to the concentration of the absorbing atoms in the vapour. The Beer-Lambert law applicable to the solution spectrophotometry can also be used in this situation. Thus, for a collimated, monochromatic beam of radiation of incident intensity I_0 passing through an atomic vapour of thickness l ,

$$I_\nu = I_0 e^{-k_\nu l}$$

where I_ν is the intensity of the transmitted radiation at frequency ν and k_ν is the corresponding absorption coefficient. The value of k_ν is determined by the concentration of the atoms which can absorb at frequency ν is given by the expression

$$\int k_\nu d\nu = \frac{\pi e^2}{m c^2} N_\nu f$$

where m and e represent the mass and charge of the electron, N_ν is the number of atoms per cm^3 capable of absorbing radiation of frequency ν and f is the oscillator strength defined as the number of electrons per atom capable of being excited by the incident radiation. Hence, for transitions from the ground state, the integrated absorption is proportional to N_ν , which approximates to the concentration of the element in the sample.

Measurement of integrated absorption requires a knowledge of the absorption line profile. At 2000 to 3000 $^\circ\text{K}$, the overall line width is about 10^{-2} nm which is extremely narrow when compared to absorption bands observed for samples in solution. This is to be expected since changes in molecular energy are accompanied by rotational and vibrational changes, and in solution, collisions

with solvent molecules cause the individual bands to coalesce to form broad band-envelopes. The overall width of an atomic absorption line is determined by a variety of factors and these will be discussed now:

(a) Natural Broadening

Natural broadening is due to finite lifetime of the atom in the excited state, and according to Heisenberg's uncertainty principle there will be some error in the energy of the state. The result is the broadening of the absorption line which is independent of the environment of the atom. For most resonance lines the natural width is of the order of 10^{-5} nm which is negligible compared to other causes.

(b) Doppler Broadening

This kind of broadening is caused by the absorbing or emitting atoms having different component velocities along the line of observation. The broadening is symmetrical about the mean wavelength of the line. The Doppler half-width is proportional to the square root of the absolute temperature and directly proportional to the wavelength of the centre of the absorption line. Hence, lines in the visible region have a greater Doppler half-width than lines in the ultraviolet. These half-widths may range from 10^{-4} to 10^{-2} nm.

(c) Collisional Broadening

Also known as pressure or Lorentz broadening, it originates due to the perturbation of the absorbing or emitting atoms by collisions with foreign gas atoms. The effect of this type of broadening varies for different foreign gases and for different

atomic states. Collisional broadening can cause a shift of the wavelength of the centre of the absorption line profile and can also cause assymetry of this profile. Thus, the centre of a given line in high-pressure system, for example a conventional flame, can be at a slightly different wavelength relative to the same line emitted by a low-pressure system such as a hollow-cathode lamp. Fortunately, these wavelength shifts are relatively small when compared to the total width of absorption line profiles in flames. The assymetry of most resonance lines in conventional flames is also thought to be small.

(d) Stark and Zeeman Broadening

Stark and Zeeman broadening effects are caused by electric and magnetic fields respectively. Although Stark broadening can cause lines to become diffuse in the arc and spark, both the Stark and Zeeman effects are negligible in flames but, can possibly be important in carbon furnace atomisation. It is also probable that the Stark effect could very slightly influence the lines from a hollow cathode lamp. But at voltages and currents employed in most lamps this is thought to be insignificant.

(e) Hyperfine Structure Broadening

Hyperfine structure, which is not an actual broadening process, can be attributed to non-zero value of the nuclear spin and/or the presence of several isotopes. Thus, each line consists of a number of separate hyperfine components each acting as an independent line. Although hyperfine splitting is negligible compared to absorption line half widths, it may not always be true.

(ii) Basic Instrumental Design

An atomic absorption spectrophotometer consists essentially of the following components:

- (a) A stable light source, emitting the sharp line of the element to be determined. The emission from the light source is modulated, so that its radiation only, and not from the flame, will be recorded in the readout signal.
- (b) A flame (or electrothermal) system which is of sufficient temperature to produce an atomic vapour of the required species from the compounds present in the solution.
- (c) A monochromator to isolate the resonance line and focus it upon the photomultiplier system.
- (d) A photomultiplier system that detects the intensity of the light energy falling upon it, and which is followed by facilities for amplification and readout of the modulated light output from the hollow cathode lamp.

Sharp-line Source

Ideally, the emission line of the source should have a half-width less than that of the corresponding absorption line. The most suitable and widely used source which fulfils this requirement is the hollow cathode lamp, although a micro-wave-excited electrodeless discharge tube can also be used.

A hollow cathode lamp consists of a sealed glass envelope with a quartz end-window, and containing a hollowed-out cylindrical cathode of some 2 mm internal diameter together with a tungsten wire anode. The cathode is fabricated from the element to be determined. By reducing the pressure inside the envelope to

about 1.3 kN m^{-2} and passing a current of 5 to 50 mA at an applied voltage of about 300V, a low-pressure glow-discharge confined to the inside of the cathode and characteristic of the cathode material is produced. The action of the gas used to fill the envelope is to bombard the cathode thereby vaporizing atoms from the surface by the process of 'sputtering'. The resulting emission spectrum contains lines from both the cathode material and the filling gas. The choice of the gas is therefore limited to those whose emission lines do not coincide with lines from the cathode itself and which will not ionise the sputtered atoms. Neon or argon are the most commonly used gases for this purpose.

In the case of the electrodeless discharge tube, radiation is derived from a sealed quartz tube containing a few milligrams of an element or a volatile compound and neon or argon at low pressure. The discharge is produced by a microwave source via a waveguide cavity and the emission spectrum of the element concerned contains only the most prominent lines with intensities up to one hundred times those derived from a hollow cathode lamp. The reliability of such sources is questionable however.

Sample Vaporization

The production of a homogeneous atomic vapour from a sample is achieved by aspirating a solution into a flame or evaporating small volumes in an electrically heated tube furnace. In all cases, the thermal energy supplied must be sufficient to evaporate the solvent and to dissociate the remaining solids into

their constituent atoms without causing appreciable ionisation.

In the flame vaporization system, the sample is drawn first into a nebulizer by the flow of support gas where it forms a mist or aerosol. Fuel gas is introduced and the mixture passed to a spray chamber where large droplets condense and run to waste. The resulting homogeneous mixture of sample droplets and gases pass to the burning chamber for combustion. The burner consists of a metal block containing a row of circular holes or one or more slots about 10 cm long. It is aligned along the optical axis of the instrument and just below the beam from the lamp so as to provide a flame of long absorption path and hence maximum sensitivity. The most generally useful flame is air-acetylene with a temperature of about 2500 °K but sometimes the cooler air-propane or the hotter nitrous oxide-acetylene flames are also used.

For flameless atomisation, two types of systems are in use i.e. the graphite tube furnace and the carbon rod or filament. In both cases, the temperature is raised rapidly to about 2500 °K by the passage of a heavy current for a period of 1 to 2 minutes. Tube furnaces, which are usually about 5 or 10 cm x 3 cm, may be flushed with argon before vaporizing the sample so as to prevent the formation of refractory oxides. The axis of the furnace is aligned along the optical path of the radiation from the lamp and as the vaporized sample is contained within this small region, maximum sensitivity can be achieved.

The sample (5 to 10 μ l) is deposited on the bottom inner surface

of the tube near the centre and the temperature raised to about 2500 °K from cold within one or two minutes. The heating cycle can be controlled so as to allow solvents to evaporate or the organic residues to be ashed before raising the temperature rapidly to that required to produce an atomic vapour.

The particular advantages of flameless atomisation as compared to the flame method are the elimination of interference effects resulting from interactions between the sample and components of the flame, increased sensitivity and the ability to handle very small samples such as clinical samples. Among the few drawbacks of the technique is the fact that elements forming carbides can not be determined.

(iii) Operation of an Atomic Absorption Spectrophotometer

In operation, the readout is adjusted to read zero absorbance when a blank solution is atomized, and the light of the hollow cathode lamp passes on to the detector. When a solution containing the absorbing species is introduced, part of the light is absorbed, resulting in a diminution of light falling upon the photomultiplier and giving rise to a change in signal which may be directly displayed on a readout or as a trace on a chart recorder.

Standard solutions of the element to be determined are employed to construct a calibration curve from which the contents of test solutions can be obtained. In some cases where the matrix of the sample presents special interference problems, the method of standard additions may be used.

(iv) Application of Atomic Absorption Spectrophotometry

Atomic absorption is one of the most widely used techniques for the determination of over sixty metallic elements in a variety of matrices. Examples of such determination include heavy metal determination in body fluids, polluted waters, foodstuffs, soft drinks and beer, the analysis of metallurgical and geochemical samples and the determination of many metals in soils, crude oils, petroleum products and plastics.

V.2. INDIRECT DETERMINATION OF ORGANIC COMPOUNDS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

Ever since the introduction of atomic absorption spectrophotometry as an analytical technique for metals, many attempts have been made to extend its applicability to the determination of non-metals and organic compounds. Thus, many examples of indirect determination of organic compounds have been reported a few of which are being quoted here:

Kumamaru et al. (57) determined phthalic acid by selectively extracting the acid with cuproine (2,9-dimethyl-1,10-phenanthroline) dissolved in MIBK from aqueous solutions containing copper (I) ions. The analysis was completed by measuring the absorbance of the copper atoms. Kumamaru et al. (58) also reported the use of neocuproine for the same determination.

Yamamoto et al. (59) found that the anions of pentachlorophenol could be extracted into nitrobenzene from aqueous solutions containing an excess of tris(1,10-phenanthroline)-iron (II) cations. The phenol was determined by measuring the absorbance

of the iron atoms in the organic extract.

Yamamoto et al. (60) also developed a method for the determination of β -hydroxynaphthoic acid, which is used mainly as an intermediate of dyestuffs. The procedure involves the selective extraction of tris(1,10-phenanthroline)nickel(II) β -hydroxynaphthoate into nitrobenzene followed by determination of the nickel content in the extract by atomic absorption.

The reaction of secondary amines with carbon disulphide to yield dialkyldithiocarbamic acids has been found to be analytically useful (61). The acid so formed can be complexed with nickel, the resulting precipitate separated from the reaction mixture by filtration. The precipitate is digested in a mixture of HCl:HNO₃ (1:1). The resultant solution is then analysed for nickel by means of atomic absorption spectrophotometry.

Atomic absorption methods for the determination of surfactants, soaps and detergents have been reported. In a method due to Sheridan et al. (62), the detergent is precipitated as a heteropoly phosphomolybdic acid-barium complex and the molybdenum in the supernatant solution is assayed by atomic absorption.

Gegiod (63) determined soap as sodium, in alkali-refined vegetable oils. The oil was treated with absolute ethanol, the mixture dissolved in ethyl methyl ketone and the resulting solution aspirated into an atomic absorption burner. Similarly, Crisp et al. (64) have described a method for the determination of anionic detergents at concentration levels below 50 μ g/l. The detergent

ions are extracted into chloroform as an ion-association complex with bis(ethylenediamine)copper(II) cation and the determination is completed by atomic absorption spectrophotometry with a graphite furnace.

Treguer et al. (65) proposed a new method for the determination of total dissolved free fatty acids in sea water. After preliminary extraction by chloroform, a copper complex is formed which is then extracted by a heptane-chloroform solution. The copper content of the extract is then determined by atomic absorption of the metal ions.

The technique of indirect determination of organic compounds by atomic absorption spectrophotometry has been found useful in clinical research. For example, the measurement of total urinary amino acids can be done by a semi-automatic method based on the formation of a copper complex followed by the determination of copper by atomic absorption (66, 67). Similarly, Menache (68) developed a method for the determination of oxalic acid in urine. The acid is precipitated with calcium ions at pH 5; the excess of calcium present in the supernatant and the total calcium added and present in the urine at pH 2 are determined by atomic absorption. The oxalic acid content of the precipitate is calculated indirectly from the difference between the two determinations of calcium.

Diaz (69) described a method for the determination of vitamin B₁₂ which involves the atomic absorption of cobalt atoms, since one

atom of cobalt is contained in each molecule of the vitamin.

Oles and Siggia (70) used silver-ammonia complex (Tollen's reagent) to oxidise aldehydes to their corresponding carboxylic acids. The reduced silver was separated from excess reagent, dissolved in nitric acid, and the resultant solution analysed for silver using atomic absorption. This resulted in the development of a new method for the determination of aldehydes.

Oles and Siggia (71) also accomplished the determination of 1,2-diols by atomic absorption spectrophotometry. They oxidised the adjacent hydroxyl groups with periodic acid. The iodate formed in the reaction is separated from the reaction medium by precipitation as silver iodate. After removal of excess silver from the reagent by filtering, the silver iodate is dissolved in ammonium hydroxide and the resultant solution is analysed for silver content by means of atomic absorption spectrophotometry.

An atomic absorption method was developed by Hurtubise (72) for the determination of disodium-edetate dihydrate in the drug streptomycin. The method involves the formation of a nickel-dihydrate complex, release of the complexed nickel by pH adjustment and the determination of nickel by atomic absorption.

Senise and Silva (73) quantitatively extracted perrhenate into MIBK from aqueous solutions containing copper(II), azide and an excess of 2,2'-bipyridine. Measurement of the extracted copper by atomic absorption then led to the determination of perrhenate.

Jones and Manahan (74) used high speed liquid chromatography in conjunction with atomic absorption to separate and determine the copper chelates of amino acids such as EDTA, NTA, EGTA and CDTA. They claim that their method is applicable to the analysis of individual chelating agents such as those used in industrial water treatment, food processing, metal cleaning and pharmaceuticals.

An indirect atomic absorption method for the determination of biuret in mixed fertilizers and urea has been reported by Woodis et al. (75).

Lastly, mention must be made of the large amount of work reported in the Japanese literature dealing predominantly with drug analysis.

The above examples indicate that the frontiers of the technique of atomic absorption spectrophotometry are being rapidly advanced to include the determination of a wide variety of organic compounds. In spite of this, there seems to be no reference in the literature dealing with the indirect atomic absorption determination of an important class of compounds, that is, the organophosphorus pesticides. In the present work, such an analytical method for one of these compounds namely malathion is described.

V.3. THE PRESENT WORK

(i) In order to gain experience in using the atomic absorption spectrophotometer, it was decided to prepare a calibration graph of absorbance against concentration of aqueous solutions of bismuth using the Perkin Elmer 303 instrument. For this purpose, a stock solution of bismuth containing 1,000 mg per litre bismuth(III) was prepared by dissolving 0.1115 g Bi_2O_3 (BDH Laboratory Reagent) in 2 ml conc. HNO_3 and diluting to 100.0 ml with deionised water. 0.0 to 500 mg/l concentration solutions were prepared by diluting 0.0 to 5.0 ml of stock solution to 10.0 ml. Using deionised water as blank, the bismuth solutions were aspirated into the burner and the percentage absorption of light (originating from an Activion hollow cathode lamp) was measured at 307 nm. The percentage absorption was converted to absorbance values by using the following relationship:

$$\text{absorbance} = \log \left(\frac{100}{100 - \text{percentage absorption}} \right)$$

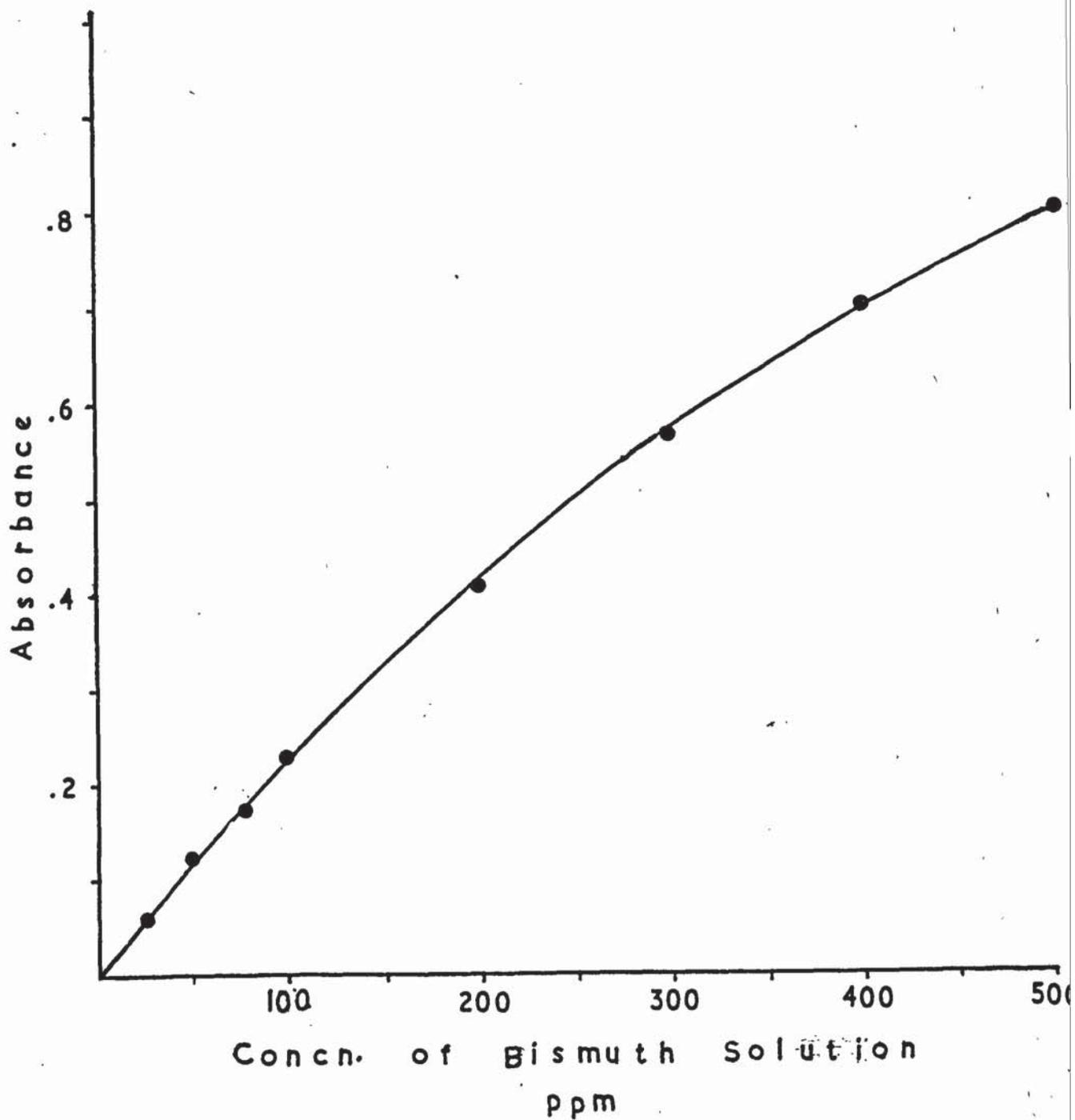
The absorbance values so obtained were plotted against the concentration of bismuth, as shown in Figure V-1.

(ii) Atomic Absorption of Bismuth as Bismuth-DMDTP Complex.

In this case, the bismuth-DMDTP complex was formed as explained in the previous pages (e.g, 43-47), and extracted into 10.0 ml of MIBK. For this purpose, 0.0 to 2.0 ml of 5×10^{-3} DMDTP solution were used. The organic solvent was then aspirated into the flame in the recommended procedure and the

Fig. V-1

Atomic Absorption of Bismuth
in Aqueous Solution



percentage absorption values noted. After converting the percentage absorption into absorbance, the latter were plotted against the volume of 5×10^{-3} M DMDTP. The result is shown in Figure V-2. (The fuel used was acetylene at a flow rate of 6.5 l/min and the support gas was air at 8.5 l/min).

(iii) Determination of Malathion by Atomic Absorption

Spectrophotometry

The reasonably linear graph of Fig. V-2 indicated the feasibility of determining malathion by indirect atomic absorption spectrophotometry. For this purpose, the 60% emulsifiable concentrate was tested. The following solutions were used. Malathion Standard Solution. Dissolve 0.1046 g malathion analytical standard (American Cyanamid Company) in 100 ml ethanol.

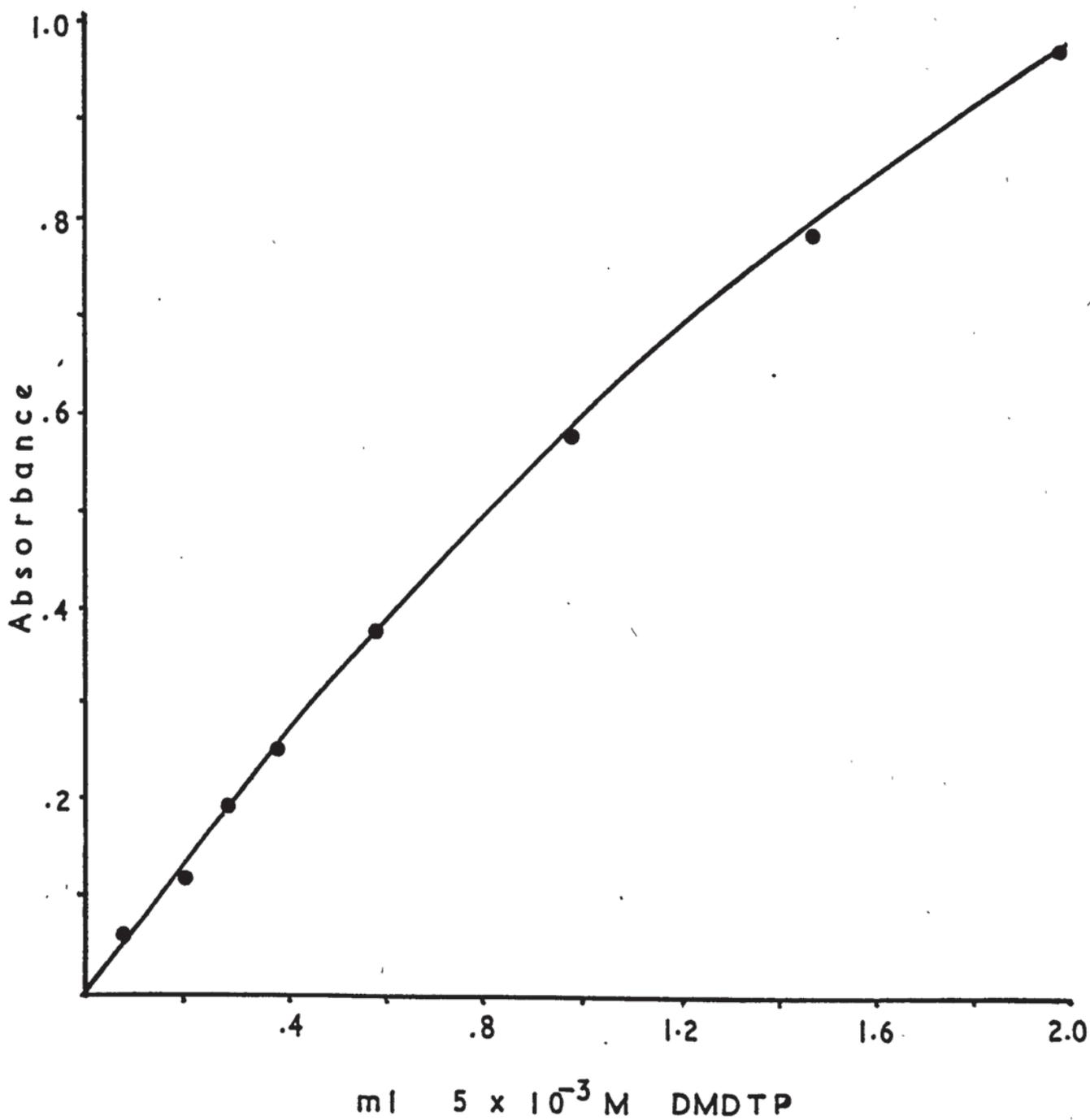
Malathion 60% Emulsifiable Concentrate Solution.

Dissolve 0.1633 gm concentrate (Murphy Chemical Ltd.) in 100 ml of ethanol.

5.0 ml of the malathion standard solution (or emulsifiable concentrate) was hydrolysed with 6 M sodium hydroxide, the resulting DMDTP complexed with bismuth and extracted into 10 ml MIEK. The absorbance of the organic extract was determined as above. Using the average absorbances of five samples of the standard and the concentrate, the concentration of the emulsifiable concentrate was found to be 60.1 %.

Fig. V-2

Atomic Absorption of Bismuth
as Bi-DMDTP Complex in MIBK



(iv) Attempts to Determine Malathion by Atomic Absorption
using Electrothermal Atomisation

In addition to determining malathion by flame atomic absorption spectrophotometry, attempts were also made to use graphite furnace atomization to achieve the same objective. The main purpose for doing this was to increase the sensitivity of the method. These attempts were not successful even after many repetitions; the reproducibility of the signal produced was extremely poor and could possibly ^{be} attributed to the fact that the instrument in use (Perkin Elmer 303) is one of the earliest models introduced by the company, and as such is not now in a very good condition after many years of use.

After many weeks of work, using up about two dozen graphite tubes it was thought best to postpone further work until a better instrument is available. Whereas the newer instruments are much cheaper to run the present one used graphite tubes each of which costs £5.00 (1979 price).

V.4. DISCUSSION OF RESULTS

Experiments with the aqueous solutions of bismuth gave an almost linear plot for absorbance versus concentration. Similar results with the bismuth-DMDTP complex in MIBK indicated the feasibility of using atomic absorption spectrophotometry for the indirect determination of malathion.

Application of the technique of atomic absorption to the analysis of 60% malathion emulsifiable concentrate proves the usefulness of the new technique.

A comparison of the concentration range of the DMDTP from Figures V-2, page 104 and II-3, page 46 shows that the atomic absorption method is very much less sensitive than the colorimetric method. It is hoped that with newer instruments better results might be achieved and that malathion could be determined with increased sensitivity. But, at present, the atomic absorption method does not offer any particular advantage over the colorimetric method. The technique is therefore of limited use although it does portray an important principle, that organophosphorus pesticides could be determined by atomic absorption spectrophotometry if they can be broken down to suitable metal complexing products. This could be of great importance in developing analytical methods for those compounds for which no suitable method exists at present.

C O N C L U S I O N S

The most important observations from the work reported in the foregoing pages can be summarised as follows:

1. Replacement of copper(II) by bismuth(III) in the recommended colorimetric method for the determination of malathion has distinct advantages. It overcomes the problems associated with the recommended method and has an accuracy at least equal to that of the copper method.

2. The modified colorimetric method for malathion using bismuth(III) lacks the desired sensitivity in the visible region of the electromagnetic spectrum, thus limiting the use of the method. This problem can be overcome by inclusion of an extra step to the modified method in which the bismuth-DMDTP complex is quantitatively transformed into an equivalent concentration of the bismuth-dithizone complex. This not only imparts an orange-yellow colour to an almost colourless solution but makes the method four times more sensitive. The improved method is much less precise than the copper or the bismuth methods.

3. The modified colorimetric method has been found to be feasible for the analysis of technical malathion and synthetic solutions of the pesticide in water.

4. The method of concentrating malathion from dilute aqueous solutions, by adsorption on Amberlite XAD-2 polymeric resin is to be recommended. It not only gives excellent recoveries but the pesticide does not alter its nature on the resin even after a period of four weeks.

5. When malathion from water (or any other matrix) has been concentrated into an immiscible organic solvent, it is recommended that a 1% solution of sodium in ethanol be used for the hydrolysis of the pesticide instead of the commonly used 6M sodium hydroxide solution. This not only converts malathion quantitatively into dimethyl dithiophosphate but also gives results with better reproducibility.

6. For the first time it has been successfully demonstrated that the technique of atomic absorption spectrophotometry can be used for the indirect determination of an organophosphorus pesticide. Although, for malathion, the method does not offer any particular advantages over the colorimetric method, it is an important observation in itself. Apart from offering an alternative method for malathion determination, the present work widens the scope and applicability of an important analytical technique which has previously been demonstrated to be useful in the determination of various other classes of organic compounds.

7. Although the methods described above do not compare with the techniques of GLC and HPLC in their ability to be useful for multi-residue analysis, they do provide considerable improvements in existing methods for those laboratories which are not necessarily engaged in multi residue work but require independent analytical methods for quantitative analysis of malathion.

SUGGESTIONS FOR FURTHER WORK

Many interesting points have arisen from the work reported in this thesis. Some of the work worthy of further attention, and possibly leading to some useful results, is described here.

1. In theory, all of the organophosphate pesticides should, on hydrolysis, yield species which could complex with metals. Even if the resulting complexes are not coloured, the pesticides could be estimated indirectly by the ligand exchange technique using dithizone, or by atomic absorption spectrophotometry. Thus, a comprehensive study of a large number of the pesticides might possibly lead to the development of newer analytical techniques for many of these compounds.

2. The application of the colorimetric method to the analysis of malathion residues in synthetic aqueous solutions has been demonstrated. It remains to be seen how the colorimetric method works for the analysis of actual field samples of polluted waters. Also, the method should be tested for application to the analysis of malathion residues in fruits, vegetables and cereals etc. Thus, it is critically important that a collaborative study be carried out regarding the replacement of the recommended method for malathion determination by the modified colorimetric method using bismuth.

3. The idea of ligand-exchange reaction is very important. It has rarely been used in analytical chemistry but promises much hope for the development of new methods and improvement

of the existing colorimetric methods for many organic compounds.

4. Malathion, like many other pesticides, can be determined with high sensitivity by gas chromatographic techniques. But, interferences from other organic compounds present in the matrix can be a problem. It might be possible to improve this situation by a combination of techniques, since the hydrolysis reaction of malathion is fairly selective and gas chromatography of the metallic complexes of dialkyl dithiophosphates has been reported in the literature. Thus, by linking the two processes of hydrolysis/complexation and gas chromatography, an improved method for the determination of malathion can be developed.

A P P E N D I X ISTABILITY CONSTANT OF NICKEL-GLYCINE COMPLEXESINTRODUCTION

The equilibrium constant 'K' for any reaction is related to the change in free energy of the reaction by the following relationship:

$$\Delta G = -RT \log_e K$$

The equilibrium constant is thus a direct measure of the degree of completion to which the reaction can proceed.

For the reaction $A + B \rightleftharpoons C + D$ the equilibrium constant is defined as:

$$K = \frac{a_c \times a_d}{a_a \times a_b} = \frac{\gamma_c \cdot \gamma_d}{\gamma_a \cdot \gamma_b} \cdot \frac{(C) \cdot (D)}{(A) \cdot (B)}$$

where 'a' indicates the activity of the species and 'γ' the activity coefficient. The brackets indicate the molar concentrations.

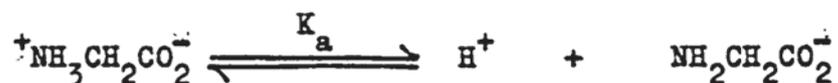
The evaluation of the activity coefficients is usually difficult and seldom done. Activity coefficients usually depend upon the ionic strength of the solution but at infinite dilution $\gamma = 1$, and concentrations and activities become equal. As it is practically impossible to work at infinite dilution, the usual procedure for the evaluation of the equilibrium constant is to keep the ionic strength of the reaction medium constant and to use the molar concentrations instead of the activities. For the determination of the true equilibrium constant, the experiment is performed at various ionic

strengths and the results extrapolated to zero ionic strength.

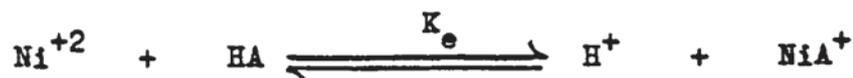
The equilibrium constant evaluated by using the molar concentrations is known as the concentration (or conditional) equilibrium constant and is defined as

$$K' = \frac{(C) \cdot (D)}{(A) \cdot (B)}$$

In cases where a metal and an ionic ligand combine to form a complex, the equilibrium constant can be called the formation constant. The experimental determination of the K' values for such reactions may be carried out by several techniques, but one of the most common solutions is that of measuring with a pH meter the H^+ concentration in a solution containing various concentrations of the metal and the ligand. For glycine ($^+NH_3CH_2CO_2^-$, denoted as HA), the H^+ concentration would be produced by the ionization of the acid as follows:

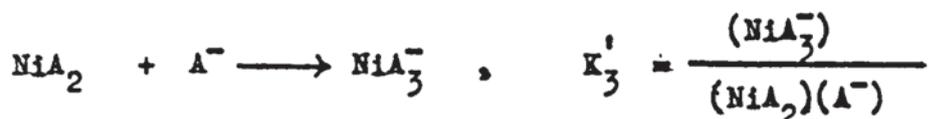
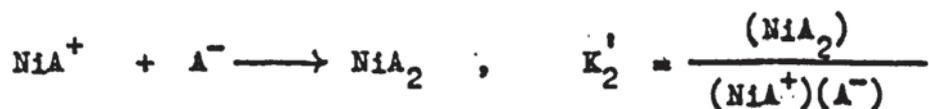


The equilibrium normally lies far to the left, but addition of Ni^{+2} to the solution results in the release of H^+ depending upon the affinity of Ni^{+2} for the chelating agent $NH_2CH_2CO_2^-$:



A knowledge of the concentration of Ni^{+2} and HA initially added, as well as H^+ concentration (from pH measurement) at equilibrium allows the calculation of the equilibrium constant, K_e , for this reaction. Since $K_e/K_a = K_1$, the value of K_1

may be evaluated. While this is the general principle, a problem is encountered, because nickel forms three types of complexes with glycine and the evaluation of their formation constants should also be considered simultaneously.



One method for the determination of K_1 , K_2 and K_3 involves the definition of a function \bar{n} as the average number of ligand molecules bound per metal ion. For the nickel-glycine system

$$\bar{n} = \frac{\text{moles of bound } \text{A}^{-}}{\text{total moles of } \text{Ni}^{+2}} = \frac{(\text{NiA}^{+}) + 2(\text{NiA}_2) + 3(\text{NiA}_3^{-})}{(\text{Ni}^{+2}) + (\text{NiA}^{+}) + (\text{NiA}_2) + (\text{NiA}_3^{-})}$$

substitutions from the above equations for K_1 , K_2 and K_3 lead to

$$\bar{n} = \frac{K_1(\text{A}^{-}) + 2K_1K_2(\text{A}^{-})^2 + 3K_1K_2K_3(\text{A}^{-})^3}{1 + K_1(\text{A}^{-}) + K_1K_2(\text{A}^{-})^2 + K_1K_2K_3(\text{A}^{-})^3}$$

For practical purposes, \bar{n} may be expressed in terms of the total glycine concentration initially added (A_{tot}), the concentration of HA and A^- , and the total Ni^{+2} concentration (M_{tot}). Thus

$$\bar{n} = \frac{A_{\text{tot}} - (\text{HA}) - (A^-)}{M_{\text{tot}}}$$

To determine (HA) and (A^-) in the above equation, an expression for the H^+ bound to the glycinate ion is introduced,

$$\begin{aligned} \text{Bound } \text{H}^+ = (\text{HA}) &= \begin{array}{l} \text{added } \text{H}^+ \\ \text{from } \text{HNO}_3 \end{array} + \begin{array}{l} \text{H}^+ \text{ from} \\ \text{diss. of } \text{H}_2\text{O} \end{array} - \text{free } \text{H}^+ \\ &= C_{\text{H}} + (\text{OH}^-) - (\text{H}^+) \end{aligned}$$

where C_{H} is the concentration of the nitric acid added to the Ni^{+2} solution. Now, for the acid HA,

$$K_a = \frac{(\text{H}^+)(A^-)}{(\text{HA})}$$

$$\text{i.e. } (\text{HA}) = \frac{K_a}{(\text{H}^+)(A^-)}$$

$$\text{or } (A^-) = \frac{K_a}{(\text{H}^+)} (C_{\text{H}} + (\text{OH}^-) - (\text{H}^+)).$$

The expression for \bar{n} , then becomes,

$$\bar{n} = \frac{A_{\text{tot}} - \left(1 + \frac{K_a}{(\text{H}^+)}\right) (C_{\text{H}} + (\text{OH}^-) - (\text{H}^+))}{M_{\text{tot}}}$$

Thus, (A^-) and \bar{n} may be calculated from experimentally known quantities. The values of \bar{n} are then plotted against $\log(A^-)$. From such plots it is possible to estimate the values of $\log K_1$, $\log K_2$ and $\log K_3$. For example, from the expression

$$K_1 = \frac{(NiA^+)}{(Ni^{+2})(A^-)}$$

it is noted that when (NiA^+) equals (Ni^{+2}) , then K_1 equals $1/(A^-)$. Since the condition $(NiA^+) = (Ni^{+2})$ means that the average number of ligands per metal ion is 0.5, the value of (A^-) at $\bar{n} = 0.5$ is the reciprocal of K_1 , i.e. $\log K_1 = -\log(A^-)$ at $\bar{n} = 0.5$. Similarly, at $\bar{n} = 1.5$ and 2.5, the values of (A^-) allow the estimation of K_2 and K_3 .

EXPERIMENTAL

Reagents:

Glycine 0.4M solution.

Potassium nitrate, 0.2M solution.

Nitric acid, 0.1M, 0.2M.

Sodium hydroxide, 0.5M.

Procedure:

After standardization of the pH meter with buffers of pH 4, 7 and 9, the pK_a of glycine was determined as follows:

To a 400 ml beaker containing 90 ml of distilled water, 100 ml of 0.2M nitric acid and 100 ml of the 0.4 M glycine solution

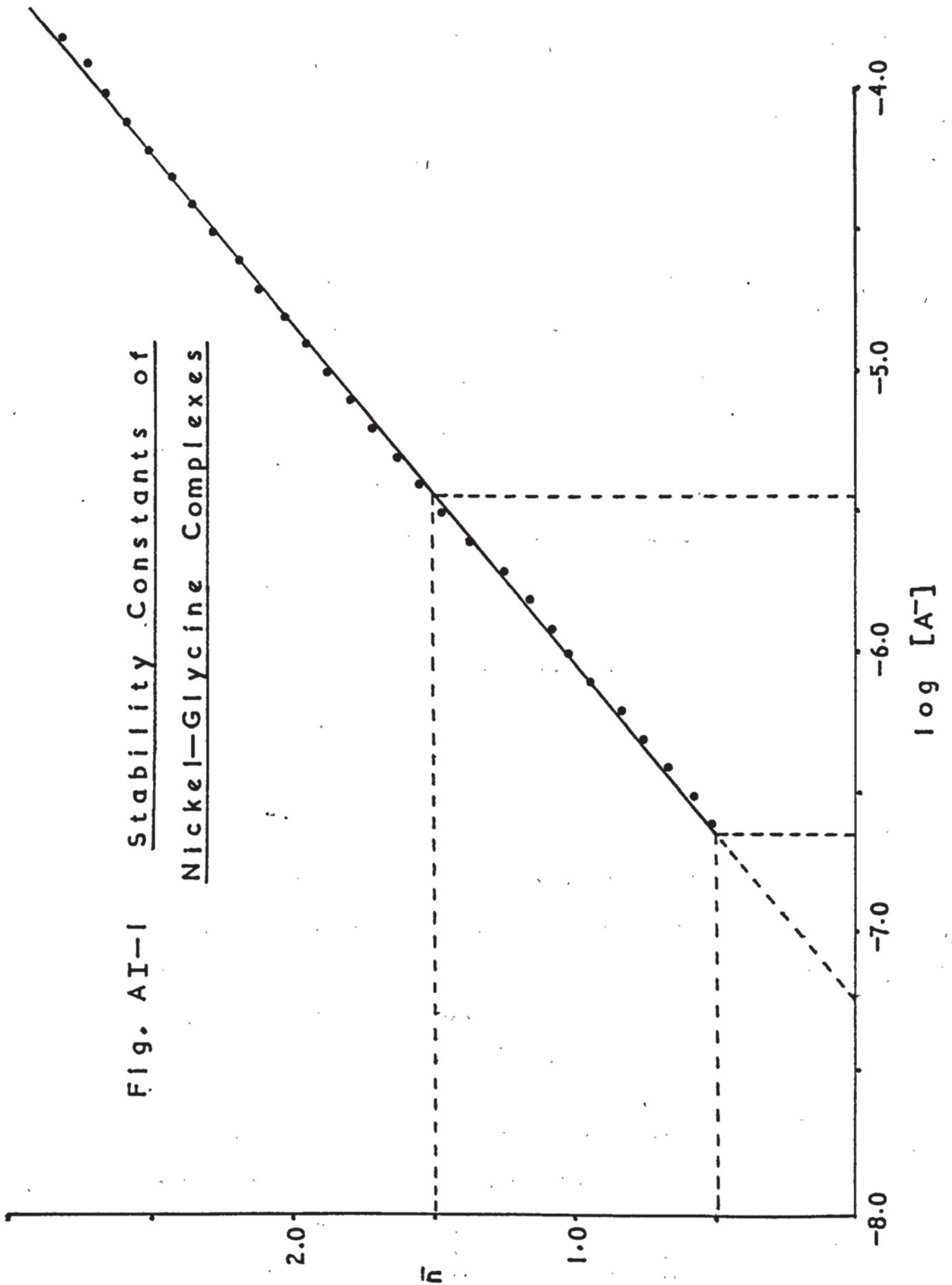
were added. The mixture was titrated with 0.5 M NaOH from a 10 ml burette. 0.2 ml aliquots of the alkali were added with constant stirring, using a magnetic stirrer. After each addition, the pH was recorded. From this information the value of pK_a was calculated using the relationship

$$pK_a = -\log(H^+) + \log \frac{A_{tot} - (Na^+) - (H^+) + (OH^-)}{(Na^+) + (H^+) - (OH^-)} .$$

The data necessary for calculating the stability constants was collected as follows.

20 ml of the 0.4 M glycinate solution was prepared by mixing together equal volumes of 0.8 M glycine and 0.8 M NaOH solutions. The resulting solution was transferred to a burette and used to titrate a solution of 100 ml of 0.2 M KNO_3 soln., 1 millimole of $NiCl_2 \cdot 6H_2O$, 10 ml of 0.1 M nitric acid and 90 ml of distilled water in a 400 ml beaker with constant stirring. After each 0.2 ml aliquot of the glycinate was added, the pH of the solution was recorded. The full 10 ml solution was used. From the titration data, (A^-) and \bar{n} were calculated at each of the 50 points. A plot of \bar{n} versus $\log(A^-)$ was made and from the values of $\log(A^-)$ at $n = 0.5, 1.5$ and 2.5 , the values of $\log K$, $\log K$ and $\log K$ were determined. The plot of \bar{n} versus $\log(A^-)$ is shown in figure AI-1.

Fig. AI-1 Stability Constants of
Nickel-Glycine Complexes



A P P E N D I X IIFORMATION CONSTANT OF THE COPPER-DIMETHYL DITHIOPHOSPHATE
COMPLEXINTRODUCTION

The formation constant of the copper-dimethyl dithiophosphate complex was determined using the method of Likussar and Boltz (38) which is based on the method of continuous variation.

When using the continuous variation method, it is necessary to prepare mixtures of equimolar solutions of metal and reagent and to maintain a constant final volume. The mole fraction is then defined by the following relation:..

$$\text{mole fraction} = x = \frac{C_M}{C_M + C_R} = \frac{C_M}{k}$$

where C_M is the total concentration of the metal, C_R is the total concentration of the ligand and 'k' denotes the constant sum of these concentrations. These mixtures, with the total absorbance A_T are measured against a reference solution containing only the ligand or the metal (if either of these absorbs at the wavelength of interest). Thus, the experimental absorbance reading at a certain wavelength is equivalent to $A_T - (A_T)_R$.

In the conventional approach, the experimental absorbances are plotted against the mole fraction, but Likussar and Boltz (38) proposed a more^{un}conventional approach. This involves the use of a normalized scale, a 'y' scale instead of the absorbance scale. This 'y' scale is based on using the quotients obtained

when the experimental absorbances are divided by the absorbance of a solution containing a large excess of ligand and a metal concentration $(C_M)_{\max}$, against a reference solution containing an identical ligand concentration with the absorbance $(A_T)_{R,ex}$. The divisor absorbance is known as ABS-MAX. The metal concentration selected for $(C_M)_{\max}$ is equivalent to the mole fraction of the metal at the stoichiometric composition of the complex, i.e. the mole fraction which has the highest absorbance in the absorbance versus mole fraction plot. The 'y' values thus obtained are plotted against 'x', the maximum value of 'y' being known as 'y_{max}'. In such plots only complexes with large formation constants give two straight lines intersecting at a point with y_{max} very close to 1. Weak complexes show a curve with y_{max} less than 1.

Once the y_{max} is found and an indication about the stoichiometry has been obtained, equations can be used to obtain the formation constant of the complex. The derivation of these equations has been discussed in detail by Likussar and Boltz (43) and these equations are presented in Table AII-1.

From the 'y' vs. 'x' curve, the 'm' and 'n' values of the complex can be found. Substituting these values, along with those of 'k' and y_{max} in the appropriate equation, $\log K'_E$ or $\log K'_f$ can be calculated.

TABLE AII-1

General Equations for Calculation of Conditional
Extraction and/or Formation Constants

Complex $M_n R_m$		$\log K'_E$ or $\log K'_f$
<u>m</u>	<u>n</u>	
1	1	$0.30 - \log k + \log y_{\max} - 2 \log (1 - y_{\max})$
1	2	$0.35 - 2 \log k + \log y_{\max} - 3 \log (1 - y_{\max})$
1	3	$0.38 - 3 \log k + \log y_{\max} - 4 \log (1 - y_{\max})$
1	4	$0.39 - 4 \log k + \log y_{\max} - 5 \log (1 - y_{\max})$
2	5	$0.76 - 4 \log k + \log y_{\max} - 5 \log (1 - y_{\max})$

EXPERIMENTAL

Reagents: 5×10^{-3} DMDTP solution.
 5×10^{-3} Copper(II) solution.

Procedures:

An amount of methanol slightly less than 9 ml was added to a series of 10 ml graduated flasks. To these were added amounts of copper(II) solution ranging from 0.0 to 0.5 ml followed by DMDTP (0.5 to 0.0 ml). The volume was made up to the mark with methanol, the flasks stoppered and shaken. The absorbance of the contents was measured at 420 nm in 1 cm cells using the UNICAM SP6-100 spectrophotometer with 95% methanol as a reference solution.

From the absorbance data it was seen that the maximum absorbance occurred at $x = 0.33$, i.e. a copper(II) solution volume of 0.16 ml and DMDTP solution 0.34 ml. Attempts were therefore made to determine ABS-MAX by using 0.16 ml of the copper(II) solution and an excess of DMDTP, but these attempts were not successful. The absorbance at $x = 0.33$ fell instead of rising (later work indicated that this must be due to the reduction of copper(II) to copper(I) by the large excess of DMDTP).

The problem was resolved by using the theoretical amount of DMDTP at $x = 0.33$ (i.e. 0.33 ml) and an excess of copper. Thus, to a fixed amount of 5×10^{-3} DMDTP (0.33 ml) were added different amounts of 10^{-1} M copper(II) solution. In order to check the results, ten concentrations of copper(II) were used, i.e. 0.001 to 0.01 ml.

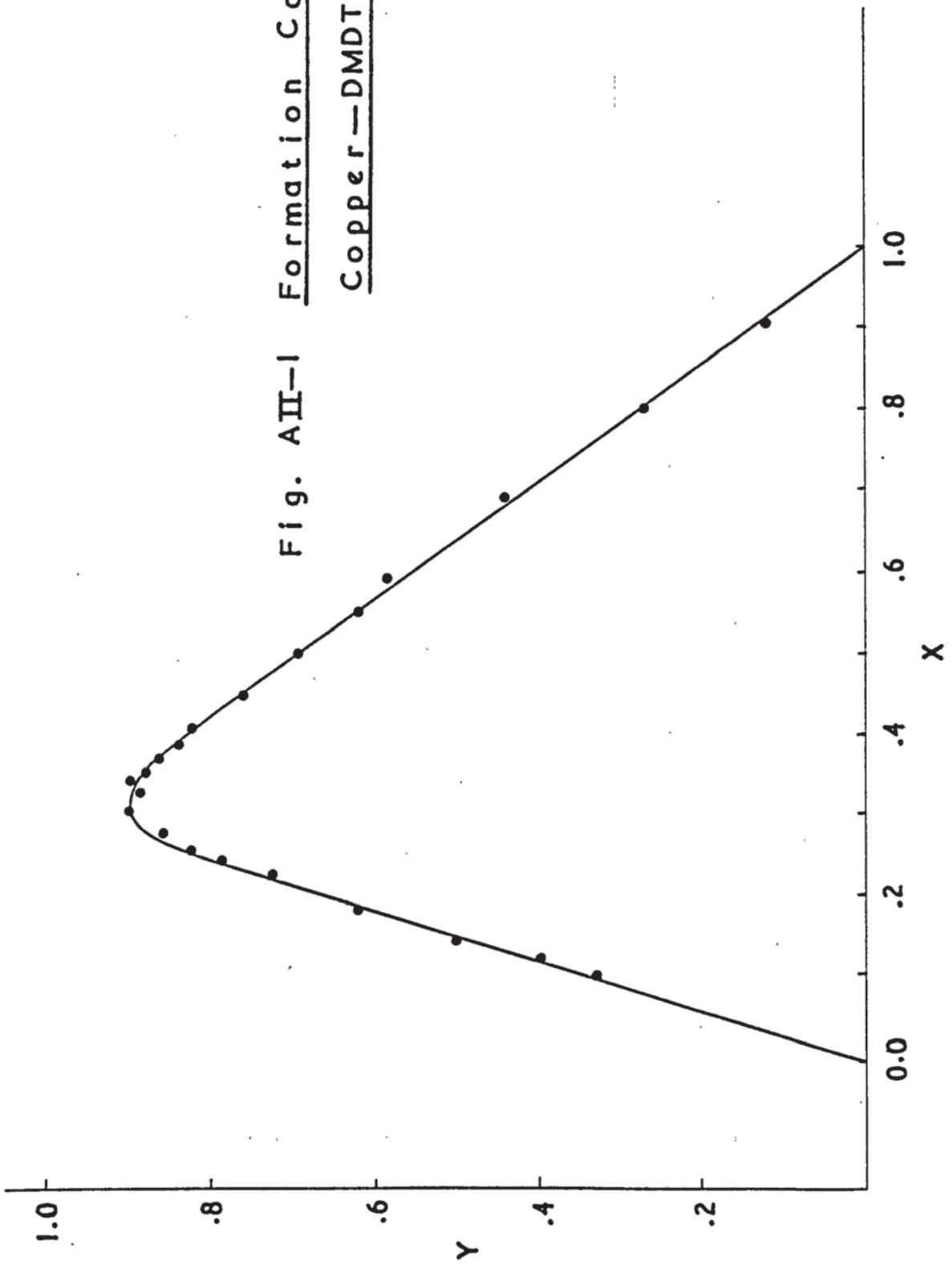
In these experiments, exactly 9.5 ml of methanol were added to the flask, and after addition of the reagents, the volume was made up to the 10 ml mark with distilled water. The following results were obtained

Volume of Copper(II) Solution (ml)	Absorbance at 420 nm 1cm cells
0.001	0.300
0.002	0.280
0.003	0.280
0.004	0.290
0.005	0.280
0.006	0.320
0.007	0.305
0.008	0.290
0.009	0.295
0.010	0.285
	average 0.293

Using the average value of 0.293 as ABS-MAX, the 'y' values were calculated and plotted versus the 'x' values as shown in Fig. AII-1. The figure shows that the value of y_{\max} is 0.9 and that the values of 'm' and 'n' are 1 and 2 respectively. Also, it is known that $k = 2.5 \times 10^{-4}$ (0.5 ml of 5×10^{-3} M solution diluted to 10 ml), thus using the second equation from Table AII-1, we have

$$\begin{aligned} \log K'_f &= 0.35 - 2 \log (2.5 \times 10^{-4}) + \log 0.9 - 3 \log 0.1 \\ &= 0.35 + 7.2 - 0.05 + 3.0 = \underline{10.50} \end{aligned}$$

Fig. AII-1 Formation Constant of
Copper—DMDTP Complex



A P P E N D I X IIIExtraction Constants of Copper(II) and Bismuth(III)
Complexes of DMDTP from Water into CCl_4 .

Using the procedure recommended by Likussar and Boltz (38) which is described above, attempts were made to determine the conditional extraction constants for the copper(II) and bismuth(III) complexes of DMDTP.

Reagents:

10^{-4} M Copper(II) solution

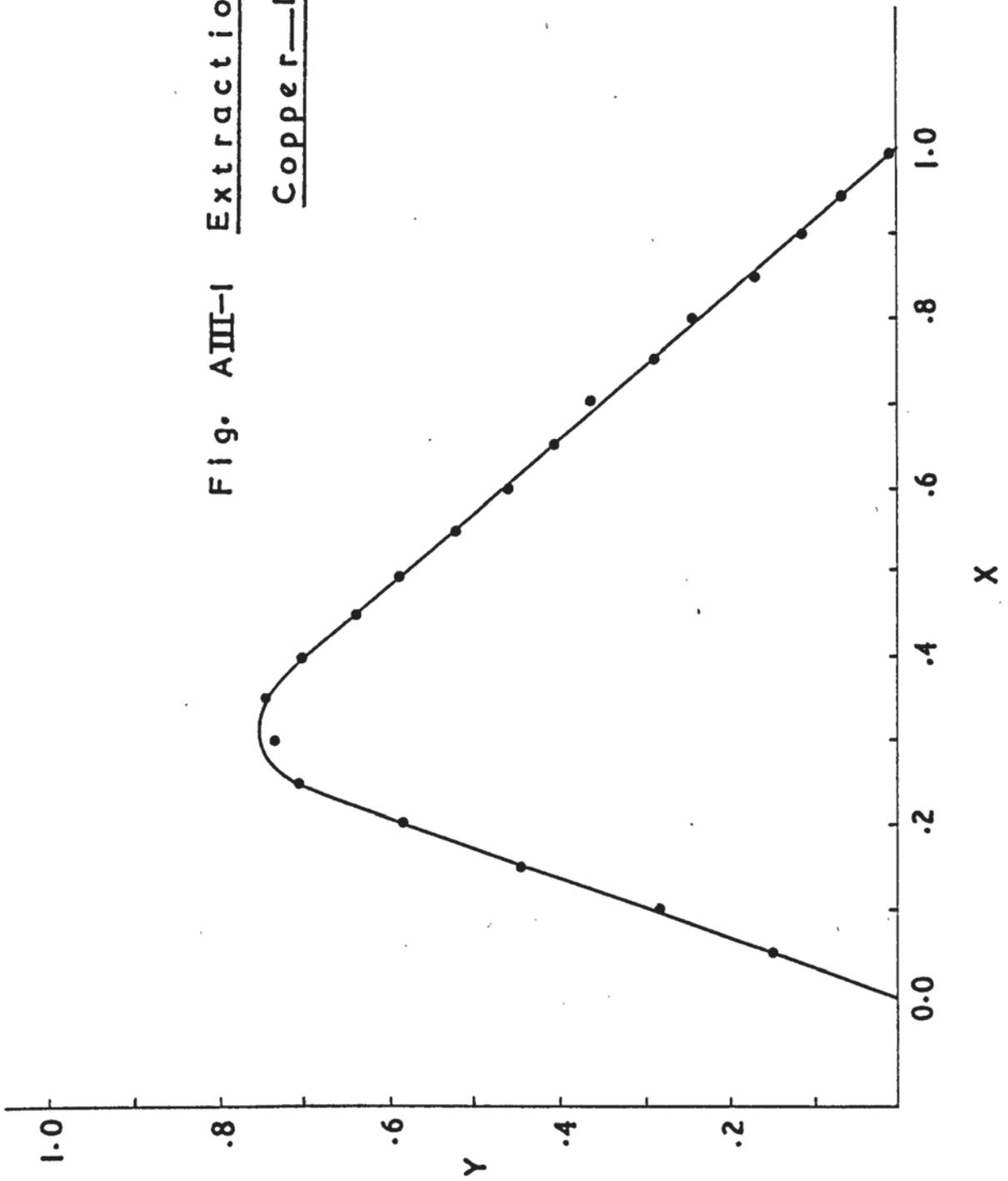
10^{-4} M DMDTP solution

Procedure:

To a 100 ml separating funnel were added 8 ml of distilled water, followed by copper solution (0.0 to 1.0 ml) and DMDTP solution (1.0 to 0.0 ml). 1 ml of 1 M nitric acid was added followed by 10.0 ml of CCl_4 and the contents of the funnel were shaken for exactly 1 min. after which time the organic layer was transferred to 1 cm quartz cell via a cotton wool plug placed loosely in the stem of the funnel. The absorbance of the organic extract was immediately read at 420 nm.

ABS-MAX was found by using 0.66 ml of 10^{-4} DMDTP and 0.05 ml of 10^{-1} copper(II) solution. The average value of five such determinations was 0.67. Using this value of ABS-MAX, the 'y' values were calculated and plotted against 'x' as shown in Figure AIII-1.

Fig. AIII-1 Extraction Constant of
Copper—DMDTP Complex



From the figure, it can be seen that $y_{\max} = 0.94$ and that $m = 1$ and $n = 2$. Also, it is known that $k = 10^{-5}$. Thus, the value of the extraction constant can be calculated (again using the second equation from Table AII-1) as

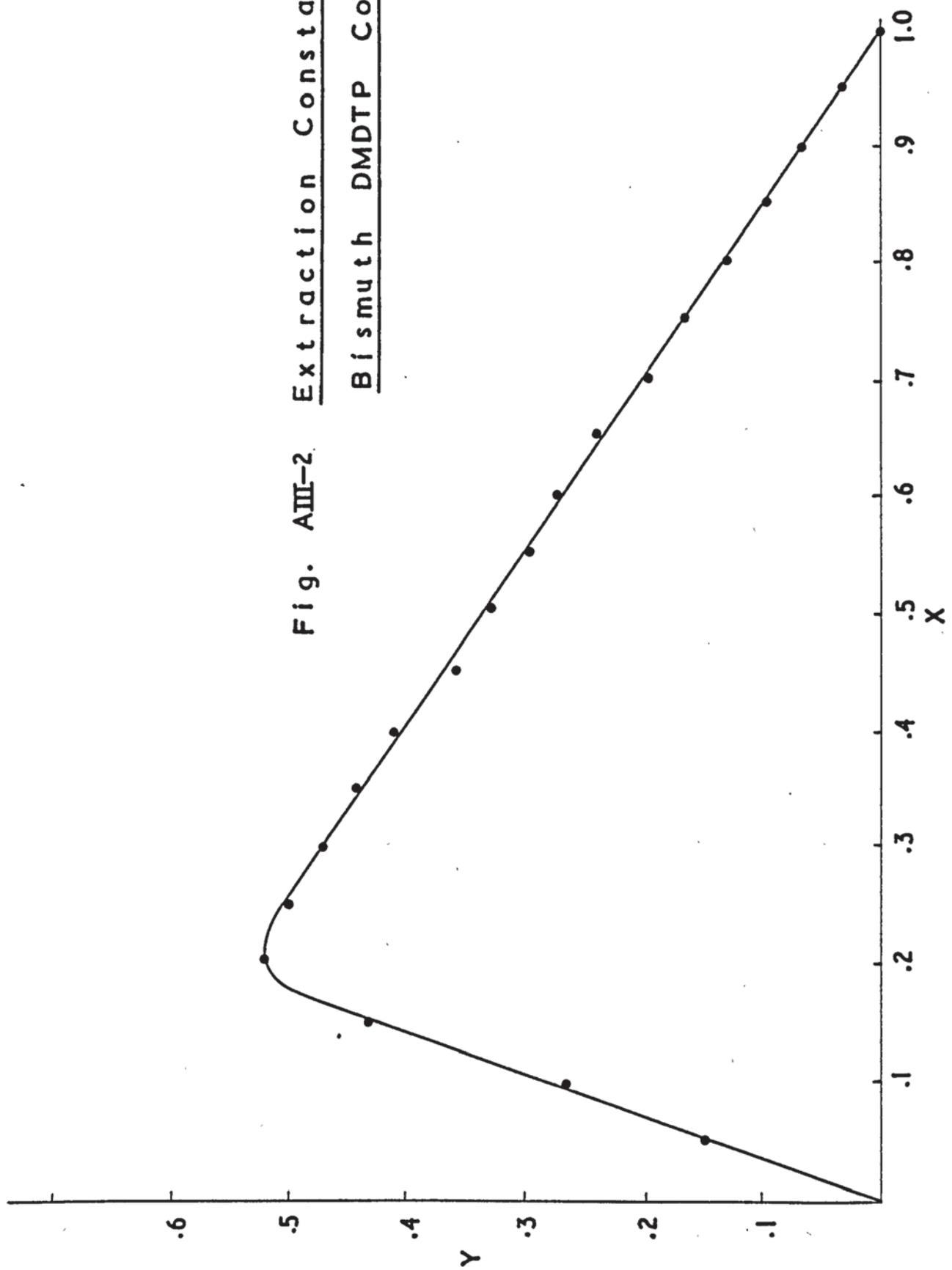
$$\begin{aligned} \log K_E^1 &= 0.35 - 2 \log k + \log y_{\max} - 3 \log (1 - y_{\max}) \\ &= 0.35 - 2 \log 10^{-5} + \log 0.94 - 3 \log 0.16 \\ &= 0.35 + 10.0 - 0.03 + 1.6 = \underline{11.92} \end{aligned}$$

The estimation of the extraction constant of the bismuth-DMDTP complex was carried out by a similar procedure using 0.0 to 1.0 ml of bismuth(III) and 1.0 to 0.0 ml of DMDTP solutions each of concentration 5×10^{-3} M. The wavelength of absorbance measurements was 325 nm. ABS-MAX was found by using 0.25 ml of the bismuth solution and 1 ml of 10^{-1} M DMDTP. The average of ten determinations was 0.61. Using this value, the 'y' values were calculated and plotted against the values of 'x' as shown in Figure AIII-2.

From the figure it is clear that $y_{\max} = 0.87$, $m = 1$ and $n = 3$. Also, $k = 5 \times 10^{-4}$. Thus,

$$\begin{aligned} \log K_E^1 &= 0.38 - 3 \log 5 \times 10^{-4} + \log 0.87 - 4 \log 0.13 \\ &= 0.38 + 9.90 - 0.06 + 3.54 = \underline{13.88} \end{aligned}$$

Fig. AIII-2. Extraction Constant of
Bismuth DMDTP Complex



A P P E N D I X IVREAGENTS FOR THE COLORIMETRIC DETERMINATION OF BISMUTH

1. Acid alizarin black SN complex of bismuth(III) can be extracted into butanol and bismuth determined by measuring the absorbance at 600 nm, (76).
2. Amaranth. At pH 2 to 3, bismuth(III) gives a precipitate with the dye. The precipitate is dissolved in 1% solution of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and the absorbance of the free dye is measured at 521 nm, (77).
3. o-Aminobenzenethiol forms a yellow complex with bismuth in dilute acid solutions, (77).
4. 4-Amino-naphthalene-1-sulphonic acid. The diazotized reagent forms a 1:2 complex with bismuth in slightly alkaline conditions which can be measured at 500 nm, (78).
5. Arsenazo III in H_2SO_4 (or HClO_4) medium of pH 0.1 to 3 forms a 1:2 complex with bismuth, the absorbance being measured at 610 or 655 nm, (79).
6. 2,2'-Bipyridyl with I^- can be used to determine bismuth by forming an ion association complex, the absorbance of which is measured at 455 nm, (80, 81).
7. Bis(thioantipyrinyl)methane forms a 2:1 complex with bismuth(III) in 0.1 to 1M H_2SO_4 medium with maximum absorbance at 535 nm, (82).
8. Bromopyrogallol Red. In acid medium of pH 2 containing gelatin, bismuth(III) forms a blue colour with the reagent, the absorbance of which is measured at 635 nm, (83).
9. Catechol Violet. In acidic medium and in presence of 2,6-dinitrophenol, catechol violet gives a colour with

- bismuth measureable at 580 nm, (84) It is also known that $\text{Bi}(\text{OH})_2^+$ forms a 1:1:2 complex with catechol violet and diphenylguanidine at pH 5 to 7. The complex shows a maximum absorbance at 630 nm, (85).
10. Chlorpromamine hydrochloride. In presence of I^- , the reagent gives a coloured complex which can be extracted from acidic solutions into chloroform. The absorbance peak is at 485 nm, (86, 87).
 11. Chlorosulphenol S reacts with bismuth(III) in the pH range 4.2 to 5.1 to form a 1:1 blue complex having the absorbance maximum at 646 nm, (88).
 12. m-Cresol-phthalexon. With this and other phthalexons, at pH 1 to 2, bismuth forms a complex with absorption peaks ranging from 540 to 580 nm, (89, 90).
 13. Dalzin. Optimum conditions for the formation of a orange-yellow coloured complex with bismuth include the adjustment of the pH to 2.5 with HClO_4 and NaOH , (98).
 14. D-PDTP. A yellow coloured complex is extracted from acidic solutions into immiscible organic solvents. The absorption peak is at 410 nm, (91).
 15. Di-mercaptothiopyrones form with bismuth(III) coloured complexes suitable for the photometric determination of bismuth. The colour of the complexes is most intense in 0.2 M to 1 M HCl and is measured at 533 nm, (93).
 16. β -Dicarbonyl compounds. An example of such compounds is 2-(thiobenzyl)acetophenone which forms a complex with bismuth(III) at pH 12.6 and extractable into heptane or cyclohexane. The maximum absorbance is at 410 nm, (92).

17. 1,2-Dimorpholinoethane gives a precipitate with bismuth which is soluble in dimethylformamide; measurement of the absorbance is done at 436 nm, (95).
18. Di-(2-naphthyl)thiocarbazono. This reagent is very similar to dithizone (see below) with absorbance peak of the bismuth complex at 525 nm, (96).
19. Diselenocarbamates. Bismuth complexes of dimethyl and diethyl compounds can be extracted into organic solvents and measured at 410 nm, (97).
20. Dithiocarbamate. Apart from dithizone, this is the second most important reagent for the determination of bismuth. It forms a complex with bismuth at practically all pH values which can be extracted into organic solvents. Although the absorption peak of the complex is in the ultraviolet region, at 400 nm the colour intensity is sufficient for routine use, (94).
21. Dithizone. One of the most well-established reagents, it can extract bismuth from aqueous solutions ranging from pH 2 to 10 into organic solvents. The bismuth-dithizone complex is orange-red in colour and the absorbance is measured at 490 nm, (94).
22. Ethyl-xanthate. Potassium ethyl xanthate forms at low pH values a precipitate with bismuth(III) which can be extracted into carbon tetrachloride. The yellow complex is measured at 400 nm, (99).
23. Fast grey RA. Bismuth can be determined in 0.01 M HNO_3 by measuring the absorbance of the violet complex formed with the reagent at 570 nm, (100).

24. Gallein. In presence of gelatin, and in acetic acid media the reagent gives a colour with bismuth measureable at 570 nm, (101, 102).
25. Haemotoxyline oxidised by hydrogen peroxide forms with bismuth(III) at pH 2-3 a 1:1 chelate exhibiting maximum absorbance at 550 nm, (103).
26. Hydroxyazo compounds. Heterocyclic hydroxyazo compounds form extractable complexes with bismuth in acidic media (pH 2-6) showing absorbance peaks in the region of 520 to 600 nm, (104).
27. Hydroxyfluorones. Hydroxyfluorones in presence of certain anions (and also antipyrine) may form coloured complexes with bismuth which can be extracted by organic solvents from acidic solutions. The complexes absorb in the region 500 to 560 nm, (105, 106, 107).
28. Hydroxyquinoline derivatives. 8-Hydroxy-7-(α -(2-methoxy-carbonyl-anilino)benzyl)quinoline forms a complex with bismuth which is extracted into chloroform at pH 12 and measured at 400 nm, (108).
29. N-Methylanalbasine- α '-azodiethylaminophenol forms a 1:1 complex with bismuth having maximum absorbance at 580 to 590 nm. The complex is extractable into organic solvents at pH 5, (109, 110, 111).
30. Methyl green. With I^- and methyl green, bismuth(III) forms a ternary complex extracted into benzene from 2N H_2SO_4 with maximum absorbance at 650 nm, (112).
31. Methyl-thymol blue. Bismuth can be extracted as a 1:2:2 ion-association complex from ammonium acetate buffered medium of pH 5 to 7 into butanol. The complex exhibits

- maximum absorbance at 560 nm (113).
32. Resorcinol derivatives. 4-(3-(1-methyl-2-piperidyl)-2-pyridylazo)resorcinol in acid medium (pH 1 to 3) forms a red 1:3 complex exhibiting maximum absorbance at 530 nm, (114).
 33. 8-Mercaptobenzimidazole. A ternary complex of bismuth with the reagent can be extracted into chloroform-ethanol with absorption peaks at 350 and 490 nm, (115).
 34. 2-Mercaptopyridine forms a precipitate with bismuth in solutions of pH 1.2 to 1.7 and absorbs strongly at 430 nm, (116).
 35. 8-Mercaptoquinoline derivatives. At least one derivative of the reagent has been reported which forms a complex with bismuth and when extracted into chloroform, benzene etc., absorbs at 430 nm. The colour of the solution is orange-red, (117).
 36. 1,10-Phenanthroline. In presence of I^- , an ion association complex with bismuth is extracted into cyclohexane at a pH of 2.5. The absorbance is measured at 465 nm, (118).
 37. Phenyl substituted thioureas. In contrast to thioures, the phenyl substituted derivatives extract bismuth into organic solvents from perchloric acid solutions. The absorbance of the resultant solution is measured at 455 nm. Two of the most important derivatives are diphenyl and di-o-tolyl thioureas, (119).
 38. Pyrazoline derivatives. The precipitate with bismuth formed in acetic acid media is soluble in chloroform

- with absorbance maximum at 495 nm, (120).
39. Pyronine G forms a 1:1 complex with bismuth which is extractable into benzene-ethyl methyl ketone (2:1) from H_2SO_4 solution (1:1). The absorbance is measured at 530 nm, (121).
 40. 1,2-Pyridylazo-2-naphthol. The bismuth complex of this reagent has been extracted into MIBK from 0.1 M HNO_3 medium and the absorbance is measured at 560 nm, (122).
 41. 1,5-Pyridylazo-2-naphthol. The compound forms a 1:1 complex with bismuth(III) in nitric acid media containing 20% of 1,4-dioxan. The wavelength of maximum absorbance depends on the concentration of the acid and for 0.05 M HNO_3 it is 505 nm, (123).
 42. Quinoxaline-2,3-dithiol. In 4 to 5 M sulphuric acid medium bismuth forms a 1:5 complex with this reagent. The absorbance can be measured at 490 nm, (124).
 43. Rhodamine B. The dye forms an ion-association complex with bismuth in presence of iodide ions. The complex is extracted into MIBK-benzene (3:2) and the absorbance is measured at 560 nm, (125, 126).
 44. Ruhemann's purple. When to an acidic solution of pH 4 the reagent is added, it forms a complex with bismuth extractable into chloroform and measureable at 530 nm (127).
 45. Stilbazol. This reagent, at pH 2, gives a coloured complex with bismuth, the absorbance of which is measured at 582 nm, (128).
 46. Tetraphenyl-phosphonium dyes. These dyes, in presence of an anion like iodide, chloride or bromide, extract

bismuth from acidic solutions into organic solvents.

The absorbance is measured at 505 nm, (129, 130).

47. 4-(2-Thiazolylazo)resorcinol. The bismuth complex of the reagent is extracted from 0.1 to 0.3 M nitric acid medium into tributyl phosphate, measuring the absorbance at 530 nm, (131, 132).
48. Thiocyanate. The bismuth-thiocyanate complex exhibits maximum absorbance at 470 nm. Optimum conditions for the formation of the complex include a solution of pH 1 to 2, (133).
49. Thiolactams. These compounds extract bismuth into organic solvents from acidic solution (1 M in HCl), the resulting complex having an absorption maximum at 490 nm, (134, 135, 136, 137).
50. Thioethyl trifluoroacetone. The reagent extracts bismuth into carbon tetrachloride at pH 6. The absorbance of the extract is measured at 460 nm, (138).
51. Thiourea. The yellow bismuth-thiourea complex, stable in acid medium, is the basis of measurement at 470 nm, (98).
52. Thoron. In perchloric acid medium (0.02 to 0.04 M), thoron gives a coloured complex with bismuth, the absorbance of which is measured at 535 nm, (139).
53. Xylenol Orange forms a 1:1 complex with bismuth(III). The optimum pH value for the complex formation is 1 and the measurement of absorbance is done at 530 nm, (140, 141, 142).

REFERENCES

1. Eto, M.
'Organophosphorus Pesticides: Organic and Biological Chemistry'
CRC Press, Cleveland, 1974.
2. O'Brien, R.D.
'Toxic Phosphorus Esters'
Academic Press, London, 1960.
3. Green, M.B.
'Chemicals for Crop Protection and Pest Control'
Pergamon Press, London etc., 1977.
4. De, A.K., Khopkar, S.M. and Chalmers, R.A.
'Solvent Extraction of Metals'
Van Nostrand Reinhold, London, 1970.
5. Cremlyn, R.
'Pesticides: Preparation and Mode of Action'
John Wiley & Sons, Chichester etc., 1978.
6. Sutherland, G.L. in Zweig, G. (ed.)
'Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives'
Academic Press, Volume II, London, 1964.
7. Thomson, W.T.
'Agricultural Chemicals Book I - Insecticides'
Thomson Publications, Indianapolis, 1973.
8. Fifield, F.W. and Kealey, D.
'Principles and Practices of Analytical Chemistry'
International Textbook Company Ltd., London, 1975.
9. Burger, K.
'Organic Reagents in Metal Analysis'
Pergamon Press, Oxford etc., 1973.
10. Norris, M.V., Vail, W.A. and Averell, P.R.
'Colorimetric Estimation of Malathion Residues'
J. Agric. Food Chem., 2, (1954), 570-573.

11. Norris, M.V., Easter, E.W., Fuller, L.T. and Kuchar, E.J.
'Colorimetric Estimation of Malathion Residues in Animal Products'
J. Agric. Food Chem., 6, (1958), 111-119.
12. Norris, M.V. and Kuchar, E.J.
'Colorimetric Estimation of Malathion in Cottonseed'
J. Agric. Food Chem., 7, (1959), 488-489.
13. Rossouw, S.D.
'The Determination of Malathion Residues'
S. Afr. J. Agric. Sci., 4, (1961), 435-437.
14. Bates, A.N.
'Removal of Plant Extractives Interfering in Barley and Rice Bran'
The Analyst, 87, (1962), 643-649.
15. Bates, A.N. and Rowlands, D.G.
'Interferences by Extractives in the Determination of Malathion Residues in Rice Bran'
The Analyst, 89, (1964), 286-287.
16. Bates, A.N. and Rowlands, D.G.
'Recovery of Malathion from a Range of Stored Products'
The Analyst, 89, (1964), 288.
17. Rowlands, D.G.
'Recovery of Malathion from Pimento'
The Analyst, 89, (1964), 498-500.
18. Piekacz, H. and Mazur, H.
'Colorimetric Determination of Malathion Residues in Corn and Cereal Products'
Anal. Abstr., 23, (1971), 2008.
19. Report by Malathion Panel
'The Determination of Malathion Residues in Cereals and Oilseeds'
The Analyst, 85, (1960), 915-921.
20. Association of Official Analytical Chemists
'Official Methods of Analysis of the Association of Official Analytical Chemists of the United States'
Washington, D.C., 1975.

21. Raw, G.R. (ed.)
'CIPAC Handbook Volume-I, Analysis of Technical and Formulated Pesticides'
Collaborative International Pesticides Analytical Council Limited, 1970, pp:443-462.
22. WHO
'Specification for Pesticides'
World Health Organisation, Geneva, 4th edition, 1973.
23. Snell, F.D. and Snell, C.T.
'Colorimetric Methods of Analysis' Vol. IV-AA
Van Nostrand Reinhold, London, 1970, pp: 174-178.
24. Handley, T.H. and Dean, J.A.
'O,O'-Dialkyl Phosphorodithioic Acids as Extractants for Metals'
Analyt. Chem., 34, (1962), 1312-1315.
25. Getz, M.E.
'Preliminary Investigation into the Quantitative Determination of Organophosphate Pesticide Residues by Conversion into Orthophosphate Ion'
J. Ass. Off. Agric. Chem., 47, (1964), 1103-1105.
26. Getz, M.E. and Watts, P.R.
'Application of 4-(p-Nitrobenzyl)pyridine as a Rapid Quantitative Reagent for Organophosphates'
J. Ass. Off. Agric. Chem., 47, (1964), 1094-1096.
27. Mora, G.A.
'Colorimetric Determination of Malathion' (in Spanish)
Analyt. Abstr., 13, (1966), 3880.
28. Long, D.E.
'Colorimetric Determination of Malathion and Parathion by using Fluoroboric Acid'
Analyt. Lett., 1, (1967), 61-65.
29. Hill, A.C.
'Nature of the Yellow Copper Complex Produced in Certain Analytical Methods for the Determination of Malathion'
J. Sci. Food Agric., 20, (1969), 4-7.
30. Wayne, R.S., Groth, W.C., Miles, J.W. and Guerrant, G.O.
'Nonaqueous Copper Colorimetric Method for Determination of Malathion in Technical Grade Malathion and in Malathion Formulations'
J. Ass. Off. Analyt. Chem., 55, (1972), 926-938.

31. Wayne, R.S.
'Collaborative Study of the Nonaqueous Copper Colorimetric and Silver Titrimetric Methods for the Determination of Malathion in Technical Grade Malathion and in Malathion Formulations'
J. Assoc. Off. Analyt. Chem., 56, (1973), 579-585.
32. Stiles, R.S., Miles, J.W., Wayne, R.S. and Newton, L.H.
'Nonaqueous Copper Colorimetric and Silver Titrimetric Methods for Determining Malathion in Technical Grade Malathion and Malathion Formulations from Domestic and Foreign Sources: Interlaboratory Study'
J. Ass. Off. Analyt. Chem., 60, (1977), 1148-1153.
33. Visweswriah, K. and Jayaram, M.
'Colorimetric Method for the Estimation of Malathion'
Agric. Biol. Chem., 38, (1974), 2031-2033.
34. Syoyama, M., Miyachi, Y. and Sakakibara, J.
'Spectrophotometric Determination of Organothiophosphorus Pesticides with Palladium Chloride and Diethyl-4-Nitrosoaniline' (in Japanese)
Analyt. Abstr., 31, (1976), 4G19.
35. Syoyama, M. and Yuza, M.
'Colorimetric Determination of Organothiophosphorus Pesticides with Palladium Chloride and NN-Diethyl-4-Nitrosoaniline' (in Japanese)
Analyt. Abstr., 33, (1977), 6G35.
36. Angelici, R.J.
'Synthesis and Techniques in Inorganic Chemistry'
W. B. Saunders Company, Philadelphia, 1977.
37. Li, N.C., White, J.M. and Yoest, R.L.
'Some Metal Complexes of Glycine and Valine'
J. Am. Chem. Soc., 78, (1956), 5218-5222.
38. Likussar, W. and Boltz, D.F.
'Theory of Continuous Variation Plots and a New Method for Spectrophotometric Determination of Extraction and Formation Constants'
Analyt. Chem., 43, (1971), 1265-1272.
39. Cavell, R.G., Day, E.D., Byers, W. and Watkins, P.M.
'Metal Complexes of Substituted Dithiophosphinic Acids' II. Complexes of Trivalent Vanadium'
Inorg. Chem., 10, (1971) 2716-1719.

40. Adams, D.M. and Cornell, J.B.
'Metal-Sulphur Vibrations. Part II. Dithiolate and Dithiophosphate Complexes and Correlations for $\nu(M-S)$ '
J. Chem. Soc., (A), (1968), 1299-1303.
41. Cavell, R.G., Byers, W., Day, E.D. and Watkins, P.M.
'Metal Complexes of Substituted Dithiophosphinic Acids. IV. Complexes of Divalent Nickel, Palladium and Platinum'
Inorg. Chem., 11, (1972), 1598-1606.
42. Corbridge, D.E.C.
'Infrared Analysis of Phosphorus Compounds'
J. Appl. Chem., 6, (1956), 456-465.
43. Cavell, R.G., Byers, W. and Day, D.
'Metal Complexes of Dithiophosphinic Acids. I. Complexes of Trivalent Chromium'
Inorg. Chem., 10, (1971), 1710-2715.
44. Askew, J., Ruzicka, J.H. and Wheals, B.B.
'A General Method for the Determination of Organophosphorus Pesticide Residues in River Waters and Effluents by Gas, Thin-layer and Gel Chromatography'
The Analyst, 94, (1969), 275-283.
45. Ripley, B., Wilkinson, R.J. and Chau, S.Y.
'Multiresidue Analysis of Fourteen Organophosphorus Pesticides in Natural Waters'
J. Ass. Off. Analyt. Chem., 57, (1974), 1033-1042.
46. Riley, J.P. and Taylor, D.
'The Analytical Concentration of Traces of Dissolved Organic Materials from Sea Water with Amberlite XAD-1 Resin'
Analyt. Chimica Acta, 46, (1969), 307-309.
47. Burnham, A.K., Calder, G.V., Fritz, J.S., Junk, G.A., Svec, H.J. and Willis, R.
'Identification and Estimation of Neutral Organic Contaminants in Potable Water'
Analyt. Chem., 44, (1972), 139-142.
48. Glaze, W.H., Henderson, J.E., Bell, J.E. and Wheeler, V.A.
'Analysis of Organic Materials in Wastewater Effluents after Chlorination'
J. Chromat. Sci., 11, (1973), 580-584.

49. Kennedy, D.C.
'Treatment of Effluents from Manufacture of Chlorinated Pesticides with a Synthetic Polymeric Adsorbent Amberlite XAD-4'
Env. Sci. & Technol., 7, (1973), 138-141.
50. Junk, J.A., Svec, H.J., Vick, R.D. and Avery, M.J.
'Contamination of Water by Synthetic Polymer Tubes'
Env. Sci. & Technol., 8, (1974), 1100-1106.
51. Osterroht, C.
'Development of a Method for the Extraction and Determination of Non-polar, Dissolved Organic Substance in Sea Water'
J. Chromat., 101, (1974), 289-298.
52. McNeil, E.E., Otson, R., Miles, W.F. and Rajabalee, F.J.M.
'Determination of Chlorinated Pesticides in Potable Water'
J. Chromat., 132, (1977), 277-286.
53. Kim, B.R.
'Adsorption of Organic Compounds by Synthetic Resins'
J. Wat. Poll. Cont. Fed., 48, (1976), 120-133.
54. Coburn, J.A., Valdmanis, I.A. and Chau, A.S.Y.
'Evaluation of XAD-2 for Multiresidue Extraction of Organochlorine Pesticides and Polychlorinated Biphenyls from Natural Waters'
J. Ass. Off. Analyt. Chem., 60, (1977), 224-228.
55. Berkane, K., Caissie, G.E. and Mallet, V.N.
'The use of Amberlite XAD-2 Resin for the Quantitative Recovery of Fenitrothion from Water - a Preservation Technique'
J. Chromat., 13, (1977), 386-389.
56. Fritz, J.S. and Lisicki, N.M.
'Titration of Acids in Non-Aqueous Solvents'
Analyt. Chem., 23, (1951), 589-591.
57. Kumamaru, T., Hayashi, Y., Okamoto, N., Tao, E. and Yamamoto, Y.
'Determination of Phthalic Acid by Atomic Absorption of Neocuproine-copper(I) Chelate'
Analyt. Chimica Acta, 35, (1966), 524-525.

58. Kumamaru, T.
'A New Application of Atomic Absorption Spectrophotometry
—Determination of Phthalic Acid by Solvent Extraction
with Neocuproine-copper(I) Chelate'
Analyt. Chimica Acta, 43, (1968), 19-25.
59. Yamamoto, Y., Kumamaru, T. and Hayashi, Y.
'New Method for the Determination of Pentachlorophenol by
Atomic Absorption Spectrophotometry'
Talanta, 14, (1967), 611-613.
60. Yamamoto, Y., Kumamaru, T., Hayashi, Y. and Matsushita, S.
'Indirect Determination of β -Hydroxynaphthoic Acid by
Atomic Absorption Spectrophotometry'
Bull. Chem. Soc. Japan, 42, (1969), 1774-1775.
61. Oles, P.J. and Siggia, S.
'Atomic Absorption Method for Determining Aliphatic
Secondary Amines'
Analyt. Chem., 45, (1973), 2150.
62. Sheridan, J.C., Lau, E.P.K. and Senkowski, B.Z.
'Determination of Non-ionic Surfactants by Atomic
Absorption Spectrophotometry'
Analyt. Chem., 41, (1969), 247-250.
63. Gegiod, D.
'Determination of Soap in Refined Vegetable Oils by
Atomic Absorption Spectrophotometry'
The Analyst, 99, (1974), 745-748.
64. Crisp, P.T., Eckert, J.M., Kirkbright, G.F. and West, T.S.
'The Determination of Anionic Detergents at p.p.b. levels
by Graphite Furnace Atomic Absorption Spectrophotometry'
Analyt. Chimica Acta, 87, (1976), 97-101.
65. Treguer, P., Corre, P.L. and Couto, P.
'A Method for Determination of the Total Dissolved
Free Fatty-acid Content of Sea Water'
J. Mar. Biol. Ass. U.K., 52, (1972), 1045-1055.
66. Bousquet, B., Bouvier, J.L. and Dreux, C.
'Urinary Excretion of Amino Acids Determined by Atomic
Absorption Spectrophotometry'
Clin. Chimica Acta, 42, (1972), 327-334.

67. Gawrgious, Y.A., Besada, A. and Hassouna, M.E.M.
'Microdetermination of α -Amino Acids by Colorimetry and Atomic Absorption Spectrophotometry'
Chem. Abstr., 86, (1977), 164884 t.
68. Menache, R.
'Routine Micromethod for the Determination of Oxalic Acid in Urine by Atomic Absorption Spectrophotometry'
Clin. Chem., 20, (1974), 1444-1445.
69. Diaz, F.J.
'Determination of Cyanocobalamin by Atomic Absorption Spectrophotometry with a Pre-mix Air-Acetylene Flame'
Analyt. Chimica Acta, 58, (1974), 911-914.
70. Oles, P.J. and Siggia, S.
'Atomic Absorption Method for Determining Micromolar Quantities of Aldehydes'
Analyt. Chem., 46, (1974), 911-914.
71. Oles, P.J. and Siggia, S.
'Atomic Absorption Method for Determining 1,2-Diols'
Analyt. Chem., 46, (1974), 2197-2200.
72. Hurtubise, R.J.
'Determination of Sodium Edetate Dihydrate in Streptomycin by Atomic Absorption Spectrophotometry'
J. Pharm. Sci., 63, (1974), 1131-1133.
73. Senise, P. and Silva, L.G.
'A New Analytical Reaction for the Determination of Perrhenate'
Analyt. Chimica Acta, 80, (1975), 319-326.
74. Jones, D.R. and Manahan, S.E.
'Aqueous Phase High Speed Liquid Chromatographic Separation and Atomic Absorption Detection of Amino Carboxylic Acid Copper Chelates'
Analyt. Chem., 48, (1976), 502-505.
75. Woodis(Jr.), T.C., Hunter, G.B. and Johnson, F.J.
'An Indirect Determination of Biuret in Mixed Fertilizers and Urea by Atomic Absorption Spectrophotometry'
J. Ass. Off. Analyt. Chem., 59, (1976), 22-25.

76. Gagliardi, E. and Khadem-Awal, M.
'Acid Alizarin Black-SN as a Reagent for Extraction-
Photometric Determination of Metals'
Analyt. Abstr., 19, (1970), 1005.
77. Grotheer, M.P. and Labert, J.L.
'Colorimetric Determination of Bismuth in Alloys'
Analyt. Chem., 30, (1958), 1997-1999.
78. Armeanu, V.
'Spectrophotometric Determination of Traces of Bismuth
in Commercial Lead'
Analyt. Abstr., 25, (1973), 125.
79. Barkovskii, V.F. and Povet'eva, Z.N.
'Photometric Determination of Bismuth in Copper Ores
and Concentrates with Arsenazo III' (in Russian)
Analyt. Abstr., 19, (1970), 63..
80. Buhl, F. and Kania, K.
'Colorimetric Determination of Bismuth as a Complex
with 2,2'-Bipyridyl and Iodide' (in Russian)
Analyt. Abstr., 26, (1974), 106.
81. Buhl, F. and Kania, K.
'Analytical Properties of Ion-Association Complexes of
Metals with 1,10-Phenanthroline and 2,2'-Bipyridyl.
Determination of Iron and Bismuth' (in Russian)
Analyt. Abstr., 30, (1976), 2541.
82. Dolgorev, A.V., Lysak, Y.G. and Lukoyanov, A.P.
'Bis(thioantipyrinyl)methane as a new Selective Reagent
for Bismuth' (in Russian)
Analyt. Abstr., 27, (1974), 2541.
83. Suk, V. and Smetanova, M.
'Bromopyrogallol Red as a Colorimetric Reagent. I.
Photometric Determination of Bismuth' (in Russian)
Analyt. Abstr., 13, (1966), 6819.
84. Malat, M.
'Photometric Determination of Bismuth with Catechol
Violet' (in German)
Analyt. Abstr., 9, (1962), 4644.

85. Shestidesyatnaya, N.L.
'Spectrophotometric Study of Complexes of Bismuth with Catechol Violet and Diphenylguanidine' (in Russian)
Analyt. Abstr., 30, (1976), 4B94.
86. Baniska, H. and Tarasiewicz, M.
'Analytical Application of Chlorpromazine-hydrochloride. I. Its Properties and Application to the Qualitative and Quantitative Determination of Bismuth' (in Polish)
Analyt. Abstr., 17, (1969), 820.
87. Baniska, H. and Tarasiewicz, M.
'Analytical Application of Chlorpromazine-hydrochloride. II. Spectrophotometric Determination of Bismuth' (in Polish)
Analyt. Abstr., 18, (1970), 2352.
88. Ishikura, F. and Tonosaki, K.
'Spectrophotometric Determination of Bismuth with Chlorsulphophenol-S' (in Japanese)
Analyt. Abstr., 34, (1978), 1B155.
89. Grunin, A.V.
'Spectrophotometric Determination of Bismuth in Copper and Copper Alloys with m-Cresol-Phthalexon-S' (in Russian)
Analyt. Abstr., 26, (1974), 2004.
90. Grunin, A.V. and Cherkesov, A.I.
'Comparative Spectrophotometric Study of Complex Formation of Bismuth with some Phthalexons' (in Russian)
Analyt. Abstr., 27, (1974), 90.
91. Navaratna, M.R. and Iyer, C.S.P.
'Dalzin (1,6-Diallyl-2,5-Dithiobiurea) as an Analytical Reagent for Estimation of Trace Quantities of Bismuth in Lead and Nickel'
Talanta, 24, (1977), 396-398.
92. Busev, A.I. and Panova, A.K.
'Spectrophotometric Study of the Interaction of Bismuth with Potassium O,O-Bis-(α -(3-methyl-5-oxo-1-phenyl-2-pyrazolin-4-ylidene)-p-tolyl)phosphorodithioate'
Analyt. Abstr., 28, (1975), 5B121.
93. Arishkevich, A.M. and Usatenko, Y.I.
'Dimercaptothiopyrones - New Analytical Reagents. III. Photometric Determination of Bismuth'
Analyt. Abstr., 11, (1964), 4235.

94. Eberhard, L., Morgaenstern, R. and Uhlemann, E.
'Chelates of β -Dicarbonyl Compounds and their Derivatives. Liquid-Extraction-Spectrophotometric Determination of Zinc and Bismuth with 2-(Thiobenzyl)acetophenone'
Analyt. Chimica Acta, 90, (1977), 173-178.

95. Amus, E., Habne, H. and Ohls, K.
'1,2-Dimorpholinoethane as a Reagent in Quantitative Analysis. III. Gravimetric and Precipitation-Photometric Determination of Bismuth' (in German)
Analyt. Abstr., 11, (1964), 3058.

96. Grzhegorzhevskii, A.S.
'Certain Properties of Di-2-Naphthylthiocarbazon and its Bismuth Complexes'
Analyt. Abstr., 4, (1957), 3822.

97. Kirspun, K.K. and Busev, A.I.
'Analytical Properties and uses of Diethyl and Diethyl-diselenocarbamates' (in Russian)
Analyt. Abstr., 17, (1969), 2554.

98. Marczenko, Z.
'Spectrophotometric Determination of Elements'
Ellis Horwood Ltd., 1976.

99. Chakrabarty, T. and De, A.K.
'Liquid-Liquid Extraction of Arsenic, Antimony and Bismuth with Potassium Ethylxanthate'
Z. Analyt. Chem., 242, (1968), 152-159.

100. Khalifa, H.
'Fast Grey RA as a new Colorimetric Reagent for the Microdetermination of Bismuth'
Analyt. Chimica Acta, 17, (1964), 941.

101. Cherkesov, A.I., Akchurina, G.S. and Aleksanorovich-Mel'nikova, A.S.
'Use of Gallein for the Photometric Determination of Bismuth' (in Russian)
Analyt. Abstr., 11, (1964), 941.

102. Kekedy, L. and Balogh, G.
'Photometric Determination of Bismuth with Gallein. Rapid Determination of Bismuth in Lead'
Analyt. Abstr., 11, (1964), 5421.

103. Prakash, O. and Mushran, S.P.
'Spectrophotometric Determination of Bismuth with Oxidised Haematoxylin'
Indian. J. Chem., 6, (1968), 270-271.
104. Nevskaya, E.M., Shelikhina, E.I. and Antonovich, V.P.
'Heterocyclic Hydroxyazo Compounds as Photometric Reagents for Bismuth' (in Russian)
Analyt. Abstr., 31, (1976), 1B136.
105. Tataev, O.A. and Shakhabudinov, A.S.
'Reaction of Bismuth(III) with some trihydroxyflurones in the Presence of Antipyrine' (in Russian)
Analyt. Abstr., 27, (1974), 89.
106. Tataev, O.A. and Shakhabudinov, A.S.
'Comparative Study of Bismuth(III) Complexes with some trihydroxyfluorones' (in Russian)
Analyt. Abstr., 28, (1975), 6B146.
107. Antonovich, V.P., Shelikhina, E.I., Nevskaya, E.M. and Yakovleva, T.P.
'Trihydroxyfluorones as Reagents for Spectrophotometric Determination of Bismuth' (in Russian)
Analyt. Abstr., 31, (1976), 1B135.
108. Roebisch, G.
'Photometric Determination of Traces of Bismuth with use of 8-Hydroxy-7-(α -(2-methoxy-carbonyl-anilino)-benzyl)quinoline' (in German)
Analyt. Abstr., 19, (1970), 4834.
109. Martirosov, A.E., Talipov, T. and Dzhilyanbaeva, R.K.
'Possible use of N-Methylanabasine- α' -azodiethylamino-phenol in Photometry' (in Russian)
Analyt. Abstr., 18, (1970), 6.
110. Martirosov, A.E., Talipov, S.T., Dzhilyanbaeva, R.K. and Tatarskaya, A.Z.
'Absorptiometric Study of Complex Formation Between Bismuth and N-Methylanabasine- α' -azodiethylamino-phenol' (in Russian)
Analyt. Abstr., 18, (1970), 144.
111. Talipov, S.T., Dzhilyanbaeva, R.K. and Martirosov, A.E.
'Use of MAAF(II) in Photometric Analysis' (in Russian)
Analyt. Abstr., 18, (1970), 2227.

112. Shestidesyatnaya, N.L., Kish, P.P. and Merenich, A.V.
'Formation and Extraction of the Iodo-Bismuthate-Methyl Green Complex' (in Russian)
Analyt. Abstr., 22, (1972), 745.
113. Shestidesyatnaya, N.L., Milyaeva, N.M. and Kotelyanskaya, L.I.
'Extraction of Bismuth as a Ternary Complex with Methylthymol Blue and Diphenylguanidine' (in Russian)
Analyt. Abstr., 32, (1977), 1B118.
114. Zigai, K.G., Talipov, S.T. and Ivanova, I.Y.
'Photometric Determination of Bismuth with 4-(3-(1-Methyl-2-piperidyl)-2-pyridylazo)resorcinol' (in Russian)
Analyt. Abstr., 13, (1966), 619.
115. Uvarova, K.A. and Usatenko, Y.I.
'Study of the Interaction of Bismuth with 2-Mercaptobenimidazole and Perchloric acid'
Analyt. Abstr., 26, (1974), 831.
116. Izquredo, A. and Bosch, E.
'Spectrophotometric Determination of Palladium(II) or Bismuth(III) with 2-Mercaptopyridine' (in Spanish)
Analyt. Abstr., 25, (1973), 872.
117. Sturis, A.K., Tsirule, Y.A. and Bankovskh, Y.A.
'Investigation of 8-Mercaptoquinoline and its Derivatives. LVI. Determination of Bismuth using 1,2,3,4-Tetrahydro-8-mercaptoquinoline' (in Russian)
Analyt. Abstr., 19, (1970), 3007.
118. Buhl, F. and Skibe, H.
'Extraction-Colorimetric Determination of Bismuth as a Complex with 1,10-Phenanthroline and Iodide ions' (in Polish)
Analyt. Abstr., 23, (1972), 3825.
119. Busev, A.I., Shevedova, N.V., Akimov, V.K. and Fursova, E.G.
'Analytical use of Complexes of Bismuth with Phenyl-Substituted Thioureas and Perchlorate ions' (in Russian)
Analyt. Abstr., 21, (1971), 151.
120. Busev, A.I., Akimov, V.K. and Saber, S.A.
'Halide Complexes of Bismuth and Cadmium with some Pyrazolinone Derivatives. Photometric Determination of Bismuth' (in Russian)
Analyt. Abstr., 21, (1971), 2526.

121. Patrescu, C. and Matache, M.
'Colour Reaction Between Bismuth and Pyronine G'
(in Romanian)
Analyt. Abstr., 29, (1975), 1B137.
122. Subrahmanyam, B. and Eshwar, M.C.
'Extraction-Spectrophotometric Determination of Bismuth
with 1-(2-Pyridylazo)-2-naphthol'
Analyt. Abstr., 32, (1977), 3B137.
123. Janik, B. and Gancarczyk, T.
'Application of the Reaction of 1-(5-Pyrazolylazo)-2-
naphthol with Bismuth ions' (in Polish)
Analyt. Abstr., 33, (1977), 2E7.
124. Chernomosrchenko, L.I. and Butenko, G.A.
'Spectrophotometric Determination of Bismuth with
Quinoxaline-2,3-dithiol' (in Russian)
Analyt. Abstr., 27, (1974), 698.
125. Sivori, R. and Guerrero, A.H.
'Direct Determination of Bismuth with Rhodamine B'
(in Spanish)
Analyt. Abstr., 16, (1969), 1212.
126. Shestidesyatnaya, N.L. and Kotelyanskaya, L.I.
'Extraction-Photometric Determination of Bismuth in
Semiconductor films' (in Russian)
Analyt. Abstr., 32, (1977), 3B214.
127. Burkina, A.Y. and Senov, P.L.
'Use of Ruhemann's Purple, Sodium Salt for the Photometric
Determination of Lead, Mercury and Bismuth' (in Russian)
Analyt. Abstr., 25, (1973), 1147.
128. Korenman, I.M. and Vorontsova, T.E.
'Spectrophotometric Determination of Bismuth' (in Russian)
Analyt. Abstr., 18, (1970), 3908.
129. Kawase, A.
'Photometric Determination of Bismuth with Tetraphenyl-
phosphonium Bromide'
Analyt. Abstr., 6, (1959), 4733.
130. Baisya, N.K. and Baruah, G.
'Extractive Spectrophotometric Method for the Determina-
tion of Bismuth with Tetraphenyl-arsonium Chloride'
Analyt. Abstr., 32, (1977), 2B124.

131. Hilickova, M. and Sommer, L.
'Reaction of 4-(2-Thiazolylazo)resorcinol with Copper(II), Lead(II), Zinc(II), Cadmium(II) and Bismuth(III)' (in German)
Analyt. Abstr., 14, (1967), 5257.
132. Tomioka, H. and Terashima, K.
'Extraction-Spectrophotometric Determination of Bismuth with 4-(2-Pyridylazo)resorcinol' (in Japanese)
Analyt. Abstr., 16, (1969), 107.
133. Rozycki, C. and Maksjan, J.
'Use of Thiocyanate in the Determination of Bismuth. I. Colorimetric Determination' (in Polish)
Analyt. Abstr., 20, (1971), 2413.
134. Sikorska-Tumicka, H.
'Thio(capro)lactam as a Reagent in Quantitative Analysis. I. Colorimetric Determination of Bismuth' (in German)
Analyt. Abstr., 14, (1967), 1942.
135. Sikorska-Tumicka, H.
'Thiolactams as Reagents in Quantitative Analysis. II. Colorimetric Determination of Bismuth' (in German)
Analyt. Abstr., 16, (1969), 633.
136. Sikorska-Tumicka, H.
'Thiolactams as Reagents in Quantitative Analysis' V. (in German).
Analyt. Abstr., 18, (1970), 3909.
137. Sikorska-Tumicka, H.
'Thiolactams as Reagents in Quantitative Analysis. VI. Colorimetric Determination of Bismuth with Thio(capro)lactam' (in German)
Analyt. Abstr., 18, (1970), 143.
138. Solanke, K.R. and Khopkar, S.M.
'Thioenoyl-trifluoroacetone as the Extracting Reagent for Bismuth'
Analyt. Lett., 6, (1973), 31-41.
139. Mottola, H.A.
'Absorptiometric Determination of Bismuth with Thoron'
Analyt. Chimica Acta, 27, (1962), 136-143.
140. Ansheeva, A.A. and Bezuglyi, D.V.
'Determination of Bismuth in Cast-iron or Steel by Absorptiometry or Complexometry with Xylenol Orange'
Analyt. Abstr., 12, (1965), 2229.

CONTAINS PULLOUTS

Modified Colorimetric Method for the Determination of Malathion.

Analyst, December, 1979, Vol. 104, pp. 1129-1134

Further Improvement of the Modified Spectrophotometric Method for the Determination of Malathion.

Analyst, June, 1980, Vol. 105, pp. 564-567

Evaluation of the Modified Colourimetric Method for the Determination of Malathion: Its Application to the Analysis of Malathion Residues in Water.

Water Research Vol. 14, pp. 1037 to 1040

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