An investigation of the Transmethylation Reaction of the Methyltin Chlorides with Inorganic Mercury

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For my parents Eugene and Dympna Brennan

Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Ph. D. is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

Signed: _____ I.D.: 95971173

Candidate

Date: Oct 2003

Man more readily believes that which he wishes to be true

Unknown

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Abstract

This work explores the consequences of the reaction between methyltin compounds and inorganic mercury which is thought to occur in the environment. This reaction has considerable environmental importance.

The introduction of organotin compounds into the environment may occur as a result of their use in various commercial products, including agricultural biocides. In this case the organotin species is applied directly into the environment. Once they have entered the environment their persistence and fate is of great importance. Some potential reactions, include UV degradation and environmental methylation, these are discussed in Chapter 1, which also includes a discussion of the various analytical techniques that are used for the determination of organotin compounds.

Metal speciation has become very important due to its impact on environmental chemistry. Much research has been centered on developing highly efficient analytical techniques that are able to determine rapidly and sensitively the chemical forms of metals in a variety of sample matrices. Capillary Zone Electrophoresis (CZE), one such technique, has been developed as a powerful technique for the rapid and highly efficient separation of metal species. Chapter 2 contains discussion on the theory of CZE along with a section describing the development of a CZE method, and its validation, for the separation of the methyltin chlorides.

Chapter 3 contains a preliminary investigation of the reactions of tetramethyltin, which may occur naturally in the environment. This includes an examination of the UV degradation of tetramethyltin and also an investigation of the transmethylation reaction between tetramethyltin and inorganic mercury.

The chemistry of trimethyltin chloride, dimethyltin dichloride and methyltin trichloride in the presence of inorganic mercury (II) is described in greater detail in Chapter 4. The parameters of the transmethylation reaction were measured. The reaction was quantified using the CZE method previously developed.

Having examined the transmethylation reaction of trimethyltin chloride and inorganic mercury in a liquid medium, the reaction was then examined in the presence of a solid support. Again, CZE was used to measure the product distributions and the kinetic parameters obtained in liquid phase were compared to those obtained in the presence of solid supports. The results are reported in Chapter 5.

1.1. Introduction

Organometallic compounds are defined as substances containing direct metal to carbon bonds. The variety of the organic moiety in such compounds is large, ranging from alkyl substituents to alkenes, alkynes, carbonyl, aromatic and heterocyclic ligands. Although some organometallic compounds have been known for many years it is only in recent decades that their industrial and environmental importance has become evident.

The study of organometallic compounds in the environment can be classified as follows:
(i) the commercial uses of organometallic compounds in industry;
(ii) the formation of these organometallic compounds in the environment;
(iii) the detection and analysis of these compounds in the natural environment.

The introduction of organometallic compounds into the environment may occur as a result of their in various commercial applications. When an organometallic compound is used as a product it is potentially available for environmental distribution, and often are applied directly into the environment, e.g. as agricultural biocides etc. Once they have entered the environment, their persistence and fate is important. Section [1.2] deals with the industrial applications of many organotin compounds.

Many organometallic compounds undergo subsequent reactions in the environment. Most important amongst these reactions is the degradation pathway which in some cases involves environmental methylation reactions. Such reactions can either lead to increasingly toxic products, or alternatively to a decrease in toxicity.
The degradation and methylation reactions of organotin compounds will be discussed in Sections [1.2] and [1.3] respectively.

The detection of organometallic compounds in environmental matrices has confirmed many of the environmental processes mentioned above. Much work has been done in developing analytical techniques to detect low concentrations of organometallic

compounds in natural samples. The main practical problem has been the low levels at which some of these compounds occur. Section [1.4] deals with the development of both direct and indirect methods of detection of organometallic species.

1.2. Organotin Compounds; Occurrence, Degradation and Fate in the Environment

Knowledge of the fate of organotins in the environment is essential for understanding the possible ecotoxicological effects of these materials. In its industrial applications, organotin compounds are extensively used as agrochemicals, as slabilisers in plastics and as additives to paints. It is therefore important to understand the effect on the environment of the introduction of these compounds to the ecosystem.

1.2.1. Input into the Environment

These organotin compounds found in the environment are generally the result of direct introduction by man, with the notable exception of methyltins which may also be produced by environmental methylation. This process will be discussed in greater detail in Section [1.3]. The first commercially registered organotin compound was marketed in 1936 for use as a stabiliser for synthetic polymers[1]. Since the biocidal properties of trialkylated tins were recognised in the 1950's, the variety of applications, products, and uses of these materials has increased significantly. To date, organotins are one of the

most widely used organometallic compounds. They are employed as pesticides, but also find important non-pesticidal applications and use as polyvinyl chloride (PVC) stabilisers and catalysts for polyurethane and silicone elastomers. The biocidal uses of the trisubstituted organotin compounds are exceeded by the applications of the di- and monosubstituted derivatives, which are used as stabilizers and catalysts[1]. The environmental impact of the trialkyl compounds is of particular concern. They have high fungicidal, bactericidal and algicidal properties.

Organotin compounds have a higher toxicity than inorganic tin, and the toxicity of organotins increases with progressive introduction of organic groups at the tin atom, with maximum toxicity for trialkylated compounds and decreasing toxicity with increased length of the organic moiety[2].

1.2.1.1. Applications of Organotin Compounds

In the overall consumption of organotin compounds, stabilisers for PVC account for 60% of the total consumption, and biocidal agents is the next largest consumer. These two represent about 90% of the total consumption of organotins. The biocidal agents are wood preservatives, antifouling agents, agrochemicals, pharmaceuticals, disinfectants, rodent repellents, protective agents of masonry and stonework, and slime preventing agents. The other uses include catalysts, glass applications, water repellents and flame retardants. PVC plastics have the second largest production volume after polyethylene: the total production in the major PVC producing countries was about 12 million tonnes in 1995.[3] Thus the amounts of added stabiliser have also increased. Lead and other organic compounds have also been used as stabilisers for PVC. The low molecular weight model compounds for PVC are stable up to 300-330°C but commercial PVC starts to decompose at 90-130°C. Hence, PVC requires stabilisers for thermal degradation during formation as well as stabilisers for photodegradation for outdoor uses. Many organotins

are able to protect the thermal decomposition of PVC effectively with only 1 g or less to 100 g of resin.

One of the characteristics of organotin compounds is a strong affinity of the tin atom to a donor ligand such as oxygen or nitrogen. The application of organotin compounds as catalysts takes advantage of this characteristic. Organotin compounds are used as catalysts for the polymerization of silicones and in esterification reactions[3]. Organotin catalysts have some advantages over traditional catalysts in that a pure product is obtained since side-reactions are greatly reduced, acid-induced corrosion of the process equipment is virtually eliminated and these organotin catalysts are highly efficient and need only be incorporated at levels of about 0.05 to 3.0%.

Another application of organotin compounds is as wood preservatives. Van der Klerk of Holland[4] first reported that organotin compounds are effective wood preservatives in 1954. He found that R_3SnX had the optimal fungicidal activity, where X = Cl, Br, etc. He also found that the X group in R_3SnX did not substantially influence their fungitoxicity; however, the R group has a great effect on the activity. Tributyltin (TBT) is widely used since it possesses a tolerable mammalian toxicity whilst it has a high toxicity to fungi and gram-positive bacteria. In particular, tributyltin oxide (TBTO) is used as a wood preservative. It is thought that Bu_3Sn oxide reacts with the hydroxyl group of cellulose of woods and the trialkyltin then remains stable on the surface of the wood.

Marine fouling can be a serious problem in the shipping industry, since it increases the surface roughness of the hull and hence its frictional resistance to movement through water. Marine animals and plants such as barnacles and algae adhere to the ships' hulls and represent a serious problem. The effectiveness of antifouling agents has led to the increased use of organotin compounds for this purpose[3]. Tributyltin gained widespread application as an effective antifouling paint biocide on pleasure boats, large ships and docks in the 1970's and 1980's. In the late 1970's, antifouling paints were found to have a detrimental environmental impact. Maguire et al.[5,6] were pioneers in studies on the

environmental chemistry and contamination of aquatic systems. As TBT leaches directly from paints into water, high contamination of coastal areas resulted. This route is the major pathway of entry of TBT into the aquatic environment. In many countries the use of TBT-containing antifouling paints has been controlled or banned since the early 1990's,.

Triorganotin compounds have a pesticidal activity towards pests of agricultural and horticultural crops. The principal advantage of organotin agrochemicals are the following:

- low toxicity to non-target organisms;
- inability of crop pests to build up resistance to inorganic tin;
- degradation in the environment.

Triorganotin compounds are extremely powerful biocides; however, their toxicity to plants is too high for practical uses in agriculture. However, the triphenyltins showed a sufficiently wide margin between fungitoxicity and phytotoxicity to enable them to be used safely. Triphenyltin compounds have very good adhesive properties and are retained firmly by leaves on which they have been sprayed, even after heavy rainfall. Thus, among the commercial organotin compounds used as biocides are triphenyltin acetate, triphenyltin hydroxide, tricyclohexyltin hydroxide, triphenyltin chloride[3]. For example triphenyltin acetate has a broad spectrum of activity against a number of fungal diseases, such as early blight and down mildew, and can be used on a variety of crops such as beans and potatoes. It also exhibits algicidal and molluscicidal properties, and is effective for use in rice paddy fields and against water snails in fish ponds. Triphenyl hydroxide is also used as a fungicide, particularly against tuber blight in potatoes, where it eradicates blight and protects the foliage from further infection. Triphenyltin chloride is also a fungicide and acts as a rodent repellant.[3]

Organotin compounds have also been developed as pharmaceuticals such as antihelmintics, disinfectants and antitumour drugs.[7,8] Dialkyltin compounds are applied as anthelmintics. For example, dibutyltin dilaurate is used to treat tapeworm in chickens or turkeys, while dibutyltin oxide is used for intestinal worms in fresh water fish such as trout. Tributyltin compounds are also effective against gram-positive bacteria. Their combination with a chemical which combats gram-negative bacteria produces a highly effective disinfectant which may be used on open areas posing a risk of infection, such as hospital floors. Common formulations contain, for example, a mixture of tributyltin benzoate and formaldehyde, or tributyltin oxide and a quaternary ammonium halide.[3]

The miscellaneous uses of organotin compounds include textile treatments, glass surface treatment, masonry protection and slime prevention on stonework. Bis(tributyltin) oxide is also useful for the prevention of fungal attack on cellulose material such as cotton textile and in cellulose-based household fillers as wood preservatives, as described earlier.[3] Monoorganotin trichloride, diorganotin chloride or their mixtures, e.g., monobutyltin trichloride and dimethyltin dichloride, are used as precursors for forming surface films of SnO_2 at high temperatures. They are vaporised and brought into contact with the glass surface in the presence of air, at about 600°C, whereupon decomposition and oxidation to tin (IV) oxide occurs on the glass surface. The fact that thin films of tin (IV) oxide less than 100 nm thickness would increase the strength and impact resistance of glassware was known.[3]

Treatments based on monoorganotin compounds conferred an adequate flame resistance to wool gaberdine fabrics. Trialkyltin or triaryltin compounds such as bis(tributyltin) oxide and triphenyltin chloride have a biocidal effect against clothes moth and carpet-beetle.[3]

1.2.2. Release into the Environment

As a result of the wide range of industrial applications of organotin chemicals there are a variety of pathways which can be envisaged for their entry into the environment.

The principal commercial use of organotins is as PVC stabilisers, and the possible routes by which these stabilisers may enter the environment are:

(a) leaching/weathering;
(b) land burial;
(c) incineration of waste material.

Of these routes, incineration is probably not significant since this method of disposal is not as common as land filling and, additionally, the organotins are likely to undergo thermal decomposition to inorganic tin compounds at the combustion temperatures. It has been estimated that a typical level of inorganic tin released into the environment from the incineration of domestic waste is approximately 1 μg per gram of suspended particles.[9]

Leaching of organotin stabilisers from PVC has been extensively studied, because of their use in food contact applications[10]. Studies have shown that the leaching rate is generally very low; although the actual rate of loss depends on the nature of the leachant (pH and aqueous or organic character), the alkyl chain length of the organotin compound present and the level of plasticiser.

With regard to the disposal of PVC waste by land burial, little is known about the environmental fate of the organotin stabilizers, which are the less toxic di- and monoorganotin compounds. It is reasonable to assume that leaching by aqueous media continues to occur, but the relationships between chemical environment, soil adsorption, leaching rates and degradation are unknown. However, it can be inferred that the PVC waste would be evenly distributed in domestic rubbish and so the expected levels of organotin compounds would be very low indeed.

The use of mono- and dialkyltins as homogeneous catalysts results in their incorporation into the finished product at very low levels, especially in terms of the final volume. A typical concentration of organotin catalysts, e.g. dibutyltin dilaurate, in a flexible polyurethane foam formulation, is of the order of 0.2 g kg^{-1}.[11] Therefore, the methods of release of these compounds will be similar to those encountered with the PVC stabilizers.

From the foregoing discussion it can be concluded that, although PVC stablilisers and homogeneous catalysts together comprise the largest consumption of organotin compounds, it is unlikely that these contribute significantly to the levels found in the environment.

Although the biocidal applications of organotin chemicals comprise only approximately 30% of the total world consumption, they probably give rise to the largest proportion of free organotins in the environment, due to their direct introduction into natural media. The use of triorganotin compounds as agrochemicals represent the greatest risk of contamination, as the manner of introduction, which usually involves spraying, increases the possibility that not only the soil, but also the air and adjacent waterways, could be contaminated by airborne particles. Volatilisation of triorganotin biocides, after application, is unlikely to be a significant problem, as these compounds are non-volatile. Triorganotin compounds have been shown to absorb strongly onto soil.[3]

Direct entry of organotins into the aquatic environment is primarily because of their use in antifouling paint systems. In agricultural applications, contamination could result from run-off water and overspray, whereas in marine antifouling paints and in antifouling rubber coatings, which utilize some 3000 tonnes of tributyl- and triphenyltin derivatives annually, the triorganotin compounds are released directly from the paint or rubber matrix into the water in the vicinity of the ships' hulls and subsequently dispersed. Consequently, the release of the triorganotin biocide in a static environment, e.g. harbours, marines and bays, is likely to be more significant than its loss when the vessel is under way in open seas.

Other possible modes of entry of organotins into the environment result from low tonnage uses, e.g. anthelmintic treatments for poultry, disinfectants, etc., and are not considered to have a significant impact on environmental levels.

In summary, tributyltin compounds are directly introduced into aquatic systems via leaching from antifouling paints, and this route is still of paramount importance. Even though triphenyltin compounds are also released via this route, applications in agriculture and runoff from agricultural fields play a more important role. Organotins can be leached from consumer products, which may be of growing importance, as the variety of material protected by TBT and the range of industrial applications is increasing. Leaching from[12] and normal weathering of PVC products leads to inputs of butyltins and methyltins into the aquatic and terrestrial environment.[13,14] In municipal waste incineration, organotins are decomposed to relatively low toxic tin oxide and to other unspecified combustion products. Although they occur, emissions into the air during application of agrochemicals, or from treated surfaces of preserved materials are not assumed to result in substantial inputs, and photodegradation decomposes these compounds relatively fast.[15]

Methyltins, in general, are found in a variety of natural waters, sediments and in rain. They may also be formed in the environment itself, by both biotic and abiotic methylation of inorganic tin. Mono-, di- and trimethyltins were found to be almost ubiquitous in the environments surveyed by Byrd and Andreae.[16] Methyltins were also recorded to be present in 5 to 10% of water and sediment samples analyzed in Canada.[17]

1.2.3. Degradation of Organotin Compounds

Owing to the widespread industrial uses of organotins, along with their biological activity and the possibility of biomethylation, their fate in the environment is of considerable interest. The possibility of their eventual degradation to a non-toxic inorganic tin species increases their potential as acceptable chemicals. Consequently, work has been carried out over a number of years to investigate the possible ways in which organotin compounds may degrade in the environment.

There is substantial evidence that organotin species found in environmental samples are related by environmental degradation pathways. A large number of studies have been conducted on the degradation of organotins in soils and water, demonstrating the progressive degradation of triorganotins to di-, mono- and inorganic tin species. Photochemical breakdown is one of the most significant modes of degradation in the environment. Certain fungi and bacteria are able to break down organotins, particularly tributyltin and triphenyltin compounds, and also are involved in biomethylation which will be discussed in Section [1.3.5]. Chemical cleavage is also a significant mechanism for degradation, encountered under usual environmental conditions and may occur in aquatic and terrestrial ecosystems.

Organotin degradation involves the sequential removal of organic groups from the tin atom which generally results in a reduction of toxicity[1,18]

$$R_4Sn \xrightarrow[-R]{+X} R_3SnX \xrightarrow[-R]{+X} R_2SnX_2 \xrightarrow[-R]{+X} RSnX_3 \xrightarrow[-R]{+X} SnX_4$$

This stepwise loss of organic groups from the tin atom is attractive to potential users of organotin compounds because it is accompanied by a progressive lowering in biological activity and, as such, the use of these compounds would be unlikely to lead to environmental pollution.

Degradation involves the breaking of a Sn-C bond and this can occur by a number of different processes. These include:

1) ultraviolet (UV) irradiation;
2) biological cleavage;
3) chemical cleavage;
4) gamma (γ) irradiation;
5) thermal cleavage.

Of these processes, γ irradiation will have little effect on environmental degradation, due to its negligible intensity at the earth's surface. Thermal cleavage is also unlikely to be of environmental significance, because the Sn-C bond is reported to be stable at temperatures up to 200°C.[19] Therefore only the first three processes mentioned above will be discussed further.

1.2.3.1. Ultraviolet Degradation

The light emitted by the sun, reaching the earth's surface, consists mostly of wavelengths above 290 nm. Therefore, with regard to the degradation of organotins, the shorter wavelengths may have a long-term effect. Ultraviolet light (UV) of wavelength 290 nm possesses an energy of approximately 300 kJ mol^{-1} and the mean bond dissociation energies for some Sn-C bonds were found[20] to be in the range 190-220 kJ mol^{-1}. Consequently, provided that absorption of the light occurs, Sn-C bond cleavage is possible, and, in fact, the maximum absorption wavelength of organotin compounds is generally within the UV region.

Getzendaner and Corbin[21] studied the UV degradation of tricyclohexyltin hydroxide, suggesting that dicyclohexyltin, monocyclohexyltin and inorganic tin species were produced, but no further details were given.

Barnes et al.[22] showed that triphenyltin acetate was degraded to inorganic tin by UV light. The study of the UV degradation of organotins in aqueous solution has also received some attention. Crosby et al.[23] reported the action of ultraviolet light on triphenyltin hydroxide in water, showing that, under simulated environmental conditions, a diphenyltin species was produced which underwent further breakdown, possibly forming a polymeric monophenyltin derivative $(PhSnO_xH_y)_n$. It was not, however, demonstrated that this polymeric species eventually degrades to inorganic tin. The effect of pH on the photolysis of Ph_3Sn^+ was also investigated in the same study. It was found that photolysis seemed to proceed slightly more rapidly under more basic conditions, and both pH extremes showed more rapid loss than neutral photolysis. No explanation was offered for this result.

The gradual disappearance of triphenyltin acetate in freshwater, seawater and sewage water has been noted by Odeyemi and Ajulo,[24] but the breakdown products were not identified. Maguire et al.[6] studied the UV degradation of tributyltin species in water in which it was observed that the tributyltin species dissolved in water neither volatilises nor loses butyl groups over a period of two months in the dark at 20 °C. In sunlight, however, it underwent a slow photolytic decomposition involving a sequential debutylation process to inorganic tin.

Blunden[15] studied the UV degradation of the methyltin chlorides in carbon tetrachloride and water with analysis by ^1H NMR spectroscopy. In the organic solvent trimethyltin chloride degraded to an inorganic tin species, via di- and mono-methyltin intermediates. In water, however, a monomethyltin derivative was not observed. The final product of the reaction was shown to be hydrated tin (IV) oxide, The ultraviolet breakdown of dimethyltin dichloride and monomethyltin trichloride in these solvents was also studied, and the approximate relative rates of degradation were established.

In conclusion, the general occurrence of tributyltin degradation products in water and sediments indicates that biotic and abiotic processes will result in the removal of this compound. TBT does not seem to be a persistent compound in water, as a rapid breakdown with half-lives on the order of seven to thirty days was found at summer temperatures. However, degradation rates were much slower at winter conditions, with half-lives of two months or longer. The loss of TBT from aquatic ecosystems by volatilization is very limited, although found relevant in model ecosystems.[25] Hydrolysis is negligible, and TBT is chemically stable in natural waters. Half-lives of ultraviolet (UV)-mediated photodegradation of TBT are longer than three months,[6] and because penetration of UV light into deeper layers is prevented, this degradation pathway is not significant. When exposed to sunlight or UV light in the laboratory, aqueous TPT hydroxide was readily degraded by homolytic cleavage of the tin-carbon bond to diphenyltin oxide and further to a water-soluble polymeric tin species.[23] Trimethyltin was also quantitatively demonstrated to be photodegraded.[15]

1.2.3.2. Biological Cleavage

The question of biodegradation of organotins is particularly important in situations where, for example, the compounds are not directly exposed to light, e.g. in the soil or on the sea bed. Biodegradation appears to be enzymatically controlled, and the alkyl group usually is converted to the corresponding hydrocarbon.

It has been shown by Barnes et al.[22] that ^{14}C-labelled triphenyltin acetate in soil degrades to inorganic tin. Since carbon dioxide was evolved and breakdown did not occur in sterile soil, it was concluded that degradation was due to the ability of certain microorganisms to metabolize the organotin compound.

Barug and Vonk[26] have shown that bis(tri[1-^{14}C]butyltin)oxide undergoes breakdown in soil due to the action of micro-organisms and Barug[27] demonstrated that the gram-negative bacteria have been found not to degrade bis(tributyltin)oxide. The degradation in wood, of bis(tributyltin)oxide and tributyltin naphthenate[28] has been examined and di- and monobutyltin species have been detected as breakdown products. This process could be due to fungal degradation, since bis(tributyltin)oxide has been shown[27] to be degraded by various individual microfungi, such as *Coniophora puteana*, *Coriolus (Trametes) versicolor* and *Chaetomium globosum.*

The biodegradation of tributyltin species in Toronto harbour was reported by Maguire et al.[29] It was found that tributyltin initially present in a freshwater harbour sample was found to degrade, in the dark, to monobutyltin and dibutyltin with an estimated half-life of 20 weeks. In samples which contained sediment the half life was found to decrease to approx. 16 weeks and in this case inorganic tin was detected and found to be the major species at the end of the experiment. A sequential debutylation pathway was shown for this water-sediment mixture.

Seligman and co-workers[30] examined tributyltin degradation in marine coastal waters. They found that while microbial metabolism was the principal degradative process, algal metabolism may have played a lesser role while photolysis was not considered to be significant. Calculated half-lives in water collected from a yacht harbour were 6 and 7 days for light and dark experiments. Half-lives from a clean-water site were 9 and 19 days for light and dark experiments respectively. The principal degradation product was again shown to be dibutyltin with lesser amounts of monobutyltin found.

Seligman[31] later determined the relative importance of marine microalgae in degrading tributyltin species in estuarine species. They found that when water lacked biota, photolysis did not contribute to the degradation of the tributyltin species. They found that tributyltin degradation was more rapid in sunlit estuarine waters compared to incubations in the dark. They also found that (hydroxybutyl)dibutyltins were formed as degradation products only in those reactions carried out in sunlight.

1.2.3.3. Chemical Cleavage

Metal-carbon bonds may be susceptible to attack by environmental/chemical means without microbiological intervention although in some circumstances, biological activity may enhance the environmental conditions necessary for abiotic attack, e.g. alteration of pH, redox potential, etc. The Sn-C bond is capable of polarization in either direction ($Sn(\delta^+)$-$C(\delta^-)$ or $Sn(\delta^-)$-$C(\delta^+)$),[32] and is, therefore, susceptible to attack by both nucleophilic and electrophilic reagents. Hence, for reactions of the type

$$\equiv Sn\text{-}C + A\text{-}B \longrightarrow \equiv Sn\text{-}A + \equiv C\text{-}B$$

A-B may be one of a wide variety of compounds, e.g. mineral acid, carboxylic acid, alkali, etc. In addition, the trimethyltin cation has been shown to transmethylate with various hydrated metal cations, e.g. Pd^{2+}, Au^{3+}, Hg^{2+}, forming a dimethyltin species and the corresponding monomethyl derivative.[33] Mercury salts can demethylate other compounds:

$$HgCl_2 + (CH_3)_3SnCl \longrightarrow CH_3HgCl + (CH_3)_2SnCl_2$$

Free radical processes can cause homolytic Sn-C bond fission, the Sn-C bond being fairly good radical trap.[32] Consequently, a very wide range of chemical reactions result in Sn-C bond cleavage. Some of these reactions may result in the disappearance of the organometal from a given location; other reactions give rise to more toxic products and exacerbate toxic effects.

From these breakdown studies it may be concluded that, generally, organotins will degrade in natural media, and this has been demonstrated for triphenyltin,[6] tributyltin[22,30,31] and tricyclohexyltin[21] compounds. In one of the aqueous degradation studies,[23] inorganic tin was not detected and it was thought that a monoorganotin species might be the end-product of the breakdown process. However, the rate of breakdown of monoorganotins has been suggested,[19] in some cases, to be slower than that of tri- and diorganotins, and it is possible that the timescale was not adequate for inorganic tin to be

detected. With regard to the degradation studies in wood[28] only di- and monoorganotin species have so far been positively identified as breakdown products. The failure, as yet, to detect inorganic tin may be due to the problems associated with extracting the total tin content from the wood sample.

No mention has been made of the timescales involved in the degradation processes, since although some of the studies have reported half-lives, they relate only to the specific laboratory experimental conditions and so are not directly comparable to environmental degradation. Additionally, it can be misleading to relate experimental timescales to an environmental situation, where the actual rate of breakdown will be dependent on many factors, e.g. intensity of sunlight, concentration of suspended matter in waterways, etc.

Clearly, many mechanisms of organometallic degradation result in detoxification and occupy significant positions in the biogeochemical cycles of the elements involved.

1.3. Formation of Organotin Compounds in the Environment

Organometallic compounds arise in the environment as a result of natural processes, including those mediated by living organisms, or because of accidental or deliberate introduction. Deliberate environmental introduction results from the widespread use of many organometals as biocides as discussed in Section [1.2]. Methylated derivatives of several elements naturally arise in the environment as a result of chemical and biological methylation, microorganisms playing highly significant roles in the latter process.[34]

Biological alkylation (bioalkylation) refers to the mechanism by which alkyl groups are linked to metal atoms, thus forming metal-carbon bonds, as a result of direct or indirect biological action. The most common alkyl group transferred is the methyl group, this process being termed biological methylation (biomethylation).

Available evidence indicates that the natural transformation of organometals under environmental conditions has a very considerable significance. The presence of a metal-carbon linkage alters the toxicity quite substantially, and also alters the accessibility of the compound. The number and type of organic groups attached to a metal or metalloid affect the ease with which the compound is absorbed or excreted. Similarly, the mobility of compounds in soil, in water, or through the air depends very much on the ligands attached to it. Obviously, then, changes in these ligands create corresponding changes in the biological absorption and/or geochemical mobility of the compounds.

Methyltin compounds detected in the environment are thought to be the result of methylation of various tin species, particularly inorganic tin, which are produced by the degradation of other organotin compounds released into the environment.

This section summarizes methyl transfer reactions of environmental significance from organic reagents, methylcobalamin(CH_3CoB_{12}) and synthesized metal complexes to organotin compounds. Most of the reactions described are in water, reflecting environmental observations.

These reactions are important because organometallic compounds are much more toxic than their inorganic counterparts. The enhanced toxicity arises from the ability of neutral organometallic compounds to permeate cell membranes and modify intracellular chemistry. Studies of organometallic compound formation by reactions of metals and metalloids with naturally occurring metabolites or known biochemical methylating agents in aqueous media under laboratory conditions are also important. These studies are steps toward the goal of understanding such processes in the environment and distinguishing between chemical and enzymatic methylation of metals and metalloids. The research on methylation of tin compounds is classified into two types: abiological and biological methylation.

1.3.1. Abiological Methylation

Abiological methylation is the methylation with chemical methylating reagents originating in the environment. The major abiological methylating agents are methylcobalamin (CH_3CoB_{12}), S-adenosylmethionine (SAM) and methyl iodide (CH_3I). The methyl group may be transferred to the metal as either a carbanion (CH_3^-), a radical ($CH_3^{\cdot}$) or as carbonium ions (CH_3^+). However, the methyl group is usually transferred to the metal as a carbanion. Carbanion transfer may also arise from other organometallic species present in the external environment e.g. $(CH_3)_3Sn^+$ to Hg(II)[35]. As well as these, many other biomolecules e.g. humic and fulvic acids may oxidise and methylate metal species.

Methyltin compounds are widely found in natural environments although it is usually difficult to ascertain the relative proportions that arise from anthropogenic sources, abiotic or biotic methylation reactions or degradation of more complex organotin molecules.[1,36,37,38,39]

There have been a number of laboratory investigations using methylcobalamin (CH_3CoB_{12}) and methyl iodide, as chemical methylating agents for tin (II) and tin (IV) compounds.

1.3.1.1. Methylcobalamin

Methylcobalamin is one of the coenzyme forms of vitamin B_{12} found in bacteria and animals. It is a crystalline cobalt complex synthesized by microorganisms and has the ability to transfer its methyl group to metals. This methylation reaction may occur by the addition of carbocation (CH_3^+), carbanion (CH_3^-) or methyl radical ($CH_3^{\cdot}$).[36,40,41]

Methylcobalamin ($MeCoB_{12}$) has been proposed as the main methylating agent for tin compounds. The proposed mechanism involves a stannyl radical, Sn(III)$^{\cdot}$ arising from the oxidation of Sn(II)$^{\cdot}$, Fe(III) being used as the oxidising agent. The stannyl radical reacts with CH_3CoB_{12} to give homolytic cleavage of the Co-C bond and produces CH_3SnCl_3 under reaction conditions of high chloride concentration[36]. Available evidence indicates that the methyl group is transferred as a carbanionic species and that acceptor atoms must be electrophilic.[36]

$$\text{Sn(II)} \xrightarrow{\text{Fe(III) or } O_2} \text{Sn(III)} \xrightarrow{CH_3CoB_{12} + H_2O} \text{Methyltin (IV)} + H_2OCoB_{12}$$

Methyltins were produced from $Sn(II)Cl_2$ and $MeCoB_{12}$ (with aquocobalamin) under HCl acidified conditions with Fe(III) as oxidising agent. No methyltins were formed without the presence of either aquocobalamin or Fe(III). Methylation to Sn(II) was also observed in the presence of O_2 as the substitute for Fe(III).[42] Although it is known that CH_3^- from $MeCoB_{12}$ would add to Hg(II),[36,41] methyl group species in tin methylation were thought to be carbonium ion (CH_3^+) or methyl radical ($CH_3^{\cdot}$) and methyltins would not be produced with addition of CH_3^- to inorganic tin under the possible environmental conditions.[43]

Both chemical forms of inorganic tin and anionic ions (counter ion) in methylating systems with inorganic tin (II) by $MeCoB_{12}$ were investigated by Dizikies et al.[44] SO_4^{2-} and ClO_4^- were found to inhibit methyltin production. Also Sn(IV) failed to produce methyltin compounds. The reaction rate was accelerated with an increase in chloride ion concentration. It was suggested that $[SnCl_3]^-$ was first produced, and CH_3^+ from $MeCoB_{12}$ transferred to $[SnCl_3]^-$ complexes.[45]

Rapsomanikis and Weber[46] studied the reaction by the addition of dimethyl cobalt complex $[(CH_3)_2Co(N_4)]+$; N_4 is 2,3,9,10-tetramethyl-1,4,8,11-tetraazacyclotetradeca-1,3,8,10-tetraene. They found that $(CH_3)^-$ of the dimethyl cobalt complex could be transferred to mono-, di- and trimethyltin.

Wood and his co-workers[42,44] have reported that CH_3CoB_{12} is demethylated by $SnCl_2$ in aqueous HCl solution, in the presence of an oxidising agent, to form a monomethyltin species. Thayer[47] found that finely divided tin (IV) oxide, SnO_2, reacts with CH_3CoB_{12} in aqueous HCl solution to form methyltin derivatives but the reaction appears to be very slow. Thayer[48] also studied the reaction of $(CH_3)_3SnOAc$, $(C_2H_5)_3SnOAc$, $(C_6H_5)_3SnOAc$, and $(CH_3)_2SnCl_2$ with CH_3CoB_{12} although the products were not identified.

1.3.1.2. Methyl Iodide

Methyl iodide (MeI) is produced by macro algae and kelp,[49] and exists widely in coastal air and seawater.[50] Methyl iodide reacts with some metals and metal sulphides in the environment to produce methyl-metal compounds.[51] Brinckman et al. have demonstrated the oxidative methylation of Sn (II) sulphide by methyl iodide in aqueous solution, under mild anaerobic or aerobic conditions, to form CH_3SnI_3. The methyltin production rate from SnS was higher than that from $SnCl_2$ or SnI_2.[52]

Methyltins were also produced by the reaction between $SnCl_2$ and CH_3I with manganese dioxide.[46] From these studies, oxidative methylation of carbocation (CH_3^+) from CH_3I was ascertained and the mechanism of methylation was proposed as shown:

$$CH_3I + Sn(II)Y_2 \xrightarrow[\text{Slow}]{} [CH_3Sn(IV)Y_2^+I^-] \xrightarrow[\text{Fast}]{} CH_3Sn(IV)Y_2I$$

1.3.1.3. S-Adenosymethionine

Another important environmental methylating agents is S-adenosylmethionine (SAM). The methyl group is transferred as a carbocation. Any recipient atom must therefore be nucleophilic, which usually requires an available pair of electrons.

1.3.1.4. Other Chemical Substances

Monomethyltin was produced in the reaction system with tin (IV) chloride and humic and fulvic acids extracted from sediment.[53] However, Hamasaki et al.[54] could not observe methyltin production in a reaction between inorganic tin(II), tin(IV) and humic or fulvic acid. They reported that ethanol, acetic acid and propionic acid could methylate inorganic tin. In these reactions, higher yields of methyltins were observed in the reaction of inorganic tin (II) than for tin (IV). Therefore, it may be concluded that both tin (II) and tin (IV) compounds appear to be chemically methylated under simulated environmental conditions.

1.3.2. Biological Methylation

Biological speciation is the formation and dissociation of metal-carbon bonds in organisms. Although ultimately chemical in nature, these processes require biological mediation for their occurrence. Metal-carbon bonds may be formed or broken under environmental conditions, however, without the necessity of the biological intervention, as discussed earlier. In addition to their ability to form metal-carbon bonds, many bacterial species have the ability to cleave such linkages.

Brinckman and co-workers[55] showed that in vitro biomethylation of inorganic tin (IV) by pure cultures of tin-resistant Pseudomonas bacteria produced methylstannanes, $(CH_3)_nSnH_{4-n}$, where n = 2-4. The formation of methyltin compounds in sediments has been described[56]: both tin chlorides, $SnCl_2$ and $SnCl_4$, were found to be methylated, and, in the study by Hallas,[39] the species formed from $SnCl_4.5H_2O$ were the hydrides, $(CH_3)_2SnH_2$ and $(CH_3)_3SnH$. Trimethyltin hydroxide[56] was converted to tetramethyltin in sediments and this process appears to be slow.

The study of biological tin-methylation first started with sediment or estuarine containing microorganisms. Tetramethyltin formation from trimethyltin by estuarine sediment was reported by Guard et al.,[56] and the addition of sulphide ion in their experimental system enhanced the yield of methyltins. Microorganisms in the estuarine samples could produce mono-, di- and trimethyltin from $SnCl_4$ but was not identified.[39] The mechanism of tin methylation was investigated using $(CD_3)_3SnCl$, D= deuterium.[43] $(CD_3)_4Sn$ was produced and $(CD_3)_3Sn\text{-}(CH_3)$ was not detected. The yields of $(CD_3)_4Sn$ were comparable to $(CH_3)_3Sn^+$. This showed that methylation was caused by redistribution of methyl groups (CD_3) from $(CD_3)_3SnCl$ and biological methylation, which is defined as enzymatic transferring of a methyl group in living organisms, was less than 5% of the total methylation in this experimental system.

However, there have been many reports supporting the theory of methylation of inorganic tin in the environment by organisms. Jackson et al.[55] reported Pseudomonas No. 244 isolated from Chesapeake Bay that could form $(CH_3)_3Sn^+$ from Sn (IV). Yeast, *saccharomyces cerevisiae*, could produce monomethyltin from various tin (II) compounds.[57] Methylation of inorganic tin was also observed by sulphate reducing microorganisms isolated from the sediment in Chesapeake Bay.[58] Green algae (*Enteromorpha sp.*) metabolized inorganic tin (IV) and transformed to mono- di- and trimethyltins.[59] Trimethyltin was detected in the leaves of *Spartina alterniflora* as biological methylation product and no methyltins were detected in root tissue.[60]

1.4. Detection of Organotin Compounds in the Environment

The growing awareness over the environmental fate of organotin compounds is reflected in the large number of analytical methods developed for their speciation. Most of the analytical speciation methods applied to actual environmental media have involved prior derivatization to transform organotin compounds into volatile hydrophobic analytes amenable to separation and identification by gas chromatography coupled to a selective and sensitive tin detector. However, chemical treatment prior to analysis, or high temperatures associated with gas chromatographic separation, may alter the relative amounts of organotins in samples and alter the true environmental picture. To avoid species redistribution that may occur during derivatization or gas speciation analysis, methods based on direct analysis, such as liquid chromatography have also been developed.

Tin in environmental samples has typically been determined as tin (IV) oxide. Organotin compounds can be completely decomposed by concentrated sulphuric acid to which a small amount of nitric acid is added. At low concentrations tin has largely been determined by spectrophotometric methods using various reagents such as dithiol,[61] haematoxylin,[62] phenylfluorone,[63] pyrocatechol violet[64] and Quercetin.[65] Inorganic tin has also been determined spectrofluorometrically using 3-hydroxyflavone as complexing agent.[66] Most of these reagents, however, lacked in sensitivity, with detection limits in the range 0.02-10 mg L^{-1}, and had poor selectivity for direct analysis. They also required elaborate extraction and separation procedures.

Atomic absorption spectrometry with a variety of flame types has been used for the determination of tin owing to its selectivity but its sensitivity is poor and, therefore, concentration and separation procedures are usually applied before determination. The use of hydride generation followed by flame atomic absorption spectrometry[67,68] or plasma atomic emission spectrometry[69] has lowered the detection limit approx. 1000-fold to the 0.05-25 μg L^{-1} range.

Other non-flame techniques have also been developed for determination of traces of tin, including graphite furnace atomic absorption spectrometry,[70] neutron activation analysis[71] and voltammetry.[72]

All of the available methodologies fall under two basic categories:

1 Indirect methods requiring prior formation of volatile alkyltin derivatives, by either:
   i) in situ derivatization using $NaBH_4$ with on-line cryogenic trapping of derivatized analytes followed by evaporative or gas chromatographic separation and detection;
   ii) prior extraction of ionic organotin compounds by organic solvents, either native or after complexation, followed by derivatization using alkylating reagent, such as Grignard reagents, and subsequent gas-chromatographic separation and detection.
2 Direct methods based on solution specification by liquid chromatography or other such analytical techniques.

1.4.1. Indirect Methods

1.4.1.1. Formation of Volatile Alkyltin Derivatives

The most sensitive analytical methods for speciation of organotin compounds in environmental matrices are based on derivatization of analytes in the sample prior to separation and measurement with a tin-selective detector. Derivatisation to increase the vapour pressure of analytes stems from the need to transform the compounds into volatile forms amenable to separation by evaporative or gas chromatographic methods, to

separate them from the matrix in order to reduce interferences in the sample and to concentrate the analytes in order to improve detection limits. Derivatisation is achieved by two different techniques:

(i) hydride formation and

(ii) alkylation.

1.4.1.1.1. Hydride Formation

The hydride generation technique has been well established as a routine method for speciation of organotin compounds. It is especially useful for determination of organotin compounds which form volatile or low boiling-point hydrides. Tin and organotin compounds react with sodium borohydride ($NaBH_4$) in acidic conditions to yield the corresponding volatile hydrides

$$R_nSn_{aq}^{(4-n)+} \xrightarrow[H^+]{NaBH_4} R_nSnH_{(4-n)} + H_2\uparrow$$

n = 1,2,3

R = organic group

The reaction was originally utilised for generation of trace amounts of stannane (SnH_4) from aqueous solutions of tin and determination by atomic absorption spectrometry.[67] The sample is usually mixed and allowed to react with an acidic solution of $NaBH_4$ in a reaction chamber, then the generated hydrides are scrubbed from solution by an inert gas and trapped cryogenically using liquid nitrogen in a U-trap filled with an appropriate chromatographic packing material. Upon warming they are separated on the basis of their boiling points and/or their chromatographic properties and detected on-line by a tin-selective detector. In general, the reduction is usually performed at a pH that is a few units below the pK_a of the species of interest.

The basic design of a hydride generation system, with subsequent atomic absorption, may be described as four steps. First, the generation of the hydride; second, the collection of the hydride (if necessary); third, transfer of the hydride to the atomiser; and fourth, decomposition of the hydride to the gas-phase metal atoms within the optical axis of the atomic absorption spectrophotometer.

Difficulties arise in the quantitative determination of organotins which form hydrides of higher boiling points such as di- and triphenyltin compounds, because they either remain in the reaction vessel or are caught in the trap, even if the trap is immersed in boiling Water.[73] Generated hydrides including high boiling-point derivatives can also be extracted, off-line, by suitable organic solvents.

A number of tin-selective detectors have been successfully interfaced to the hydride generation system for speciation of organotin compounds. These include the flame photometric detector (FPD),[74] quartz-furnace atomic absorption spectrometry (QFAAS)[73,75,76,77] graphite-furnace atomic absorption spectrometry (GFAAS),[74] atomic fluorescence spectrometry[78] and mass spectrometry,[79] other non-specific detectors such as the flame ionization detector (FID)[80] have also been used. Hydride generation has been utilized for derivatization of organotins from HPLC and GC effluents prior to detection by QFAAS.[81]

Detection limits with flame photometric detection (FPD) for inorganic tin and methyltins were found to be 20 pg and 15 pg respectively. These detection limits were defined as twice the baseline noise. Flame photometric detection is based on the molecular emission by the diatomic species SnH which is formed in the highly reducing hydrogen/air flame. The emission has a bandhead at 609.5 nm. Calibration graphs within the working range 0.15-100 ng L^{-1} were presented. In a comparison with graphite furnace atomic absorption detection the flame photometric detection was found to be most useful for unpolluted samples, because the concentrations of the methyltins are generally at or below the limits of detection. The furnace method provided higher detection limits than the flame

photometric detector, but was useful for highly polluted samples as the linear working range was almost an order of magnitude higher than the flame detector.

Parts per trillion concentrations (ppt, ng L^{-1} or pg ml^{-1}) of tin and methyltin compounds have been determined by emission spectrometry, using a tin selective flame photometric detector after on-line hydride generation with 1% sodium borohydride ($NaBH_4$) at pH 6.5, in a variety of natural samples including rain water, estuarine water, tap water, seawater, human urine, digested chicken shell and sea shell.[82] The hydrides were scrubbed by helium gas and cryogenically trapped, by liquid nitrogen (-196°C), in a glass U-trap packed with 20% w/w OV-3 silicone oil on Chromosorb-W. They were then volatilized by controlled warming and separated on the basis of their boiling points. The volatilized hydrides were swept by the carrier gas into a quartz burner supporting a hydrogen-rich hydrogen-air flame, and detected utilizing the emission band of SnH at 610 nm.

Hodge et al.[73] pioneered the use of Atomic Absorption Spectrometry (AAS) to detect alkyltin compounds but the method was found to lack sensitivity. The method used involved hydride derivatization and separation on a glass wool column, followed by AAS detection. Atomic absorption spectrometry using a hydrogen-air flame supported in a quartz T-tube was used for determination of ng L^{-1} concentrations of, among others, dimethyltin (DMT) and trimethyltin (TMT). Analysis was carried out in seawater, lake water and digested marine algae and sediment. The quartz-tube burner was located axially in the beam path of a tin hollow cathode lamp used for the atomic absorption measurements at a wavelength of 286.3 nm. Organotin compounds with boiling temperatures above 100°C were volatilised by immersing the trap in a hot water bath.

Donard et al.[75] speciated inorganic tin, methyl- and butyltin compounds by volatilisation from water samples by hydride generation, cryogenic trapping, separation by a chromatographic packing material, and detection by atomic absorption spectrophotometry in an electrothermal quartz furnace at 224.61 nm wavelength. They obtained detection limits of 20-50 pg as Sn and linearity of calibration curves up to 30 ng

as Sn. This method avoided the use of preconcentration by extraction and allowed direct determination of the methyl- and butyltin compounds from environmental waters. Randall et al.[76] further developed the chromatographic/AAS technique for aqueous solution by using a column attached directly to the quartz furnace to obtain simultaneous detection limits of 11 pg for mono-, 14 pg for di- and 45 pg for tributyltin with linear calibrations from sub-nanogram to nanogram quantities. The detection of methyltin compounds was also possible, by using a slower rate of heating, but with a slight loss of sensitivity for the butyltin compounds. The technique incorporates the flexibility and selectivity of AAS as the detector with detection limits lower than those reported previously by Donard et al.[75]

Queuvillier et al.[83] studied the leaching of organotin compounds from polyvinyl chloride material using hydride generation/cryogenic trapping/gas chromatographic separation and detection in a quartz cell of an atomic absorption spectrometer. Detection limits measured in tap water were 0.5 ng/l (as Sn) for inorganic tin, 0.6 ng/l (as Sn) for monobutyltin and 0.5 ng/l (as Sn) for dibutyltin. The repeatability of the method was assessed and relative standard deviations were found to range between 6 to 10%.

In the determination of tin species in natural waters Andreae and Byrd[74] described the application of the hydride generation technique coupled with cryogenic trapping and separation. Inorganic tin, methyltin, dimethyltin and trimethyltin, which are present in aqueous solution as cations or hydroxide complexes, are reduced to the corresponding stannanes with sodium tetrahydroborate. The stannanes are stripped from solution by a helium stream and are trapped at liquid nitrogen temperatures on a chromatographic packing. The trap is then warmed and the stannanes are eluted in order of increasing boiling points. Graphite furnace detection gave a detection limit of 50 pg.

D'Ulivo[78] used direct hydride generation combined with non-dispersive atomic fluorescence spectroscopy to determine eleven organotin compounds of the type R_nSnX_{4-n} (n = 1,2,3, R= methyl, ethyl, butyl and phenyl). The method was used to determine total dissolved tin in natural uncontaminated waters, in which only tin and

methyltin species were present. The method could also be used to determine these tin species in contaminated waters if a bromination pretreatment step was used. Inorganic tin was used as the calibration standard.

Woolins and Cullen[80] used hydride generation in the determination of organotin compounds contained in aqueous samples. In the preparation of low-boiling hydrides, such as Me_2SnH_2, Me_3SnH and Et_2SnH_2, the reduction was performed in dibutyl ether and the tin compounds were then distilled off leaving the dibutyl ether behind. Gas chromatography was chosen as the method for separating the many different types of organotin compounds analysed, with flame ionisation detection. It was found that the flame ionisation detector was more sensitive to butyltin hydrides than to phenyl species and most sensitive to biphenyltin hydrides.

Hydride generation has been used in the determination of butyltin compounds in sediment using gas-chromatography atomic absorption spectrometry.[81] The procedure consisted of reacting the butyltin species in the sediment extract with $NaBH_4$ or $NaBEt_4$ to convert the ionic butyltin species into the corresponding butyltin hydride or butylethyltin in a glass reaction vessel. The butyltin hydride or butylethyltins were stripped from solution with a stream of helium and then trapped using liquid nitrogen. The species were then separated chromatographically in order of increasing boiling points by heating the trap to + 200°C, and were subsequently detected using an electrically heated quartz furnace in an atomic absorption spectrometer. Monobutyltin and dibutyltin were determined quantitatively by this hydride generation method.

1.4.1.1.2. Alkylation

Chemical derivatization by alkylation depends on the reaction of organotin compounds with a Grignard reagent (R'MgX; R'= organic group, X= anion), to convert the ionic mono-, di- and tri-organotins, in environmental samples, into their corresponding non-polar tetrasubstituted compounds:

$$R_nSn^{(4-n)+} \xrightarrow[\text{solvent}]{R'MgX} R_nSnR'_{(4-n)}$$

n = 1,2,3

R, R' = organic groups

The usual procedure for trace determination of organotin compounds with alkylation involves five basic steps

i) acidification of samples;
ii) extraction with an organic solvent;
iii) derivatisation;
iv) clean-up and preconcentration;
v) analysis.

Acidification is usually accomplished by either HBr or HCl to transform the organotin species into the respective halides which are suitable for extraction by an organic solvent and/or to dissolve solid phases or particles in order to release the compounds from inclusions in the sample, e.g. from biological tissue and suspended particulates in water or inorganic matter in sediment and sludge. Extraction with an organic solvent is necessary because reaction with the Grignard reagent has to be carried out in aprotic solvents, usually diethyl benzene, toluene, ether or hexane. Protic solvents such as dichloromethane or chloroform can still be used for extraction but should be removed under reduced pressure and replaced prior to the derivatization step as they react with the Grignard reagent. A complexation reagent, such as tropolone or diethyldithiocarbamate, is added to the organic solvent to recover inorganic tin and organotin compounds with

fewer and shorter alkyl chains attached to the tin atom, as they exhibit lower solubilities in organic solvents, like monobutyltins.[19] Methyltins which are highly solvated in aqueous solution are usually extracted after saturating the aqueous samples with sodium chloride to increase the ionic strength and induce "salting out".[84,85] Chau et al.[84] used this "salting out" method to extract butyltins from sewage samples. Tropolone was used as the complexation reagent. Harino et al.[85] used a similar method to extract butyltin and phenyltin compounds from water samples. The mixture was extracted with a tropolone-benzene solution. An increase in the amount of NaCl added to the aqueous layer by over two fold greatly enhanced recoveries.

Derivatisation of organotin compounds to tetrasubstituted species requires care in handling of the Grignard reagents because they are extremely reactive. After alkylation the excess reagent is destroyed by the addition of water or dilute acid solution and the extract dried with a suitable drying agent. Finally, after a clean-up step using alumina, silica gel, or Sep-Pak C_{18} columns, the sample is concentrated by evaporation under a gentle stream of nitrogen and the organotin compounds separated by gas chromatography and detected by a tin selective detector.

A number of Grignard reagents have been used to convert the ionic organotins in environmental samples into volatile tetrasubstituted alkyltin derivatives; they include methyl-,[86,87] ethyl-,[14,84,88,89] propyl-,[85] butyl-,[90] pentyl-,[5,91,92,93] and hexyl-magnesium chlorides/bromides.[94] The choice of alkylating reagent depends on the analytes being sought. Ethylation and pentylation are usually employed as they allow the determination of methyl-, propyl-, butyl- and phenyl-tin species which are of environmental concern. Specific detectors used for identification of alkyltin derivatives after GC separation include mass spectrometry (MS),[86,87,88] flame photometric detection (FPD),[14,82,85,88,89,93] quartz furnace atomic absorption spectrometry (QFAAS)[84,90] and microwave-induced plasma-atomic emission spectrometry (MI-PAES).[92]

Tolosa et al.[86] described an analytical procedure for the determination of tributyltin in seawater, sediments and biota. The tributyltin was first extracted and then derivatised

with the Grignard reagent MeMgCl. The organotin fraction was isolated from the derivatised extract by column chromatography. The final determination was accomplished by on-column capillary gas chromatography coupled to a flame photometric detector and mass spectrometry confirmation. The reproducibility of the method was found to be around 15%.

Stab et al.[87] systematically studied the method used to analyse organotin compounds involving alkylation combined with GC analysis with the aim of improving the reproducibility of the method, and found that the use of ammonium chloride as a quenching agent led to greater recoveries. The use of well chosen internal standards helped to improve the reproducibility of the optimised method.

Methylation has been used for derivatisation of TBT in seawater, sediment and mussel tissue, followed by GC-FPD determination of the hexane extract. Ionic organometallic compounds such as the butyltins and inorganic Sn (IV) species generally have rather high boiling points; a derivatization step is required to lower their boiling points to values appropriate for GC separation. Ethylation with ethylmagnesium bromide has been found to give derivatives with appropriate boiling points for GC operation. This derivatization does not change the identity of the original alkyl group, thus making it possible to identify the methyl- and butyltin species.

Ethylation, using ethylmagnesium bromide, was used by Fent to speciate between butyltins (MBT, DBT, TBT), phenyltins (mono-, di-, triphenyltin), dioctyltin and tricyclohexyltin, in environmental water and sludge samples.[14] Prior to derivatisation the samples underwent acid digestion and a detailed extraction procedure. Small portions of the Grignard reagent were then added to the extracts and allowed to react for 10 minutes. The resultant mixture was dried and reduced in volume to prepare for GC-FPD analysis.

Chau et al.[84] also used ethylation in the determination of butyltin species in sewage and sludge. The butyltin species were first extracted into tropolone, followed by ethyl-derivatisation to the tetraalkyl-substituted forms to be determined by gas

chromatographic atomic absorption spectrometry (GC-AAS). This system makes use of the separation power of the gas chromatograph and the element-specific atomic spectrometer as a detector. The GC-AAS system is element- and species-specific, and is highly sensitive.

Alkylation is often preferred over hydride formation as the resulting tetrasubstituted organotin compounds can easily be purified and concentrated, which is necessary for low-level samples and complex matrices such as animal tissue or sewage sludge. Muller[88] used ethylmagnesium bromide in a similar method to Fent[14] described above to ethylate surface water, sewage, sludge and sediment extracts in the determination of organotins. Analysis was performed by both GC-FPD and GC-MS. The photometric detection mechanism was attributed to light emission of the excited organotin species in the detector flame. Two different wavelengths were related to excited Sn-O and Sn-H bonds at 485 and 611 nm, respectively. The response was strongly dependent on the flame conditions, and the hydrogen rich flame was found to enhance selectivity and sensitivity.

Dachs et al.[89] also used ethylation, as the derivatisation step, in the determination of organotin compounds in sediments. Analytical determination was performed using GC-FPD. It was found, however, that most of the variability in the proposed method could be associated with the derivatisation step

Propylation has also been used as a method to transform organotins into a form suitable for GC analysis. Harino et al.[85] proposed a method for the simultaneous determination of butyl- and phenyltin compounds in the aquatic environment. The analytes in water, sediment and biological samples were extracted into 0.1% tropolone-benzene solution after adding hydrochloric acid diluted with tetrahydrofuran. With sediment samples, inorganic sulphur-containing species co-extracted with the analytes were removed with tetrabutylammonium hydrogensulphate-sodium sulphide, which brought about a decrease in many of the acompanying peaks. After propylation with *n*-propylmagnesium bromide, the analytes were determined by gas chromatography with flame photometric detection.

The recoveries were in the range 70-100% and the detection limits for a signal-to-noise ratio of 10 were 3 ng L^{-1} in water samples and 0.5 μg kg^{-1} in sediment and biological samples. It was found that the organotin compounds immediately reacted with the *n*-propylmagnesium bromide after its addition.

Butylation was used for derivatisation of methyltin compounds and inorganic tin in water from lakes, rivers, marinas and harbours. Experiments were carried out on 100 ml water samples saturated with sodium chloride to improve the recovery of the highly polar and volatile methyltins and acidified with 10 ml of HBr to prevent hydrolysis and adsorption of Sn (IV) species on the container walls. Extraction was performed with 5 ml of 0.1% tropolone-benzene and butylation was carried out with BuMgCl. The butylated methyltin species were analysed by GC-QFAAS.[90]

Butyltin species were determined in water samples from lakes, rivers and harbours after reaction of the tropolone-benzene extract with pentylmagnesium bromide and analysis by GC-FPD, with a detection limit of 0.02 μg L^{-1} for an eight litre extracted sample.[5]

Lake sediments were analysed for butyltin compounds after heating to reflux temperature samples with a benzene-tropolone solution followed by derivatisation of the extract with pentylmagnesium bromide.[91] The excess reagent was destroyed with sulphuric acid and the organic layer separated, concentrated by drying and cleaned-up on activated florisil with hexane as the eluent. The final solution was concentrated and analysed by GC-FPD. Results showed highest concentrations of butyltin species in areas with extensive boating and shipping activities.

Pentylation was also used for alkylation of butyltin compounds from water and sediment extracts followed by determination using gas chromatography coupled to a microwave-induced plasma atomic emission spectrometer (GC-MIPAES). The method gave an absolute detection limit of 0.05 pg (as Sn), and the accuracy of the method was confirmed by independent analysis by GC-AAS.[92]

Chiavarini et al.[93] used spiked sea samples to analyse for butyltins by an analytical procedure consisting of a liquid-liquid extraction followed by a derivatization step with pentylmagnesium bromide, a clean-up step on silica gel and a GC-FPD determination, resulting in a butyltin concentration of below 2 ng L^{-1}.

Hexylmagnesium bromide has been utilised for alkylation/determination of butyltin compounds in oyster and mussel tissues by GC-FPD.[94] Extensive sampling and extraction procedures were used and the extracts were then treated with hexylmagnesium bromide. The hexylation reaction was carried out either overnight or for 2 hrs at 70°C.

In conclusion, it is apparent that although gas chromatography (GC) is an extremely common analytical technique for volatile analytes, its use for speciation of inorganic analytes has been relatively limited. This possibly reflects the fact that most GC detectors are not element-specific, and that there may be problems associated with transferring the analyte from the end of the GC column to the atom/ion source when atomic spectroscopy is used for detection. The GC eluent is obviously in the gas phase and is usually at an elevated temperature. This temperature must be maintained all the way along the transfer line to the detector and failure to do this leads to cool spots and condensation of the analyte. A heated transfer line is therefore obligatory. The majority of work performed coupling GC with atomic spectrometry has so far been achieved using flame spectrometry as a detector. This approach has been used most commonly for analytes that are present in relatively high concentration. A potential limitation of using GC is that often the sample must be derivatized to make it volatile enough for analysis. this can greatly increase sample preparation time, may cause loss of analyte and uncertainty about the identity of the original species in the sample.

1.4.2. Direct Methods

The most sensitive methods for the speciation of organotins, as mentioned earlier, rely upon digestion or extraction combined with various chemical means for derivatisation to form hydrophobic organotin analytes representative of the original tin-containing species, and of sufficient volatility for speciation by gas chromatography coupled to a sensitive tin-specific detector. However, any chemical manipulation can alter the relative amounts of tin and organotin compounds present in the sample, hence altering their true environmental impact.

Use of GC to separate tin species necessitates the generation of hydrides or the conversion of the relatively involatile organotin species to their corresponding methyl, ethyl or pentyl derivatives. Both of these derivatization steps are subject to interference, and involve considerable sample manipulation that can lead to analyte losses. The need to minimise the risk of decomposition at high temperatures associated with GC analysis, and to avoid derivatisation, which introduces additional handling steps that augment experimental error, has encouraged many investigators to seek alternative methods based on direct solution speciation of organotin compounds. High performance liquid chromatography is an appropriate tool, provided a sensitive and selective detector is used. The popularity of LC in the speciation of organometallic compounds is well recognised. It offers the possibility of separating ionic, polar and non-polar compounds by a variety of separation techniques, including normal and reverse-phase liquid chromatography, reverse-phase ion-pair chromatography (IPLC), size exclusion chromatography (SEC) and ion-exchange chromatography (IEC). The compatibility of liquid flow rates from LC columns with traditional sample introduction devices makes it easy to interface LC with a variety of sensitive and specific liquid detection systems for direct excitation of the HPLC effluent stream.

For high performance liquid chromatography (HPLC), detection by flame[95] or graphite furnace[96] atomic absorption spectroscopy (AAS), inductively coupled plasma mass

spectrometry (ICP-MS)[97] or spectroflurometry[98] have been reported. Articles describing the use of supercritical fluid chromatography have also been published.[99]

HPLC has three main advantages

i) derivatisation of the organotin compounds is not necessary
ii) minimum sample handling is required, and
iii) stationary and mobile phases can be varied to obtain the best separation.

However, interfacing to the detection system may be problematic.

1.4.2.1. Ion-exchange Chromatography

Ion-exchange chromatography is carried out on ionisable analytes, by using column packing materials that possess charge bearing functional groups. In the case of organotin compounds the sample cations $[R_nSn]^{(4-n)+}$ compete with mobile phase counter ions Y^+ for the ionic sites X^- of the cation exchanger. The majority of organotin speciation studies employing ion-exchange chromatography are carried out with silica based columns.

Jewett and Brinckman[96] were the first to develop the separation of organotin compounds on silica-bonded ion-exchange columns and most of the subsequent work using these columns has been based on their findings. A broad range of organotins were speciated in trace quantities by a combination of an element specific graphite furnace atomic absorption (GFAA) detector coupled with high performance liquid chromatography (HPLC) employing commercial bonded phase Strong Cation Exchange (SCX) columns. Optimisation of SCX column parameters was characterized in terms of efficiency and resolution to provide examples for the separation of organotins, $[R_nSn]^{(4-n)+}$, by class (n=2,3) and functionality (R= aryl, alkyl, etc.). SCX column performance was found to

vary for individual organotin analytes as did HPLC-GFAA system detection limits in the range 5-30 ng (as Sn). This work also instigated the development of element specific detection, to compensate for the absence of chromophores in most organotin compounds and the low detection limits associated with conventional HPLC detectors. The separation mechanism they suggested for the organotin species was based on the three main characteristics of the column, namely, cation exchange due to the sulphonate groups, reversed phase due to the bonded phase and adsorption arising from exposed silanol sites.

McLaren et al.[97] analyzed an extract of a sediment standard reference material by using a silica based cation-exchange column coupled to ICP-MS. The extraction procedure used was not effective for MBT so analysis was limited to DBT and TBT.

A considerable amount of research into the separation and determination of organotin compounds has been carried out by Ebdon and co-workers. Most of their work in this area involves the use of silica based cation-exchange columns and an eluent consisting of methanol and ammonium acetate. By using this system, coupled to a variety of detectors they were able to determine Sn(II) and TBT in harbour water[95] and TBT in estuarine water[98] and inorganic tin and different butyltin and phenyltin species in aqueous solution.[100]

There has only been one report of the use of resin based ion exchange columns for organotin speciation.[101] The work utilized a sulphonated poly(styrene-divinylbenzene) column, an eluent composed of 50 mM citric acid, 50 mM lithium hydroxide and 4 mM oxalic acid in 100% (v/v) methanol and detection via post-column hydride generation followed by electrically heated QF-AAS. With these conditions all three butyltin compounds and Sn(IV) were resolved in less than 10 min using isocratic elution.

1.4.2.2. Reversed-phase Chromatography

The reversed-phase mode involves the use of a polar eluent with a non-polar stationary phase and is particularly useful for the chromatography of polar molecules. The bonded stationary phase usually consists of an alkyl moiety, which is chemically bound to a silica support material. The eluent is usually water, containing a proportion of organic modifier such as methanol. The eluting power of the mobile phase dramatically increases with the proportion of organic solvent present. Separation of different organotin compounds has been achieved by a number of workers using this approach.

The earliest reported separation of organotin compounds by HPLC was in 1977 by Brinckman et al.[102] who analyzed mixtures of triphenyltin, tributyltin and tripropyltin. These workers employed C_2 and C_{18} bonded phase columns, a mobile phase containing 100% (v/v) methanol and GF-AAS detection. They proposed a retention mechanism based on a number of competing equilibria, involving different mobile and stationary phase ligands.

The first successful use of the reversed-phase mode for the speciation of a large number of organotin compounds differing in both the type (e.g. methyl, ethyl, butyl, etc.) and the number (e.g. mono-, di-, tri-) of substituents was the work of Kadokami et al.[103] They established that aqueous, methanol or tetrahydrofuran eluents were unsuitable because the peak shapes of the di- and tri-substituted compounds were not symmetrical, and that the mono-substituted compounds could not be eluted from the column. In an effort to overcome these problems they added tropolone or oxine (8-hydroxyquinoline) to the mobile phase. The idea for this came from the use of these reagents as complexing ligands in liquid/liquid extractions and also because oxine had been previously used to overcome adsorption interactions in the reversed phase separation of other organotin compounds and the technique was used for the analysis of TBT in seawater.

1.4.2.3. Normal-phase Chromatography

This approach involves the use of stationary phases that have a higher polarity than that of the eluent. The bonded phase columns used are made by covalent attachment of a polar organic moiety to the surface of the microparticulate silica-gel support. Non-polar organic solvents are usually employed as the eluent, although chloroform, ethanol or aqueous acetonitrile have also been used. For organotin speciation the majority of the columns employed cyanopropyl bonded phases, which are considered to be of medium polarity.

The use of cyanopropyl bonded stationary phase to separate diphenyltin and dialkyltin compounds was reported by Langseth.[104] The mobile phase used consisted of toluene, acetic acid, methanol or ethanol and morin (2',3,4',5,7-pentahydroxyflavone). The organotin compounds were thought to form stable complexes with the morin, and this helped to reduce tailing due to adsorption by residual silanol groups present on the column. Most studies employing reversed- or normal-phase separation modes encounter problems associated with adsorption of the organotin compounds onto unreacted silanol groups. A number of methods are available to overcome this unwanted interaction. One such method is the use of chelating agents such as morin or tropolone to block interactions with the silanol groups. The high fluorescence intensity of the morin complexes gave good selectivity, sensitivity and detection limits of 1.0 pg for diphenyltin and 4.0 pg for dimethyltin. Further work[105] led to the simultaneous determination of the butyltin, ethyltin and methyltin trichlorides as well as dibutyltin, monobutyltin and monomethyltin chlorides.

Astruc et al.[106] used 0.005% (m/v) tropolone in toluene with a 0-5% (v/v) gradient of methanol to separate monobutyltin, dibutyltin, tributyltin and tetrabutyltin. This method could not be used routinely because it slowly degraded the column. With isocratic conditions using tropolone in toluene as eluent, tributyltin and tetrabutyltin co-eluted, whereas dibutyltin was resolved but monobutyltin was strongly adsorbed onto the

column. The HPLC system was interfaced to a GFAAS system and the method used to determine the dibutyltin and tributyltin concentrations in river water and sediment.
It is well known that organotin compounds in environmental media are not sufficiently volatile or inert for successful direct GC separation. In fact, most organotins are either strongly complexed by natural ligands present in environmental media, such as saline fluids, or behave as classical solvated metal ions.[107]

1.4.2.4. Detectors for HPLC Speciation

Sensitive and element-specific detectors are required for organotin speciation. The conventional detectors used for HPLC analysis include fluorescence,[95,100,104,105] ultraviolet, [102,108] electrochemical[109] and refractive index.[104] The majority of alkyltin compounds contain no ultraviolet or fluorescence chromophore (the exception being phenyl-substituted compounds); consequently the commonly used photometric detection methods have to be modified to study these compounds.

In the case of fluorescence detection this is facilitated by reaction of the organotin compounds with a suitable reagent such as morin, either pre-column,[104,105] or post-column.[95,100] Direct UV detection at 254 nm has been used to determine triphenyltin.[102] The lack of a suitable UV chromophore on the alkyl-substituted organotin compounds has been overcome in a number of ways, including the use of a photometrically active counter-ion (benzyltrimethylammonium cation) in the mobile phase with indirect photometric detection of the organotins.[108] Electrochemical detection methods[109] have not been widely reported for organotin speciation work, probably because of their lack of sensitivity. The use of refractive index detection has been reported,[104] but for the methyl-ethyl- and butyl-substituted organotin compounds it showed a marked lack of sensitivity.

The most commonly used detection systems for metal speciation work are atomic spectrometric methods, such as atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES). Both of these techniques are metal-specific. In using AAS or AES the method of atomisation (flame, furnace or plasma) must be able to handle large volumes of mobile phase, solvent flow rates in the range 0.1-2.0 ml min^{-1} and eluents that may be non-aqueous in nature.

Flame atomic absorption spectroscopy readily accepts liquid samples, but does not provide low enough detection limits to determine organotin levels in environmental samples. A number of approaches have been used to overcome this problem, including the use of quartz furnace[110] or the generation of hydrides.[101,110,111] The use of a quartz furnace effectively increases the residence time of the analyte in the flame, whereas the hydride generation method overcomes the low nebulisation efficiency encountered with aspiration of liquid samples. For some species the limit of detection can be lowered by a factor of 1000 by using hydride generation and this approach also eliminates interferences from elements that do not readily form hydrides.[110]

Electrothermal atomisation offers higher sensitivity than the use of flames, but because of the temperature cycle involving drying, ashing and atomisation steps, analysis is usually off-line and discontinuous in nature.

Atomic emission spectroscopy has the advantage of long linear calibration ranges and simultaneous on-line determination of a number of elements. For these reasons it comes closest to meeting the requirements necessary for a universal HPLC detector for the determination of the organometallic species. However, the low temperature of flame atomic emission spectroscopy does not offer detection limits low enough for environmental work and therefore has not been used for organotin speciation. The use of a plasma as the method of atomisation for AES has been found to be more sensitive than the various flames used, because of the greater atomisation efficiency.

The three principal plasma sources that have been used in analytical studies include inductively coupled argon plasma (ICP), a direct current argon plasma jet (DCP) and microwave induced helium plasma (MIP). Only the first two have been coupled to HPLC for organotin speciation. Hahn et al.[69] used a hydride generation/condensation system with an inductively coupled argon plasma polychromator for the determination of tin in foods. A detection limit of 0.8 ng/ml was found. Both ICP and DCP are able to accommodate large aqueous or organic flows, whereas MIP is unable to tolerate aerosol introduction without destabilisation or extinction of the plasma. For this reason MIP has been more widely used as a GC detector.

Ebdon and Alonso[98] determined tributyltin in estuarine waters by high performance liquid chromatography with fluorimetric detection using morin in a micellar solution. A highly efficient on-column pre-concentration method for TBT was described. After the pre-concentration step conventional HPLC ion-exchange was used to separate the different organotin compounds retained on the pre-concentration column. These analytes were then detected by use of a fluorimetric procedure based on micellar systems.

1.4.2.5. Other Methods

1.4.2.5.1. Cathodic Stripping Voltammetry

Total dissolved tin in estuarine waters was determined by cathodic stripping voltammetry with adsorptive collection of complexes of tin(II) with tropolone[72]. The optimized conditions included a deposition potential at –0.8 V and re-oxidation potential of –0.4 V from which the potential change which was initiated in a negative direction. A complicated preconcentration step, involving adsorption of the complex, amalgamation, re-oxidation, re-adsorption and measurement of the reduction current of the adsorbed complexes, was required to eliminate interference from molybdenum which is also

present in sea-water. Samples were UV-photolysed prior to analysis in order to remove interfering surface-active organic compounds, simultaneously converting organotin compounds to inorganic tin. The limit of detection was 5 pM tin using a 10 min. stirred deposition prior to the scan.

1.4.2.5.2. Neutron-activation analysis

Bowen[71] used neutron activation analysis and radiochemical separation of tin-121 to determine tin in biological material. A precision of ± 10% was found with a sensitivity of 10-20 ng. In the determination of radioactivity the tin(II) sulphide samples were counted by using a scintillation counter with a thin crystal of anthracene as detector.

1.4.2.5.3. Supercritical fluid chromatography

Capillary supercritical fluid chromatography with inductively coupled plasma mass spectroscopic detection has been developed for the analysis of organometallic compounds and has been applied by Blake et al.[99] to the determination of organotin compounds.

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2. Analysis of Methyltin Chlorides by Capillary Zone Electrophoresis

2.1. Introduction

Capillary electrophoresis (CE) is a technique used in the separation of charged molecules inside a narrow tube under the influence of an electric field. Separation in CE is based on differences in solute mobilities when a strong electric field is applied across a separation buffer solution (also known as the run buffer). CE offers advantages over traditional chromatographic techniques, such as high efficiency, short analysis time, small sample and separation buffer volumes, and ease in changing the separation buffer. Additionally, selectivity in CE can easily be altered by the addition of different buffer additives.

2.1.1. Historical Perspective

Capillary electrophoresis has evolved from what now seem to be rudimentary column electrophoresis techniques that were introduced three decades ago. Early reports by Hjerten[1] employed columns having inner diameters (ID) on the millimetre scale. However, advantages of smaller diameter columns were soon discovered by Virtanen and by Mikkers et al. Virtanen[2] used Pyrex tubing with I.D.'s ranging from 200-500 μm to quantitatively analyze Li^+, Na^+ and K^+ using potentiometric detection. His report is noteworthy for its recognition of the important influence of electroosmotic flow on a solutes behaviour. Mikkers et al.[3] separated a number of inorganic and organic anions using 200 mm i.d. Teflon tubing and UV and conductometric detection schemes. The most widely accepted initial demonstration of the powers of capillary electrophoresis was that by Jorgenson and Lukacs.[4,5,6] Their pioneering work provided the first demonstration of high separation efficiency with high field strength in narrow capillaries. They solved the previously encountered injection problems using 75 μm I.D. capillaries. They separated 5-diethylamino-1-naphthylalene sulfonyl and flourescamine derivatives of several amino acids and used fluorescence as the detection method. They were able to apply large voltages across the column to achieve very high efficiencies. Most importantly they described some of the main concepts of CE,

including the conditions necessary for high efficiency and the importance of electroosmosis.

Reviews have been published by both Vesterberg[7] and Compton and Brownlee[8] who traced the history of development of capillary electrophoresis back to more than a century ago with the formulation of Kohlrausch's theories on the behaviour of ions in a solution under the influence of an electric field.[9] Kohlrausch initially described how the passage of an electric current through a solution was caused by the independent migration of ions towards the respective electrodes. The conductivity of the solution therefore was a function of the concentration of the ions and the velocity of their migration. Kohlrausch also proposed theories on the behaviour of a boundary between two salt solutions with a common counter ion in the presence of an electric field. He suggested that under the influence of an electric field the concentration ratio of two ions A and B at the boundary reached a steady state which was related to the mobilities and charges of the ions by the function

$$\frac{C_A}{C_B} = \frac{\mu_A}{\mu_A + \mu_X} \times \frac{\mu_B + \mu_X}{\mu_B} \times \frac{Z_A}{Z_B} \qquad \textbf{[Eq. 2.1]}$$

Where μ_A, μ_B and μ_x denote the mobilities of the two ions and the common counter ion X, Z is the charge on each ion and C refers to the concentration of the ions. If the mobility of A exceeds that of B and the initial concentrations are equal, the shape of the zone occupied by B ions will adjust accordingly forming a concentration gradient within the zones until the equilibrium is reached. This is when the concentration of each ion at the boundary obeys the Kohlrausch equation.

This theory prompted a series of investigations into the separation of ionised species by exploiting the differences in their electrophoretic mobilities. In the 1930's Tiselius[10] succeeded in separating proteins into specific boundaries by performing "moving boundary" electrophoresis in a quartz tube. These protein boundaries were detected by photography using UV light. A particular problem with the method was that a substantial amount of heat was generated in the quartz

tube during the separation. Without effective heat dissipation, a radial temperature gradient was established within the tube which caused inhomogenities in the viscosity of the support electrolyte. The resultant parabolic flow caused considerable blurring of the boundaries. In a later publication Tiselius[11] modified the experiment by introducing a rectangular electrophoresis cell with cooling at 4°C. With this apparatus four serum proteins were successfully separated into definite zones.

The problem of removing the excess heat generated during electrophoretic separations to improve resolution continued to be a dominant theme in the early development of capillary electrophoresis. These earlier studies were also unable to demonstrate the high separation efficiencies achievable because of sample overloading, a condition induced by poor detector sensitivity and large injection volumes.

In the 1980's many developments were made in capillary electrophoresis. In 1984 Terabe et al.[12] introduced micellar electrokinetic capillary chromatography (MECC). Major advances in detection also occurred in the 1980's, with the introduction of such techniques as fluorescence (1981), ultraviolet (1984), laser fluorescence (1985), mass spectrometry (1987), electrochemistry (1987) and indirect fluorescence (1988).[13,14,15,16,17] The first commercial instrument was introduced in 1988 and in the following year many more instruments became available from various suppliers. From this stage on progress in the area of capillary electrophoresis has been mainly in the area of method development.

2.1.2. Electrophoresis

Electrophoresis is a process for separating charged molecules based on their movement through a fluid under the influence of an electric field. The separation was traditionally performed in a medium such as a semisolid slab gel or in non-gel media such as cellulose or acetate. These media are less inert than gels since they contain charged surface groups that may interact with the sample or the run buffer. A carrier electrolyte is also required for electrophoresis. Otherwise known as the background electrolyte, or simply the run buffer, this solution maintains the requisite pH and provides sufficient conductivity to allow the passage of current necessary for the separation. Frequently, additional materials are added to the buffer to adjust the selectivity of the separation. These reagents, known as additives, can interact with a solute and modify its rate of electrophoretic migration.

Capillary electrophoresis can be considered as the electrophoretic separation of a number of substances inside a narrow tube. In capillary electrophoresis a mixture of different substances in solution is introduced, usually as a relatively narrow zone, into the separating system and induced to move under the influence of an applied potential. Due to differences in the effective mobilities (and hence migration velocities) of different substances under the electric field, the mixture then separates into spatially discrete zones of individual substances after a certain time.

The CZE system consists of a buffer-filled capillary placed between two buffer reservoirs, and a potential field which is applied across the capillary. In general, the flow of electroosmosis is towards the cathode, and hence a detector is placed at this end. Injection of solutes is performed at the anodic end.

2.1.3. Main Theoretical Concepts

The theoretical aspects of CE have been studied extensively since Jorgenson and Lukacs initial paper[4]. Here only the main theoretical concepts such as electroosmotic flow, electrophoretic migration, resolution and efficiency will be discussed.

Capillary electrophoresis comprises a family of related techniques with differing mechanisms of separation, one of which is capillary zone electrophoresis (CZE). Separations by CZE are performed in a homogeneous carrier electrolyte solution and is distinguished from other forms of capillary electrophoresis by the absence of a gel or a pseudophase, instead, the capillary is usually made of fused silica. CZE is the most commonly used technique in CE. Many compounds can be separated rapidly and easily.

2.1.3.1. Electroosmotic Flow (EOF)

Separations in CZE are based on the differences in the electrophoretic mobilities resulting in different velocities of migration of ionic species in the electrophoretic buffer contained in the capillary. The separation mechanism is mainly based on differences in solute size and charge at a given pH. Most capillaries are made of fused silica, which contains surface silanol groups. These silanol groups may become ionized in the presence of the electrophoretic medium. The interface between the fused silica tube wall and the electrophoretic buffer consists of three layers, as shown in Fig. [2.1]: the negatively charged silica surface (at pH > 2), the immobile layer (the stern layer), and the diffuse layer of cations (and their sphere of hydration) adjacent to the surface of the silica tend to migrate towards the cathode. This migration of cations is called electroosmotic (or electroendoosmotic) flow (EOF). Upon application of an electric field, cations in its outer portion flow toward the cathode and, since they are more solvated, transport bulk liquid in the same direction.

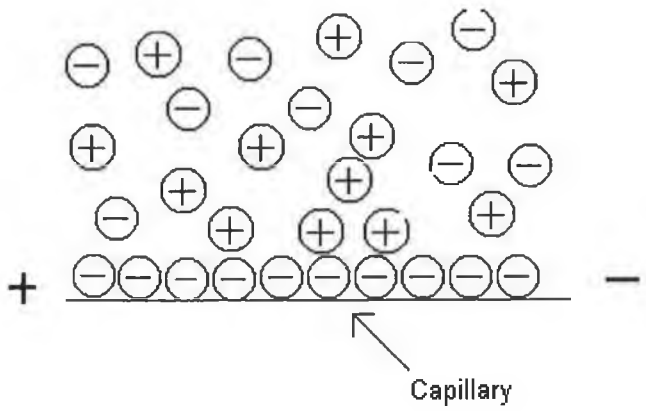


Fig. [2.1] Schematic representation of ions at a silica-solution interface in a fused silica capillary

The velocity of electroosmotic flow, v_{eo}, can be calculated using the equation:

$$v_{eo} = \frac{\varepsilon}{4\pi\eta} \times E\zeta \qquad \textbf{[Eq 2.2]}$$

where ε is the dielectric constant of the buffer, η is the viscosity of the buffer, E is the applied electric field, and ζ is the zeta potential. The electroosmotic velocity in uncoated fused silica capillaries is usually significant with most commonly used buffers. It is also significantly greater than the electrophoretic mobility of the individual ions in the injected sample. Consequently, both anions and cations can be separated in the same run. Cations are attracted towards the cathode and their speed is augmented by the electroosmotic flow. Anions, although electrophoretically attracted toward the anode, are swept towards the cathode with the bulk flow of the electrophoretic medium. Under these conditions, cations with the highest charge to mass ratio migrate first followed by cations with reduced ratios. All the unresolved neutral components are then migrated as their charge to mass ratio is zero. Finally, the anions migrate. Anions with lower charge to mass ratio migrate earlier than those with greater charge to mass ratio. The anions with the greatest electrophoretic mobility migrate last. The order of migration is depicted in Fig. [2.2].

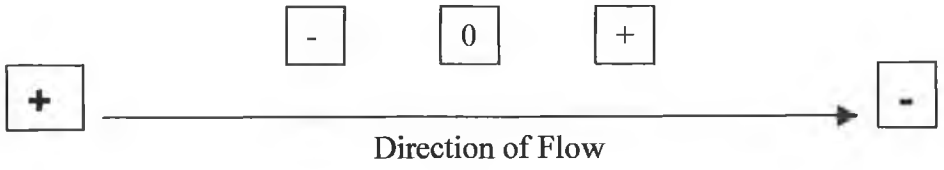


Fig. [2.2]. Order of Migration of Ionic Species in CZE.

In CE separations performed in fused silica capillaries, the electroosmotic flow is towards the negative terminal when a positive potential is applied at the injection end across the fused silica capillary to the detection end. Consequently, cations move towards the negative terminal with the apparent velocity, ν_{app}:

$$\nu_{app} = \nu_{eo} + \nu_{ep} \qquad \textbf{[Eq 2.3]}$$

where ν_{eo} and ν_{ep} are the electroosmotic flow velocity and the electrophoretic velocity, respectively. In the case of anions, due to the strong attraction by the positive electrode, they flow towards the positive terminal against the electroosmotic flow. The apparent velocity then becomes:

$$\nu_{app} = \nu_{eo} - \nu_{ep} \qquad \textbf{[Eq 2.4]}$$

Under these conditions, the separation of anions usually requires longer times compared to the separation of cations due to the smaller apparent velocity. One important point to note is that it is possible to change the charge to mass ratio of many ions by adjusting the pH of the buffer medium to affect their ionization and hence electrophoretic mobility. The electroosmotic velocity, ν_{eo}, can also be adjusted by controlling:

- The pH (since more silanol groups are ionised, both the zeta potential and the flow increase)
- The viscosity (as viscosity increases the velocity decreases)
- The ionic strength (because of its effect on the zeta potential)
- The voltage (flow increases proportionally to voltage)
- The dielectric constant of the buffer.

Rinsing the capillary can alter the ionisable silanol groups on the silica surface and hence the electroosmotic flow. The electroosmotic flow could also be reversed through the addition of a cationic surfactant, or eliminated by coating the capillary walls with reagents that result in a neutral wall surface. These will be discussed in Section [2.1.4.1]. Since electroosmotic flow is driven by the zeta potential at the capillary walls, there is a flat velocity distribution across the diameter of the capillary. This plug-like profile distinguishes CE from other separation methods in which the profile is parabolic in nature. The plug-like profile of CE is advantageous because it greatly reduces the velocity gradient across the column, decreasing band broadening due to slow mass transfer across such a gradient.[18] Consequently the only factor contributing to bandbroadening is longitudinal diffusion.

2.1.3.2. Principles of Separation in Capillary Zone Electrophoresis

The separation mechanism is illustrated in Fig. [2.3].

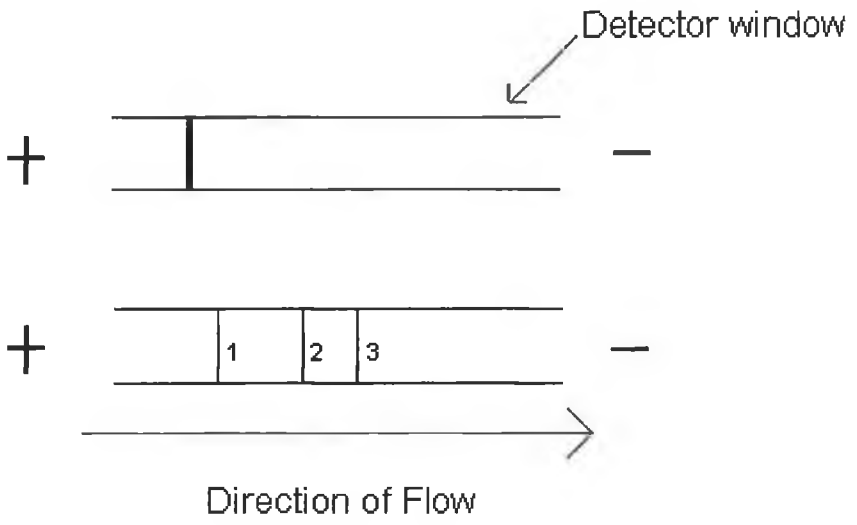


Fig. [2.3]. Schematic Representation of a three Component Separation by CZE at the moment of injection (top) and after separation (bottom).

Ionic components are separated into discrete bands when each solutes individual mobility is sufficiently different from all others. Four fundamental features are required for good separations in CZE

1. The individual mobilities of each solute in the sample differ from one another.
2. The background electrolyte is homogeneous and the field strength distribution is uniform throughout the length of the capillary.
3. Neither solutes nor sample matrix elements interact with or bind to the capillary wall and
4. The conductivity of the buffer substantially exceeds the total conductivity of the sample components.

From the earliest descriptions of capillary electrophoresis, the electroosmotic flow has been recognised as an important parameter for the optimisation of separations. Towns and Regnier[19] quantitatively studied the effect of cationic polymer analyte

adsorption to the inner wall of fused silica capillaries on electroosmotic flow and CE separation efficiency. It was found that polycationic species were more strongly adsorbed than polymers with a small number of positive charges. This adsorption of polycations caused a non-uniform distribution of the zeta potential which created complex liquid flow profiles and caused the efficiency of a separation to be reduced by 50% when 2% of the length of the capillary was fouled.

Several methods have been described to monitor EOF in a capillary. A direct image of the liquid core of the capillary was used by Taylor and Yeung[20] to study the influence of electroosmotic and hydrodynamic flow on band broadening. They carried out two types of experiments. One was to monitor the front of a fluorescence labelled solvent as it passed through the capillary and the other was an examination of the local velocities using submicron sized particles as probes.

Since the discovery of the importance of the electroosmotic flow on a separation in CE, much work has been done in the area of controlling the electroosmotic flow. Many different approaches for controlling the electroosmotic flow have been suggested, including the addition of surfactants, the use of organic solvents and coating the capillary walls. Chang and Yeung[21] implemented a self-regulating mode to dynamically control the electroosmotic flow rate. A constant electroosmotic flow was demonstrated for sample ions in CE, which was independent of a changing sample matrix composition.

Instrumental control of the electroosmotic flow has also been investigated. Wu et al.[22] studied the electrokinetic phenomena at the silica-solution interface under the influence of an applied radial electric potential gradient. The separations of peptide and protein mixtures in CZE with direct control of electroosmosis were described. The effect of such control on the separation efficiency and resolution of peptides and proteins was also discussed.

2.1.3.3. Electrophoretic Migration

The mobility, μ of an ionic solute in CE is directly proportional to its charge, q, and inversely proportional to its ionic radius, r, and the viscosity, η, of the separation buffer as given by

$$\mu = \frac{q}{6\pi\eta r} \qquad \textbf{[Eq 2.5]}$$

A comparison of the mobilities of anions, cations, and neutral solutes is shown in Fig. [2.4].

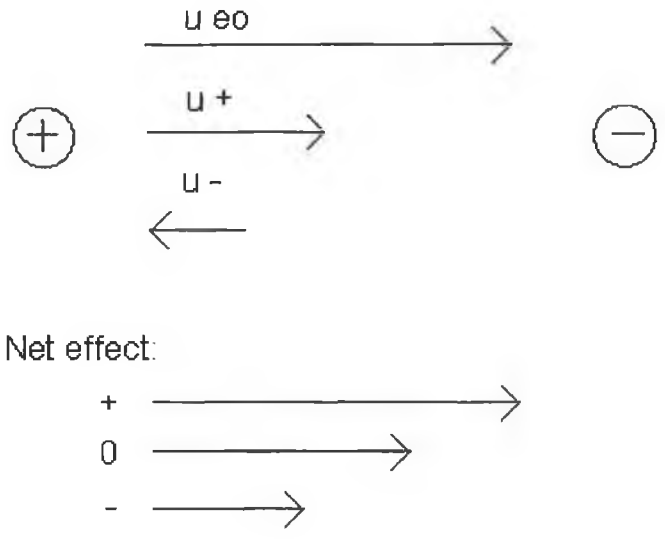


Fig. [2.4] Comparison of the mobilities of anions (-), cations (+) and neutrals (0) in a capillary electrophoresis system based on the cumulative effects of electrophoresis and strong electroosmotic flow towards the cathode.

Experimentally, an anionic solute's mobility is calculated from

$$\mu_{ep} = \left(\frac{lL}{V}\right) \times \left(\frac{1}{t_o} - \frac{1}{t_t}\right) \qquad \textbf{[Eq 2.6]}$$

where l is the distance from the point of injection to the detector, L is the total length of the capillary, V is the applied voltage, t_o is the retention time of an unretained solute (thus a measure of electroosmotic flow), and t_t is the retention time of the solute.

Under conditions in which electroosmosis does not occur, the migration velocity (v) in electrophoresis is given by

$$v = \mu_{ep}E = \mu_{ep}\frac{V}{L} \qquad \textbf{[Eq 2.7]}$$

where μ_{ep} is the electrophoretic mobility, E is the field strength (V/L), V is the voltage applied across the capillary and L is the length of the capillary. The time taken for one solute to migrate from one end of the capillary to the other is the migration time (t) and is given by

$$t = \frac{L}{v} = \frac{L2}{\mu epV} \qquad \textbf{[Eq 2.8]}$$

2.1.3.4. Coated Columns

Chemical derivatization of the capillary wall is a widely used technique for changing the properties of the silica surface in the preparation of coated columns. In their early attempts to reduce electroosmotic flow, Jorgenson and Lukacs[23] used trimethylchlorosilane (TCMS) to silylate the silica surface. Subsequently, many approaches have been adopted to improve the effectiveness and stability of the coatings for CE capillaries. Polyacryamide[24,25] and polyethylene glycol[26] coatings are two of the most commonly employed types of coatings. A charge reversal surface modification technique has also been developed.[27]

2.1.3.5. Charge reversal coating

A charge reversal surface modification technique has been used to modify the capillary surface in order to improve the separation of proteins. In this method, a positively charged polymeric coating agent is ionically adsorbed to the negatively charged silica surface, forming a neutral surface initially. Subsequently, hydrophobic interactions between surface bound polymers and free polymers produces a positively charged second polymer layer. Upon reversal of the surface

charge, the electroosmotic flow is reversed. Hence, the polarity of the system must be reversed in order to ensure that all the analytes travel past the detector. The procedure required to form the coating involved rinsing the additive, which is usually a cationic surfactant, through the capillary prior to analysis. Separation of several basic proteins has been demonstrated.[27]

The mechanism of charge reversal is illustrated in Fig [2.5]. Ion pair formation between the cationic head group of the surfactant and the anionic silanol group occurs. The hydrophobic surfactant tail extending into the bulk solution cannot be solvated by water. Its solvation need is satisfied by binding to the tail of another surfactant molecule. As a result, the cationic head group of the second surfactant molecule is in contact with the bulk solution. The capillary wall behaves with cationic character because of this treatment, and the EOF is directed toward the positive electrode.

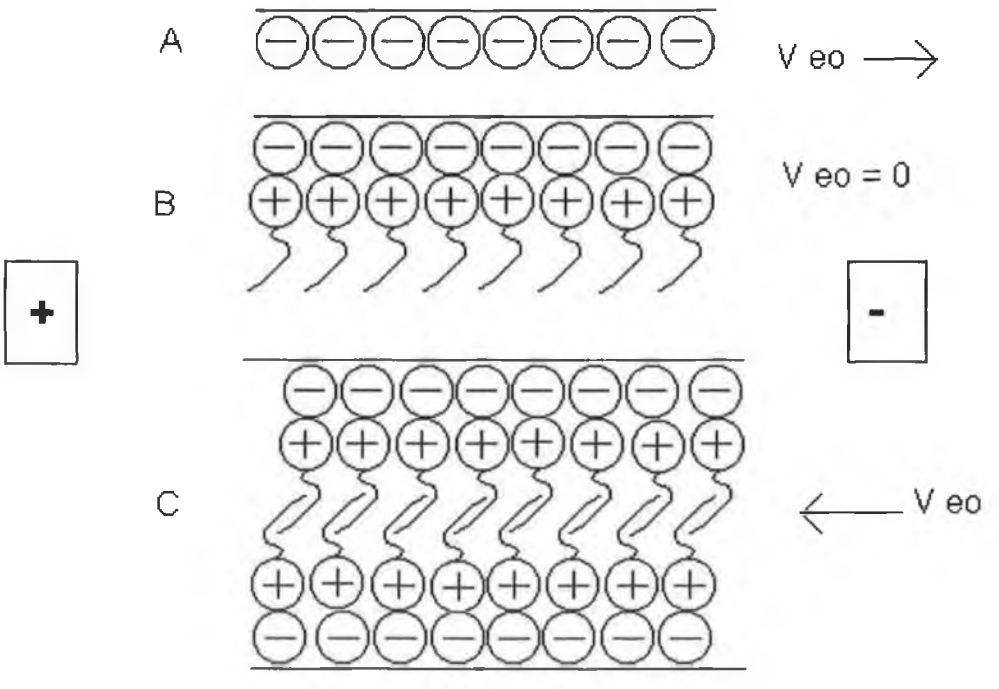


Fig. [2.5] Representation of the cationic surfactant charge-reversal at the capillary wall. **A**: No surfactant added; electroosmotic flow in normal direction. **B**: Electrostatic adsorption of the positively charged surfactant head groups to the negative silanol groups on the silica surface of the capillary inner wall. **C**: Bilayer formation by hydrophobic interaction between apolar chains, resulting in the reversal of the electroosmotic flow.

2.1.3.6. Bandbroadening / Efficiency

Under ideal conditions the only contribution to bandbroadening in CE is longitudinal diffusion, and hence the variance of the migrating zone with (σ^2) can be written as

$$\sigma^2 = 2Dt = 2DL^2/\mu_{ep}V \qquad \text{or} \qquad \sigma = (2DL/\mu_{ep}E)^{1/2} \qquad \textbf{[Eq 2.9]}$$

Where mobility, μ, was defined in Eq. [2.6], V is the applied voltage, and D is the diffusion coefficient of the solute.[28]
The number of theoretical plates (N) is therefore given by

$$N = L^2/\sigma^2 = \mu_{ep}\frac{V}{2D} \qquad \textbf{[Eq 2.10]}$$

Efficiency is most strongly influenced by the magnitude of the applied voltage and increases with applied voltage, but not capillary length. Maximum efficiency and short analysis times are obtained with high voltages and short columns, provided that there is efficient heat dissipation. At some point Joule heating actually diminishes the efficiency observed. Efficiency can also be limited by the concentration of solute injected, solute-wall interactions and temperature gradients.

2.1.3.7. Resolution

The mobilities of a pair of injected solutes have a strong influence on the resolution, R_s, obtained for them as given by

$$R_s = (N^{1/2}/4)\left[\frac{(\mu_1 - \mu_2)}{(\mu_{av} - \mu_{eo})}\right] \qquad \textbf{[Eq 2.11]}$$

where N is the number of theoretical plates, μ_1 and μ_2 are the mobilities of the two solutes and μ_{av} is their average mobility, and μ_{eo} is the electroosmotic mobility of the buffer system employed. Resolution is maximised by large differences in solute mobilities, high applied voltages, and electroosmotic flow opposing the mobilities of the solutes. However, extreme magnitudes of these variables can lead to problems such as long analysis times and Joule heating.

2.1.3.8. Joule Heating

Joule heat, the heat generated as electric current passes through the solution in the separation capillary, is a concern in all CE separations. Because this heat is dissipated at the wall of the column, the solution in the centre of the column can be warmer than that adjacent to the walls. Such a temperature gradient alters the buffer viscosity, which leads to different solute mobilities across the diameter of the capillary and band broadening, thus reducing the efficiency of the separation. A temperature difference between the capillary centre and wall as small as 5°C has been shown to cause significant band broadening.[28] The amount of Joule heat generated during CE separations can be controlled by reducing the applied voltage or utilising smaller diameter capillaries that exhibit lower current and more effectively dissipate heat.

2.1.4. Apparatus

One of the main advantages of CE is that it requires only simple instrumentation. Before 1988, all work was done on simple home made systems of a design similar to Jorgenson and Lukacs's original work.[4] A schematic diagram of the basic CE instrument is shown in Fig. [2.6]. Basically, it consists of a measured length of fused silica capillary suspended between two buffer reservoirs that are adjusted to equal heights to eliminate hydrostatic flow. The separation voltage from a high voltage power supply is applied to platinum electrodes placed in the reservoirs. A detector and some form of data acquisition are the final necessary components of

the system. Automation is particularly important in CZE. The best analytical precision is found when experiments are performed in a highly repeatable fashion. In CE automated systems, sample introduction is performed in a repeatable manner, detection is on-line and the instrumental output resembles a chromatogram.

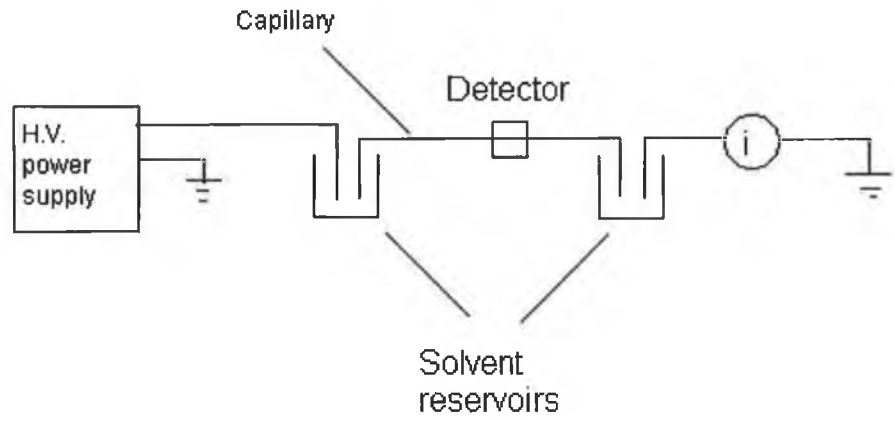


Fig. [2.6]: Diagram of a Basic Capillary Electrophoresis System.

2.1.5. Sample Injection Methods

The combination of the high efficiency of separation and the short analysis times possible with CE produces an extreme constraint on the sampling system. Samples must be introduced on-column with minimum volume in order to preserve this high separation efficiency.[29] The simplest methods of sample introduction are direct electromigration of the sample onto the column[5] and hydrodynamic injection via the formation of a small pressure gradient between inlet and outlet of the column.[30,31,32]

2.1.5.1. Electrokinetic injection

Electromigration injection is also called electrokinetic injection. Electrokinetic injection is accomplished by briefly (seconds) replacing the inlet buffer reservoir with the sample solution and applying high voltage (several kilovolts) across the

capillary causing sample to enter the end of the capillary by electromigration. The inlet end of the capillary is then returned to the inlet buffer reservoir and the operating voltage is applied to carry out a separation. Electromigration injection includes contribution from both electrophoretic migration of charged sample ions and electroosmotic flow of the sample solution. The electrophoretic and electroosmotic velocities can be represented as[33]

$$\nu_{ep} = \mu_{ep}\frac{V_i}{L} \qquad \textbf{[Eq 2.12]}$$

$$\nu_{eo} = \mu_{eo}\frac{V_i}{L} \qquad \textbf{[Eq 2.13]}$$

where μ_{ep} is the electrophoretic mobility of the sample molecule, μ_{eo} is the electroosmotic mobility of the sample solution, V_i is the injection voltage and L is the length of the column.

The length of the sample zone is given by

$$l = (\nu_{ep} + \nu_{eo})t_i \qquad \textbf{[Eq 2.14]}$$

where ν_{ep} is the electrophoretic velocity of the sample molecule, ν_{eo} is the electroosmotic velocity of the sample solution, and t_i is the injection time (i.e. time over which the injection voltage is applied).

By substituting **[Eq 2.12]** and **[Eq 2.13]** into **[Eq 2.14]**, the sample zone length becomes:

$$l = (\mu_{ep} + \mu_{eo})\frac{V_i t_i}{L} \qquad \textbf{[Eq 2.15]}$$

For on-column injection methods, the amount, w, of sample injected (in weight or number of moles, depending on unit for concentration) into the capillary is given by

$$w = \pi r^2 lC \qquad \textbf{[Eq 2.16]}$$

where r is the radius of the capillary, l is the length of the sample zone, and C is the sample concentration. Thus we can determine w, the amount of solute injected by electromigration by combining **[Eq 2.15]** and **[Eq 2.16]** to give:

$$w = (\mu_{ep} + \mu_{eo})\, \pi r^2 \left(\frac{V_i t_i C}{L} \right) \qquad \textbf{[Eq 2.17]}$$

From this equation it can be seen that the quantity of sample injected during electrokinetic injection can be controlled through the variation in the injection time t_i, and injection voltage V_i.

While electrokinetic injection is appropriate for an ionic solute, a hydrostatic injection method can be substituted for neutral solutes.

2.1.5.2. Hydrostatic injection

Hydrostatic injection requires the creation of a pressure difference between the inlet and outlet ends of the capillary. Each of the three common ways to generate a pressure differences requires the inlet end of the capillary to be moved from the inlet buffer reservoir to a sample solution vial. The first variation requires elevating the sample solution sufficiently to induce gravity flow, thus causing a plug of sample solution to be introduced onto the column. The other modes of hydrodynamic injection require the application of a positive pressure to the sample solution or a negative pressure (vacuum) to the outlet end of the capillary.

2.1.6. Detection Techniques

Perhaps the most rapidly developing aspect of capillary electrophoresis is the area of detection. The ability to detect trace amounts of a wide variety of solutes will dictate the future of capillary electrophoresis. The small capillary dimensions employed in capillary electrophoresis and the small volumes produced present a challenge to achieve sensitive detection without introducing zone dispersion. Zone broadening normally caused by joints, fittings and connectors can be eliminated by on-column detection. On-column UV adsorption and fluorescence detection have been the most commonly used detection techniques for CE applications. Many other detection techniques have been explored with varying degrees of success.[34]

To achieve on-column UV or fluorescence detection, a window has to be made on the polyimide coating of the fused silica capillary. The simplest way that can be used to form the window is by burning off a small section of the polyimide,[35] although alkaline etching[36] and mechanical scraping[37] can also be used. More elaborate devices have also been designed for this purpose, which use an electrically heated filament to remove the polyimide coating.[37]

2.1.6.1. Absorption Detection

UV-visible absorption is currently the most popular detection technique for capillary electrophoresis. The main reasons for its popularity include it being a relatively universal technique with on-column detection and it is relatively sensitive due to limitation by path length. Absorbance detection is typically carried out on-column by removing a small length of the column's protective polymer coating close to the outlet, as described earlier, and directing a light beam through that section. Fused silica tubing has a UV cut-off around 170 nm and this is suitable for UV detection. In this method, the path length is limited to the inner diameter of the capillary (typically 50-100 μm). Since absorbance is directly related to path length, by the Beer Lambert Law, many efforts have been made to

increase the path length and thus increase sensitivity. These include the fabrication of capillaries with a larger diameter "bubble" in the detection region,[38] coating the detection region with a reflective material that will allow the light beam to enter the column and be reflected several times across the diameter before exiting the column,[39] and the creation of columns with Z-shaped detection zones.[40]

Another consideration is that with small capillaries ideally only the capillary is illuminated during detection in order to reduce stray light. Much of the early work using UV detection suffered from poor sensitivity due to the limitations of unfocused light sources. Walbroehl and Jorgenson[13] described a fixed wavelength UV detector using a Cd "penray" source, which was focused onto the capillary. This produced reasonable detection limits. Terabe et al.[12] also used on-column UV detection with MECC in the separation of substituted benzenes and alcohols. Fujiwara and Honda[41] used UV detection with MECC in the determination of ingredients in analgesics preparations. Peak area ratios were found to give the best precision (1-2%), and the ingredients of a commercial preparation were determined. UV has remained the most popular method of detection in CE despite its limited sensitivity. Recently direct UV detection has been used in the speciation of metal ions in different oxidation states and detection limits in the range 1 x 10^{-6} mol L^{-1} for Fe^{II} to 8 x 10^{-6} mol L^{-1} for Cr^{VI} were reported.[42]

2.1.6.2. Fluorescence Detection

Fluorescence detection is a second frequently used detection method.[18,43] Fluorescence detection is most easily adapted for use in CE, since its sensitivity is not path-length dependent. On-column detection is accomplished simply by imaging the excitation source onto the column and collecting the emission at an angle perpendicular to the incident light via a lens or optical fibre and transmitted to an optical detector. This allows the use of much narrower columns when lasers are employed as excitation sources, known as laser-induced fluorescence. The excitation source can be as simple as an arc lamp, where the excitation wavelength

is isolated with glass filters and then focused onto the capillary. The sensitivity of fluorescence detection also depends on the intensity of the laser light.[44]

Therefore, within reason, sensitivity can be enhanced by increasing the power of the laser light source. Laser induced fluorescence (LIF) detection has been used to characterise humic substances and their interactions with metal ions by CE[45]. An indirect fluorescence method was applied by Lee and Whang for the CE analysis of organotin compounds.[46] Other techniques have been developed to detect analytes of interest that do not contain a chromophore. The more widely used ones include indirect absorbance and electrochemical measurements.

2.1.6.3. Indirect Detection

Indirect absorbance detection is performed by adding an absorbing species to the separation buffer, thus creating a background signal. As the analyte zone, which has a much lower absorptivity than the buffer additive, passes the detector a reduction in the background is evident.[42] Thus, in capillary electrophoresis, a charged chromophore can be used such that analyte ions of like charge will displace the chromophore, while ions of opposite charge may ion pair with it. This allows visualisation of many species which would ordinarily be detector inactive, since the signal produced in this manner is independent of the spectral properties of the analyte. Initial demonstrations of the feasibility of indirect detection in CE were performed by Hjerten,[47] who employed indirect UV absorbance detection of organic anions. Indirect UV detection is presently the most versatile method to solve the problem of universal detection for metal speciation by CE.[48,49] When choosing a UV-active co-ion, a close mobility match to the analyte ions is required; otherwise, asymmetrical peak shapes are generated. Various cationic and anionic co-ions such as imidazole,[44] pyridine[50] and chromate[51] have been successfully utilised for indirect detection of metal species in CE.

2.1.6.4. Electrochemical Detection

Electrochemical detection includes amperometric, potentiometric, and conductometric measurements.[42] Amperometric techniques have been more commonly employed, as they are experimentally less difficult and provide good sensitivity.[52] A major concern in the development of each of these methods is the need to isolate the electrochemical cell from the electrophoretic current. Furthermore, each of these techniques requires special electronics and careful capillary modification to be successfully executed.

2.1.7. Application of Capillary Zone Electrophoresis to the Separation of Organotins

2.1.7.1. Separation of Inorganic Ions

A number of reports on the determination of simple inorganic ions by CE have been reported. The analysis of inorganic ions by capillary electrophoresis continues to grow in importance as the problems of sample preparation and detection are addressed. Methods to optimise separation and detection of inorganic ions have been discussed in several reports.[53,54,55] When applicable, the simplest means of monitoring inorganic ions separated by CE is direct UV absorbance. However, the lack of suitable UV chromophores has led to the investigation of other detection techniques such as conductivity or indirect detection. Most reports of the separation of inorganic cations using CZE are based on the use of indirect UV detection in the presence of an organic amine as the primary electrolyte component.[56,57,58] Recently, copper electrolyte was also reported as an inorganic ionophore.[59] The CE determination of organolead and organoselenium compounds was performed with on-column direct UV detection.

The electrophoretic separation of cations depends on the equivalent ionic conductivities and the velocity of electroosmotic flow. The latter depends on the charge of the capillary walls, whereas the relative mobilities of ions in solution can be modified by changing the ionic strength and pH by addition of complexing ligands, as discussed earlier.

In recent years, metal speciation has rapidly gained importance due to its impact on environmental chemistry, toxicology and biomedical sciences. A lot of research has been carried out on developing high-efficiency analytical techniques that are able to determine rapidly and sensitively the chemical forms of metals in a wide variety of sample matrices. Conventionally, this has been largely achieved by coupling the separation power of gas chromatography (GC) and liquid chromatography (HPLC) with the sensitive responses of spectrophotometric and electrochemical detectors. Recently, capillary electrophoresis has developed to be a powerful technique for the rapid and highly efficient separations of metal species.[60] The distinctive feature in the separation of metal cations and metal-complexed ions by CE is their high charge to mass ratio and hence large electrophoretic mobility. However, anionic electrophoretic mobilities and the EOF have different directions. Such a counter-electroosmotic migration reduces substantially the range of anionic analytes that can be separated for the detection at the cathode end.

2.1.7.2. Separation of Organotins

Compared with traditional chromatographic techniques, organotin species can be separated by CE without changing the actual form of the species. Since most organotins possess poor chromophoric properties, indirect UV detection is commonly used. Pobozy et al.[61] used CE with indirect UV detection for trimethyltin (TMT), triethyltin (TET) and tributyltin (TBT) and with direct UV detection for triphenyltin (TPhT). A better separation of analytes and much lower detection limits were obtained by CE as compared with HPLC.

Five triorganotin compounds, TMT, TET, tripropyltin (TPT), TBT and TPhT were efficiently separated by CE in 10 min. by Whang and Whang.[62] Simultaneous separation of both di- and triorganotins was achieved with the addition of α-CD as a modifier in the electrolyte. The detection limits for the organotin studied were between 0.2 and 2.4 mg L^{-1} (as tin).

An indirect fluorescence detection method for the CE separation of triorganotin compounds has also been developed.[45] The five triorganotin cations (TMT, TET, TPT, TBT and TPhT) could be separated within 15 min. and detected down to 0.9 – 2.1 mg L^{-1} as tin. Although this detection scheme is more sensitive than UV absorption detection, its concentration sensitivity is still not adequate for routine environmental analysis in which a μg L^{-1} detection level is generally required.

2.2. Experimental

2.2.1. Apparatus

The capillary electrophoresis instrument used in these experiments was the P/ACE 2050 series (Beckman Instruments, Fullerton, CA) outfitted with an IBM computer. The system was equipped with a fixed wavelength UV detector, which was fitted with a 190 nm optical filter (Acton Research Corporation). The system was operated under the System Gold control, data acquisition, and analysis software (Beckman Instruments, Fullerton, CA). Polyimide-coated fused silica capillaries (Composite Metal Services, Worcester, England), 100 μm internal diameter and 57 cm total length were used throughout. A detection window was created by burning off a small section of the polyimide coating 50 cm from the injection end of the capillary. Sample introduction was carried out by hydrodynamic (pressure) injection. Before each injection the capillary was conditioned by rinsing for 0.5 minutes with 1 M NaOH, followed by a 1-minute rinse with run buffer. Optimum conditions for analysis are listed in Table [2.5].

2.2.2. Reagents

All standards and buffers were prepared using distilled deionised water (Milli-Q system, Millipore Co., USA). Trimethyltin chloride, dimethyltin dichloride and methyltin trichloride were obtained from Aldrich. The run buffers used were sodium dihydrogenorthophosphate and di-sodium hydrogenorthophosphate, and were also obtained from Aldrich. The capillary modifier used was cetylpyridinium chloride (B.D.H. Limited, Poole, England).

2.2.3. Development of a Separation Scheme of Three Methyltins using UV Detection

This method has been adapted from a previous method[63] and optimised to give the final operating conditions summarised in Table [2.5]. In the previous method the surface of the capillary was modified using the cationic surfactant cetyltrimethylammonium bromide (CTAB). It was suggested, however, that this modifier led to incomplete coating of the capillary wall, and thus to irreproducible results. Statistical validation of the method was found to have between day and within day coefficients of variability of greater than 7% Relative Standard Deviation (RSD). As the use of a surface modifier is the basis of the separation scheme, and the use of CTAB was found to be unsatisfactory, another surfactant cetylpyridinium chloride (CPC) was examined as an alternative. This modifier was found to be effective in the separation of the methyltins. These positively charged polymer molecules are ionically adsorbed onto the negatively charged silica surface, initially, to form a neutral surface. Subsequent hydrophobic interactions between surface bound polymers and free polymers produce a positively charged polymer layer. This reversal of the surface charge of the capillary leads to a reversal of the electroosmotic flow. Therefore the polarity of the system must be reversed to ensure migrating ions travel towards the detector.

Having selected cetylpyridinium chloride (CPC) as the flow modifying agent, the separation of the three methyltin compounds was optimised in terms of buffer concentration, buffer pH, voltage and injection time using a standard mixture composed of 500 ppm Me_3SnCl, 500 ppm Me_2SnCl_2, and 250 ppm $MeSnCl_3$. The CPC was added to a 15 mM phosphate buffer and its concentration was varied to give optimal separation of the methyltin chlorides at a final concentration of 0.2 mM.

2.2.4. Procedure Validation

The linearity and reproducibility of this method were both determined as per Dagadar and Smyth[64]. The term precision is used to describe the reproducibility of results. The precision of a measurement is readily determined in the form of the percentage coefficient of variation of replicate experiments performed under identical conditions.

As such, the standard procedures of intra-assay (within day) and inter-assay (between day) were used to describe the linearity and precision of this method. For the intra day variability test six samples over the entire concentration range of analysis were analyzed using the method. Each sample was injected six times and their migration times measured. The mean migration time of each methyltin and its relative standard deviation were calculated. This value was known as the coefficient of variation when expressed as a percentage and was a measure of the precision of the method within a single day. The linear regression curve obtained was used to demonstrate the linearity of the assay.

The inter-day variability test consisted of analysis of a single standard, within the aforementioned concentration range, six times a day over six days. Again, the relative standard deviation and coefficient of variation were calculated to give an indication of the long-term reproducibility of the method. A 1000 ppm standard sample was used to evaluate the between day variability.

2.2.5. Results and Discussion

2.2.5.1. Wavelength Selection

Direct UV detection was examined as a method of detection for this CZE separation. UV analysis of $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl was therefore performed to determine the optimum wavelength for the detection of these species. The UV spectra recorded are shown in Fig. [2.7]. It can be seen that sufficient absorbance occurred at 190 nm to allow this wavelength to be selected as the detection wavelength for direct detection CZE analysis.

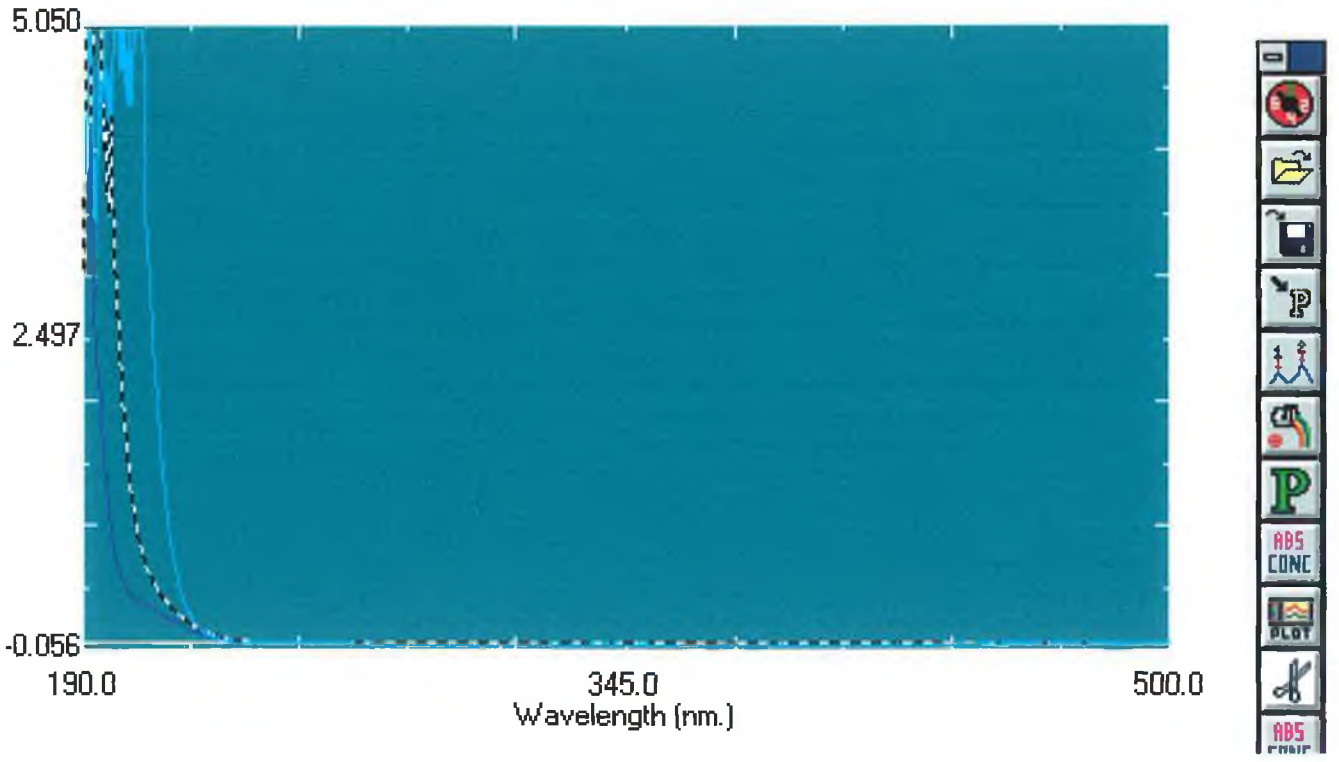


Fig. [2.7]: UV spectra of Me_3SnCl (___),Me_2SnCl_2(_ _) and $MeSnCl_3$ (___), recorded from 190 to 500 nm.

2.2.5.2. Buffer Conditions

In capillary electrophoresis the background electrolyte or run buffer is the most important variable in the development of a successful separation for most analytes. The buffer is responsible for controlling pH, which in turn controls the ionization and mobility of the analytes. The buffer also controls the ionization of the surface silanols and the magnitude of the electroosmotic flow. It carries the electrical current necessary for the system to function.

Ideal buffers should have good buffering capacity (within one pH unit of the pK_a value) and low UV-vis. absorbance. They should have low conductivity to minimize current generation and allow high voltages that speed analysis and increase efficiency. The mobility of the buffer should match that of the analytes to preserve peak shape. Very importantly, the analytes and other sample matrix components must be soluble in the buffer[65].

Consequently, in determining the method, it was necessary to optimise such conditions as buffer concentration, operating pH and the concentration of any buffer additives used – in this case cetylpyridinium chloride concentration. Several instrumental parameters, such as operating voltage and injection time must also be optimised once the run buffer has been selected.

2.2.5.3. Optimisation of Buffer pH

Buffer pH is one of the key parameters used to optimise separations in capillary electrophoresis. The applied potential causes a current that is carried by cations and anions. This conductivity is determined by both the concentration of the ions and the speed of movement of the ions in the applied electric field. This mobility of ions is governed by their charge to mass ratio, and is termed electrophoretic mobility (μ). By adjusting the pH of the buffer it is possible to alter the degree of

ionisation of species present in the electrolyte system and therefore change the charge to mass ratio of a species, resulting in a change in the mobility of the ion. As discussed in Section [2.1.4] the electrophoretic mobility of an ion is proportional to the charge of the ion by

$$\mu_{ep} = \frac{q}{6\pi\eta r} \qquad \textbf{[Eq 2.5]}$$

where q represents ionic charge, η is the viscosity of the medium and r is the ionic radius.

The above equation defines only the observed mobility. To calculate the true mobility a correction for electroosmotic flow must be made. Electroosmotic flow occurs because of charge on the surface of the capillary. The walls of fused silica capillaries possess an intrinsic negative charge due to the presence of ionizable silanol groups at its surface. Positive counterions compensate for the negative charge on the wall thus forming an electrical double layer. When a voltage is applied, the mobile positive charges migrate in the direction of the cathode. Since ions are solvated by water, the fluid in the buffer is mobilized as well and dragged along by the migrating charge. This electroosmotic flow is transmitted through the capillary.

The electroosmotic flow velocity (v_{eo}) is related to the potential at the beginning of the diffuse double layer known as the zeta potential (ζ) by:

$$v_{eo} = \left(\frac{\varepsilon}{4\pi\eta}\right)(E\zeta) \qquad \textbf{[Eq 2.2]}$$

where E is the applied electric field strength, ε is the dielectric constant of the buffer and η is the viscosity. This expression assumes that the dielectric constant and viscosity of the solvent are the same in the diffuse layer as in the bulk solution. At high pH the silanol groups are fully ionised generating a strong zeta potential and a dense double layer. As a result the EOF increases as pH increases.

Electroosmotic mobility may be measured by injecting a neutral solute such as acetone and measuring the time it takes to migrate through the capillary. The apparent mobility of a solute is therefore related to both the electrophoretic mobility and the electroosmotic flow

$$\mu A = \mu_{eo} + \mu_{ep} = \frac{lL}{tV} \qquad \textbf{[Eq 2.18]}$$

where l is the length of the capillary to the detector and L is the total length of the capillary. As the pH increases, the electroosmotic flow also increases and therefore the migration time is decreased.

In determining the optimum pH for the separation of the methyltin chlorides the main consideration was the resolution between dimethyltin dichloride and trimethyltin chloride. At low pH these compounds co-elute, so higher pH buffers are required for separation.[62] The run buffer pH was examined over the range 7.5 to 9.0. The concentration of the phosphate buffer was 15 mM and the concentration of the cetylpyridinium chloride was 0.1 mM throughout the experiment.

The variation in migration times with increasing pH can be seen in Fig [2.8]. The effect of increasing the pH from pH 7.5 to 8.0 was a slight decrease in migration time for methyltin trichloride but a significant increase in migration time for both dimethyltin dichloride and trimethyltin chloride. An increase in electroosmotic flow brought about by the increase in pH should result in the overall decrease in analysis time as was the case for $MeSnCl_3$. As for Me_2SnCl_2 and Me_3SnCl the increase in analysis time observed may be due to the incomplete ionization of the species below pH 8.0. As a result it is possible that the partially charged di- and tri-methylated species may interact with the positively charged surfactant bi-layer causing retardation of their migration thus increasing migration time.

From pH 8.0 to pH 9.0 there is little variation in migration times for any of the species analyzed. This suggests that the electroosmotic flow of each of the ions is

invariant over this pH range. Maximum separation between Me_2SnCl_2 and Me_3SnCl occurred at pH 8.25.

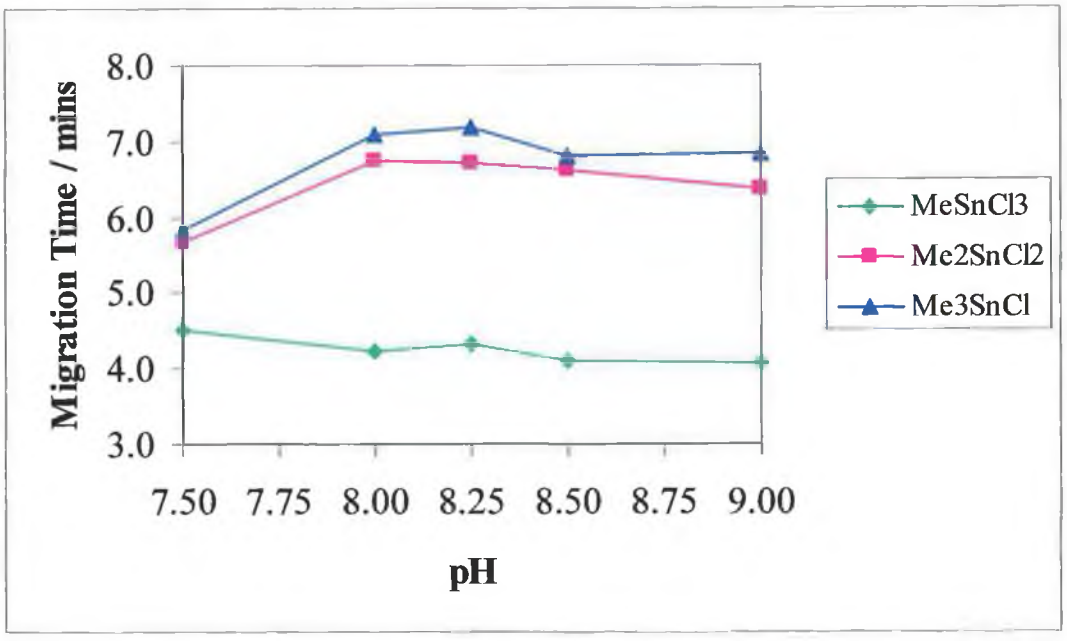


Fig. [2.8]. Variation in migration time for each methyltin species in the pH range 7.50 to 9.00.

pH	Resolution
7.50	Unresolved
8.00	2.02
8.25	2.73
8.50	2.85
9.00	2.88

Table [2.1]. Effect of increasing pH on the resolution between Me_2SnCl_2 and Me_3SnCl.

The resolution of Me_2SnCl_2 and Me_3SnCl is important in determining optimum operating conditions. At lower pH values these two species are known to co-elute

and it is only at higher pH that separation occurs. As can be seen from Table [2.1] the resolution of Me_2SnCl_2 and Me_3SnCl increased significantly from pH 7.5 where the components were unresolved to 2.728 at pH 8.25. This figure remained reasonably constant up to pH 9.0, the highest pH value examined. Therefore, any value in the range pH 8 to pH 9 would be acceptable for the separation of the methyltin chlorides. It would be expected that the resolution of Me_2SnCl_2 and Me_3SnCl would not change significantly as the migration time of these two compounds was invariant over this pH range.

Fig. [2.9] shows individual electropherograms recorded at different pH values. Increasing the pH from 7.5 to 8.5 shows a marked improvement in the resolution of dimethyltin dichloride and trimethyltin chloride. At pH 7.5 Me_2SnCl_2 and Me_3SnCl are not resolved. $MeSnCl_3$ migrates through the system in approx. 4.5 mins. By increasing the pH to 8.0 the resolution of Me_2SnCl_2 and Me_3SnCl improves significantly and the separation of $MeSnCl_3$ and Me_2SnCl_2 is also enhanced due to a decrease in the migration time of $MeSnCl_3$ and a concurrent increase in the migration time of Me_2SnCl_2. The peak that appears at approx. 2 minutes has been found to be due to chloride ions. An injection of a dilute HCl sample confirmed this identity.

The number of theoretical plates is given by the expression

$$N = 5.54\ (t_m/w_{½})^2 \qquad \textbf{[Eq 2.19]}$$

where t_m is the migration time and $w_{½}$ is the peak width at half peak height. The efficiencies calculated for each methyltin species are illustrated in Fig. [2.10]. It is clear that the optimum pH for the separation of the methyltins is in the range 8.0 to 8.5 with the maximum efficiency for each methyltin compound occurring at pH 8.25. Peak widths remain relatively constant as the pH increases, therefore ensuring a stable plate number. Further experiments in which the pH is varied along with other buffer conditions will allow discrimination between the pH values.

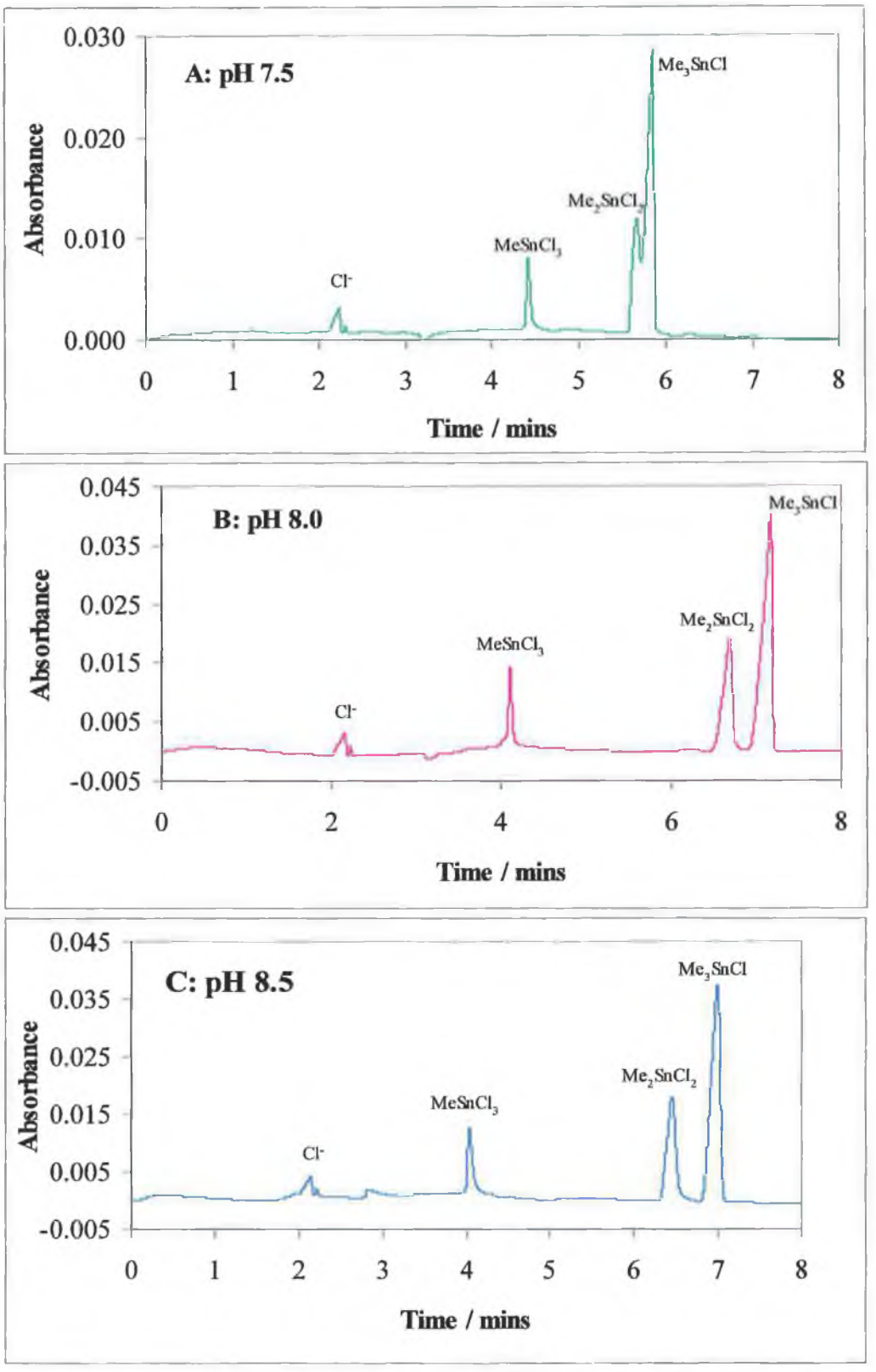


Fig. [2.9]. Electropherograms of a 1000 ppm standard mixture of $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl using a) pH 7.5, b) pH 8.0 and c) pH 8.5. Separations were carried out using 15 mM phosphate buffer with 0.01 mM CPC.

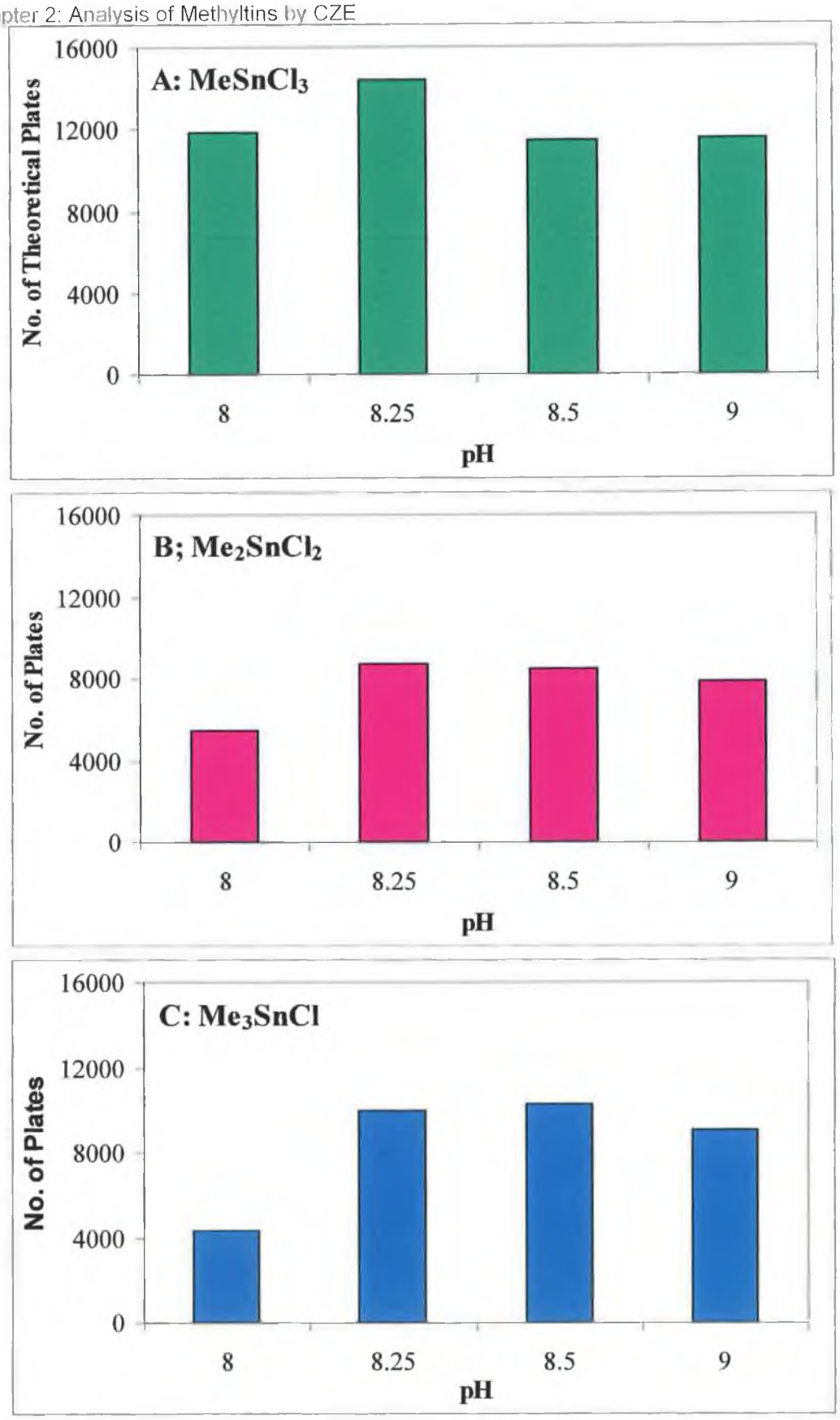


Fig [2.10]. Electropherograms of a 1000 ppm mixture of $MeSnCl_3$, $Me2SnCl_2$ and Me_3SnCl separated using 15 mM Phosphate buffer at a) pH 7.5, b) pH 8.0 and c) pH 8.5.

2.2.5.4. Optimisation of Buffer Concentration

The electrophoresis buffer is of key importance in capillary electrophoresis because its composition fundamentally determines the migration behaviour of the analytes. A suitable electrolyte system must ensure the correct electrophoretic behaviour of all individual solutes, the overall stability of the system and satisfactory separation of the analytes. Ionic strength or concentration of the buffer has significant effects on the mobilities of solutes and on separation efficiency. As the ionic strength increases the zeta potential and similarly the EOF decreases in proportion to the square root of the buffer concentration. Van Orman et al.[66] confirmed this experimentally reporting equivalent EOF for different buffer types as long as the ionic strength is constant.

The variation in migration times of the three methyltin chlorides over the concentration range 10 to 20 mM was initially investigated at pH 8.0. Experiments were subsequently repeated at pH 8.25 and pH 8.5. The cetylpyridinium chloride concentration was maintained at 0.01 mM throughout each experiment. It would be expected that as the buffer concentration is increased that the migration time of each of the species would also increase due to the reduced electroosmotic flow. This, however, was not found experimentally.

Fig. [2.11] shows the effect on migration time of each of the methyltin compounds of increasing the ionic strength of the run buffer from 10 to 20 mM at three different pH values. At pH 8.0 the migration times of Me_2SnCl_2 and Me_3SnCl first increase as expected but they then decrease. The decrease in migration time is a consequence of Joule heating becoming significant at higher buffer concentrations. This decrease in migration times is also apparent for the analytes at both pH 8.25 and pH 8.5. The effect of increasing the buffer concentration was greatest for the slower migrating compounds Me_2SnCl_2 and Me_3SnCl. The migration of $MeSnCl_3$ was relatively unaffected by Joule heating but a slight decrease in migration time with increasing buffer concentration was still observed.

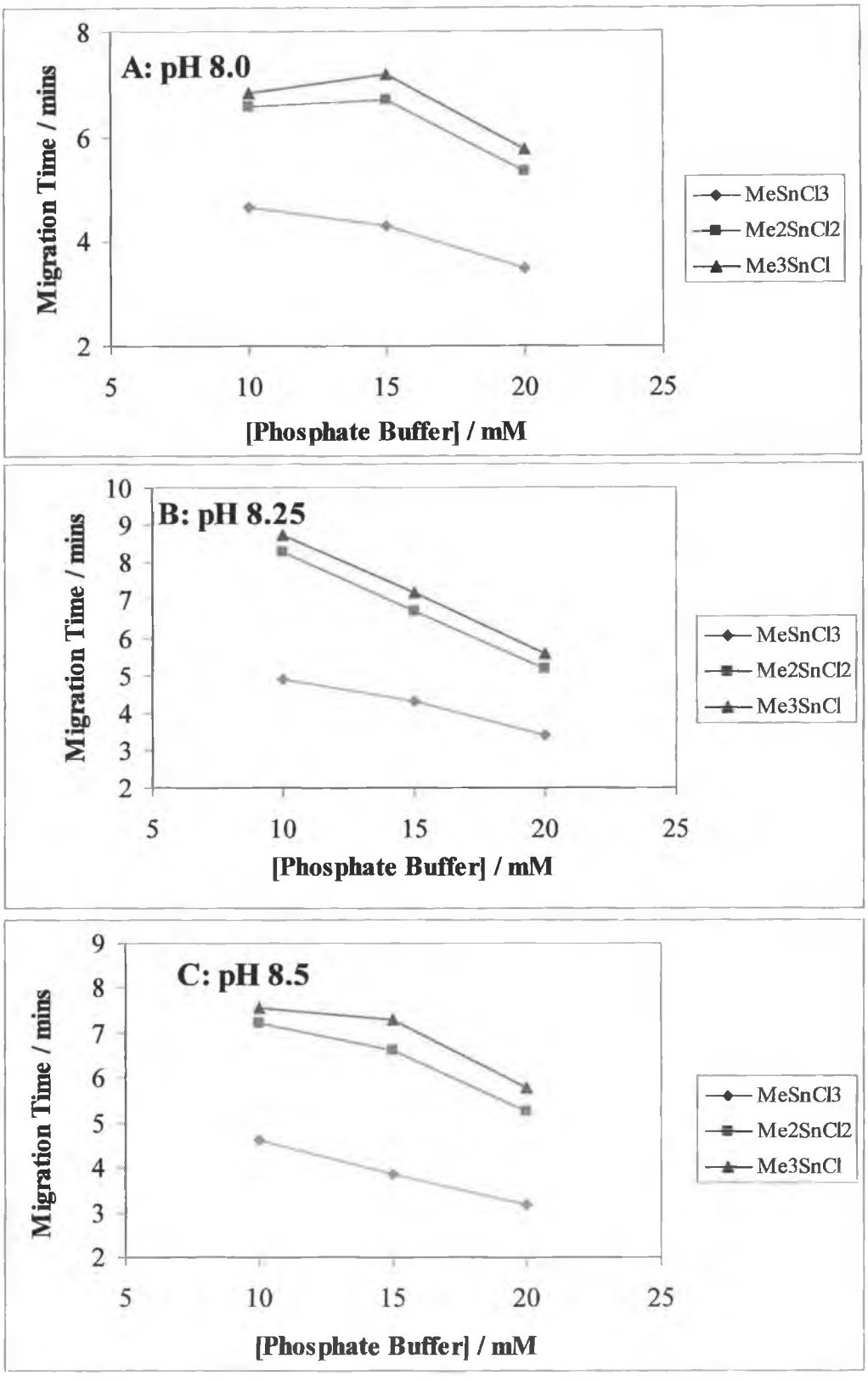


Fig. [2.11] Variation in migration time for each methyltin with phosphate buffer concentration at a) pH 8.0, b) pH 8.25 and c) pH 8.5.

The use, therefore, of a dilute buffer permits the use of wider-bore capillaries but the loading capacity of the separation is reduced. The ionic strength of the buffer influences not only the EOF and electrophoretic mobility, but indirectly the viscosity of the medium. More concentrated buffers have greater conductivity and generate more heat when the voltage is applied. The viscosity is dependent on the temperature, so there is also a dependence on the capillary diameter.[67]

It can be seen that at each pH level increasing the concentration of buffer led to a decrease in migration time. From **[Eq 2.14]** this decrease in migration time may be due either to an increase in EOF or an increase in electrophoretic mobility. It has already been determined that there is no change in EOF in the pH range being examined (Fig. [2.8]). As ionic strength increases the ionic charge (q) must also increase. May be related to pKa values of the phosphate buffer.

pH	15 mM	20 mM
8.00	2.022	2.503
8.25	2.728	2.397
8.50	2.846	3.060

Table [2.2]. Resolution of Me_2SnCl_2 and Me_3SnCl with increasing buffer concentration.

The resolution of Me_2SnCl_2 and Me_3SnCl was seen to increase as buffer concentration increased in an effect similar to that seen by Nielen.[68] Calculations in Table [2.2] show that for both 15 and 20 mM buffers the resolution of these two compounds increased to a maximum at pH 8.5. No figures were obtained for the 10 mM buffer as the species were not resolved at this concentration.

Electropherograms obtained using 10 mM, 15 mM and 20 mM phosphate buffers at pH 8.25 are compared in Fig [2.12]. The decrease in migration time for each species is apparent. The separation efficiencies for each pH were calculated for both 15 and 20 mM phosphate buffer. It can clearly be seen in Fig. [2.13] that for each species the highest number of plates was achieved at 15 mM run buffer.

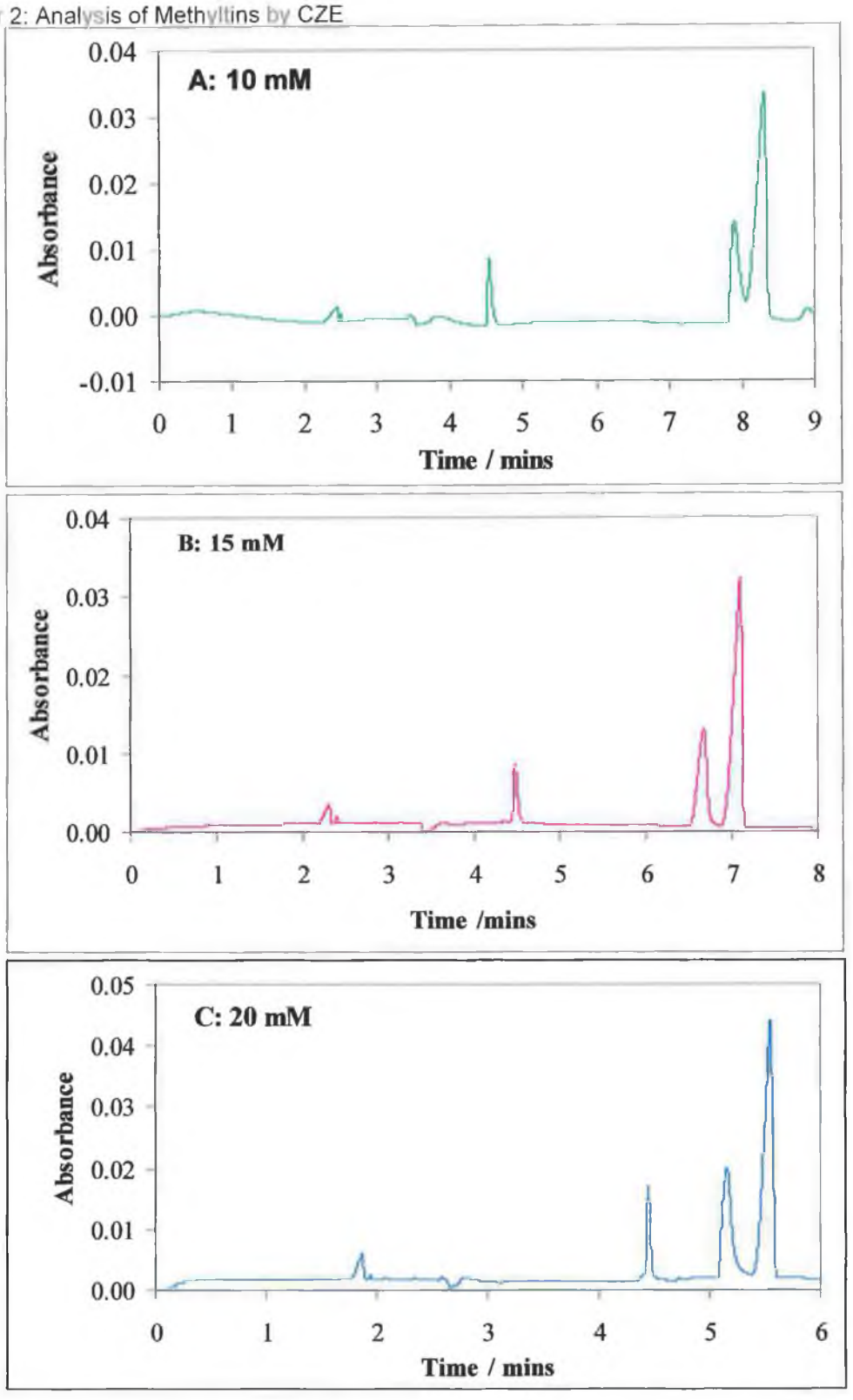
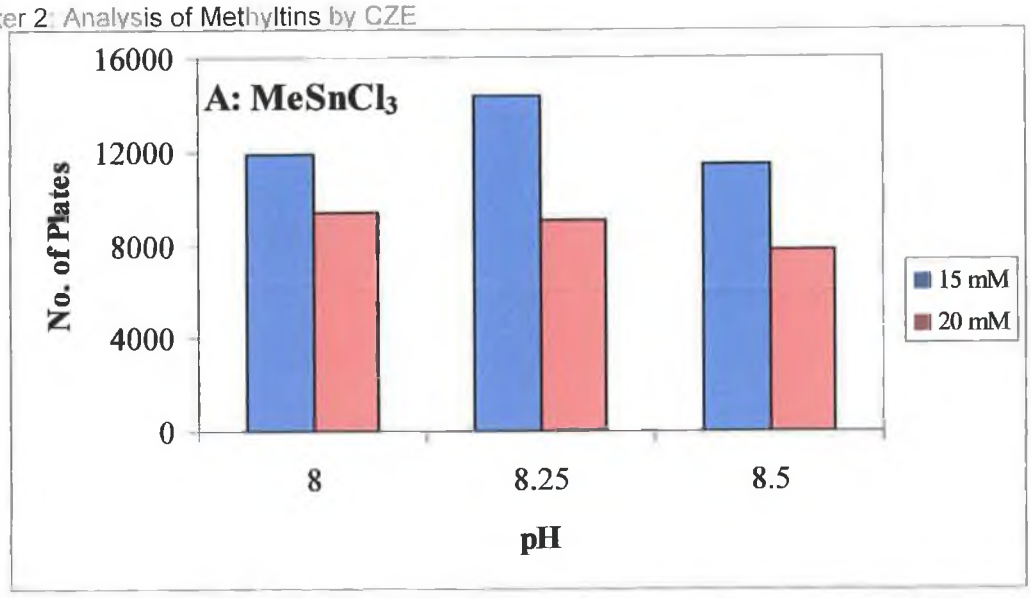
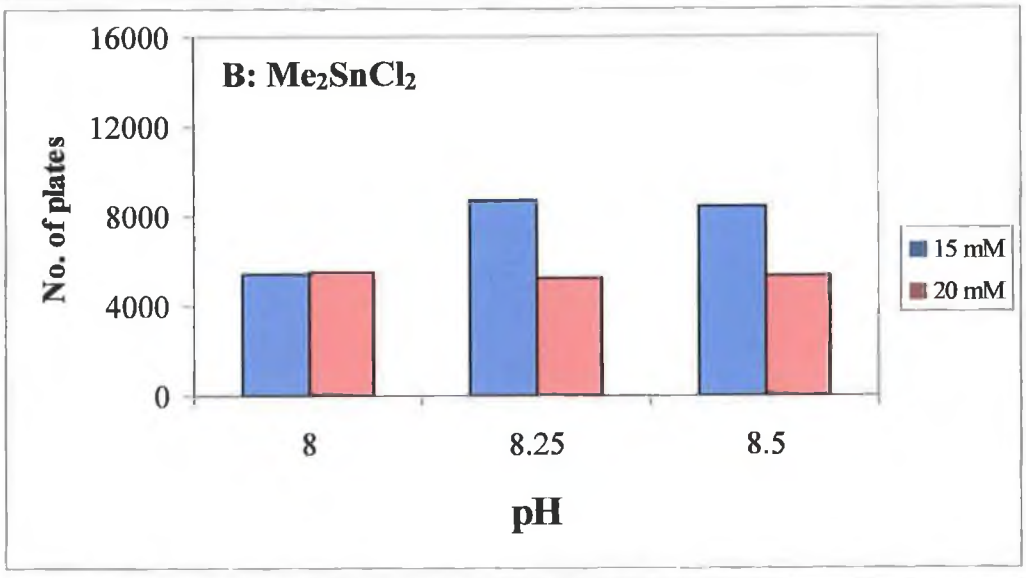


Fig. [2.12]. Electropherograms of a 1000 ppm standard mixture of $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl using a) 10 mM, b) 15 mM and c) 20 mM phosphate buffer. Analysis was carried out at pH 8.25 with 0.01 mM CPC.





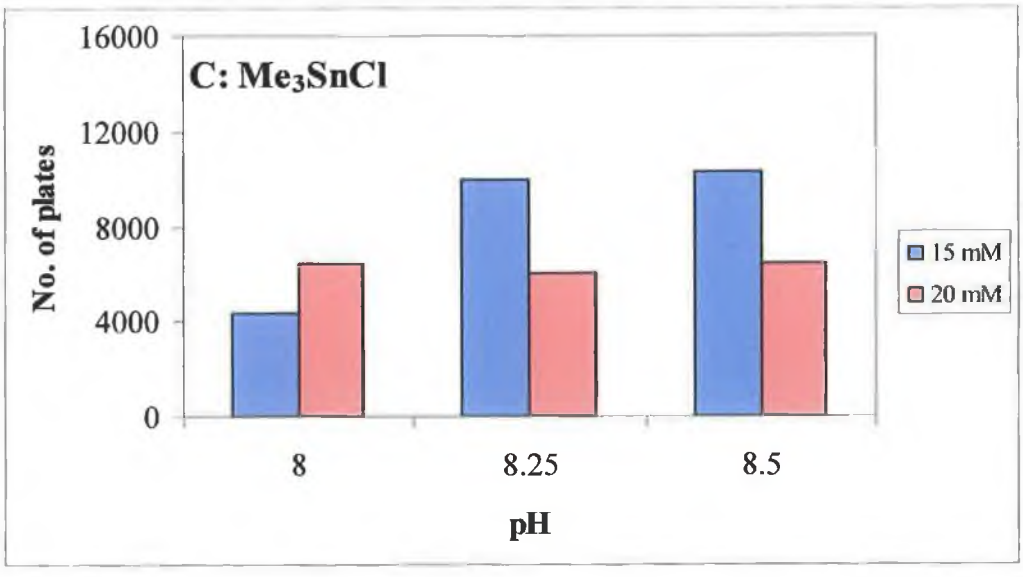


Fig [2.13]. Comparison of calculated efficiency values for each tin species over the pH range 8.0 to 8.5, for both 15 mM and 20 mM phosphate buffer.

It was therefore decided that 15 mM was the optimum phosphate buffer concentration for analysis of the methyltin chlorides. Optimum pH was determined to be pH 8.25 because at this value a higher number of plates were achieved for both $MeSnCl_3$ and Me_2SnCl_2 than at pH 8.5.

2.2.5.5. Optimisation of Cetylpyridinium Chloride Concentration

The use of ionic surfactants can lead to an effective way to achieve better selectivity in capillary electrophoresis. The resulting separations resemble reversed-phase HPLC in that the analytes partition between a mobile phase (i.e. the background electrolyte) and a pseudo stationary phase (the surfactant). The concentration of surfactant used is below its critical micelle concentration to prevent micelle formation. The polarity of the instrument is reversed because the EOF changes direction and this then ensures that all analytes travel past the detector. This occurs because the cationic surfactant binds to the negatively charged silanol sites on the fused-silica surface and effectively changes the charge on the wall from negative to positive. The procedure required to form the coating involved adding the cationic surfactant to the buffer and rinsing through the capillary prior to analysis.

The influence of the cetylpyridinium chloride concentration on EOF and migration time of the methyltin compounds was investigated at concentrations of 0.1 mM, 0.2 mM, and 0.3 mM in both 15 mM and 20 mM phosphate buffer systems at pH 8.25. In all cases an increase in the EOF and apparent mobility of each ion was observed with increasing concentration. This is due to the gradual neutralisation of the silica surface charge through the adsorption of surfactant monomers followed by bi-layer formation. At lower surfactant concentrations not all the surface sites are taken up by surfactant adsorbents and hence a local EOF might exist in the opposite direction consequently resulting in a net reduction in the EOF towards the cathode. As the CPC concentration increases less sites are exposed and thus the EOF continues to increase until the silica surface becomes

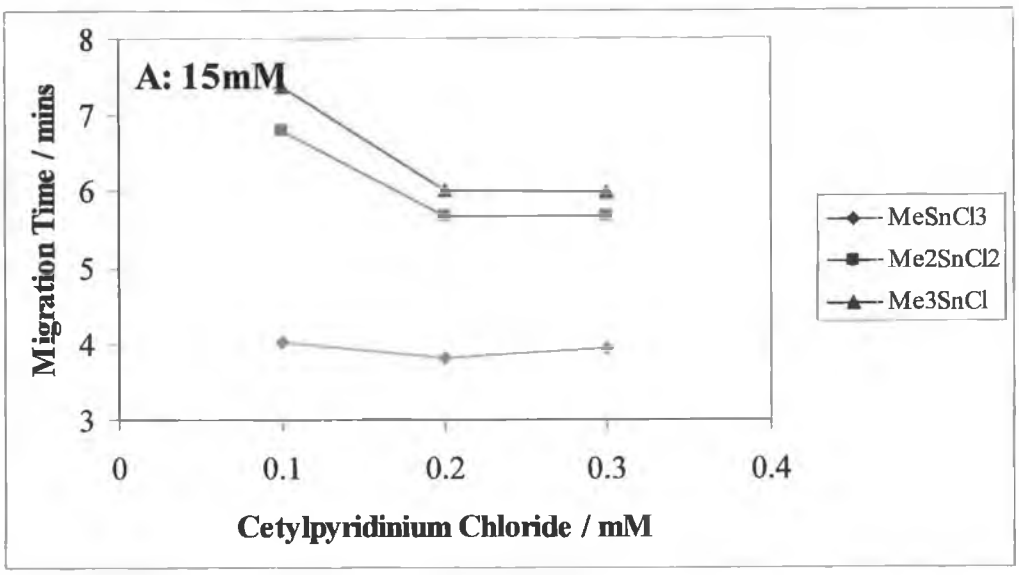
completely saturated after which the EOF remains constant. Experimentally, an increase in EOF leads to a decrease in migration time.

From Fig [2.14] the effect of increasing the concentration of cetylpyridinium chloride on the migration time of the methyltins can be seen. As the concentration of the surfactant is increased more surface sites are occupied, and thus the EOF increases, which leads to a decrease in the migration time. On increasing the CPC concentration from 0.1 mM to 0.2 mM a significant decrease in migration time of each methyltin, at both 15 mM and 20 mM, was observed. Except for $MeSnCl_3$ in a 20 mM phosphate buffer, increasing the CPC concentration to 0.3 mM did not lead to further decrease in migration time for any of the species analyzed. Instead the migration times remained constant, indicating no EOF change. Therefore, it would seem that, at a CPC concentration of 0.2 mM all the surface sites were occupied and any further increase in surfactant concentration would not lead to any increase in EOF, and thus the migration time of each species would remain constant.

	Phosphate Buffer Conc.	
[CPC] / mM	**15 mM**	**20 mM**
0.1	3.433	1.846
0.2	1.958	1.398
0.3	1.432	2.251

Table [2.3]. Resolution of Me_2SnCl_2 and Me_3SnCl with increasing cetylpyridinium chloride concentration

The resolution of Me_2SnCl_2 and Me_3SnCl was seen to be higher for the 15 mM buffer than for the 20 mM buffer. The resolution also decreased as the CPC concentration increased for both buffers. The decrease in resolution with increase in CPC concentration is a result of the reduction in migration time due to higher CPC levels.



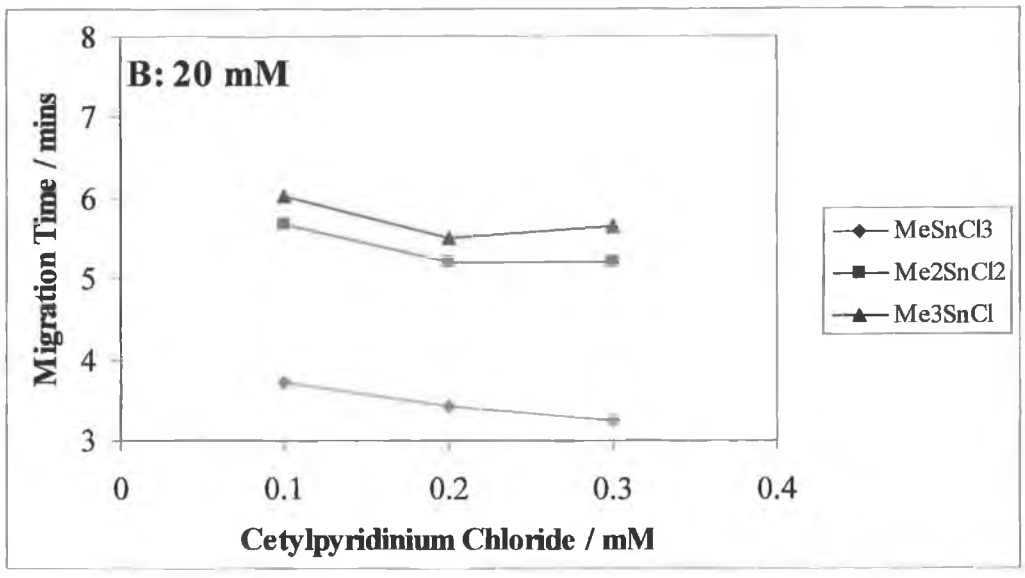


Fig [2.14]. Variation in migration time for each methyltin compound with Cetylpyridinium chloride concentration at A) 15 mM and B) 20 mM phosphate buffer concentration.

At a concentration of 0.1 mM CPC in a 15 mM phosphate buffer, not all the available surface sites are occupied by cationic surfactant, so the time taken for the tin compounds to migrate through the capillary is longer than at higher CPC levels. The migration time is longer, but the resolution is better. At 0.2 mM CPC the migration time is reduced at the expense of reduced resolution. The resolution obtained at this value however is sufficient for a valid separation. These results confirm that 15 mM is the optimum buffer concentration, and indicate that 0.2 mM is the optimum cetylpyridinium chloride concentration.

Fig. [2.15] shows individual electropherograms of the separation of the methyltin chlorides in a 15 mM buffer using 0.1 mM, 0.2 mM and 0.3 mM cetylpyridinium chloride. The decrease in migration time for each species is apparent on moving from 0.1 mM to 0.2 mM CPC concentration, whereas similar migration times are apparent for 0.2 mM and 0.3 mM CPC. The separation efficiencies for each CPC concentration were calculated for both 15 mM and 20 mM phosphate buffer concentration. It can be seen in Fig. [2.16] that the number of theoretical plates is highest for 15 mM buffer concentration. The number of plates drops significantly on moving from 0.1 mM to 0.2 mM, with an even further drop on moving to 0.3 mM CPC concentration. A 15 mM phosphate buffer with 0.2 mM cetylpyridinium chloride at pH 8.25 was ultimately chosen as the optimum run buffer composition.

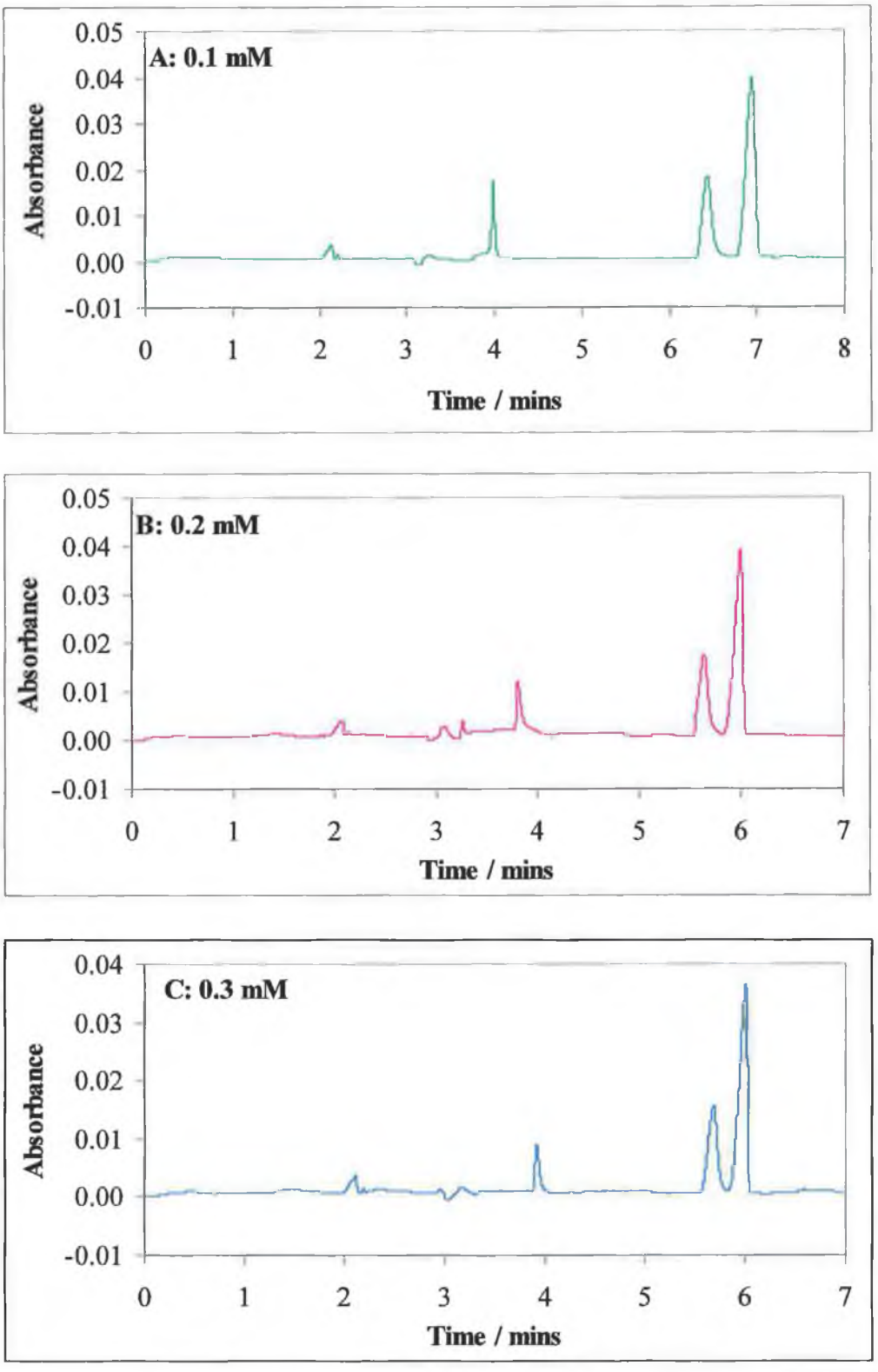
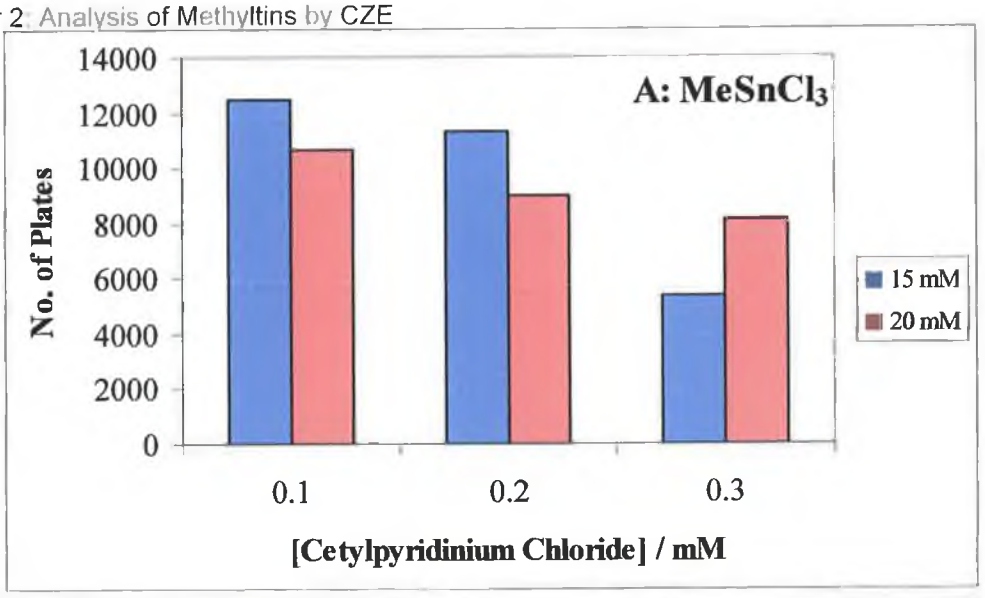
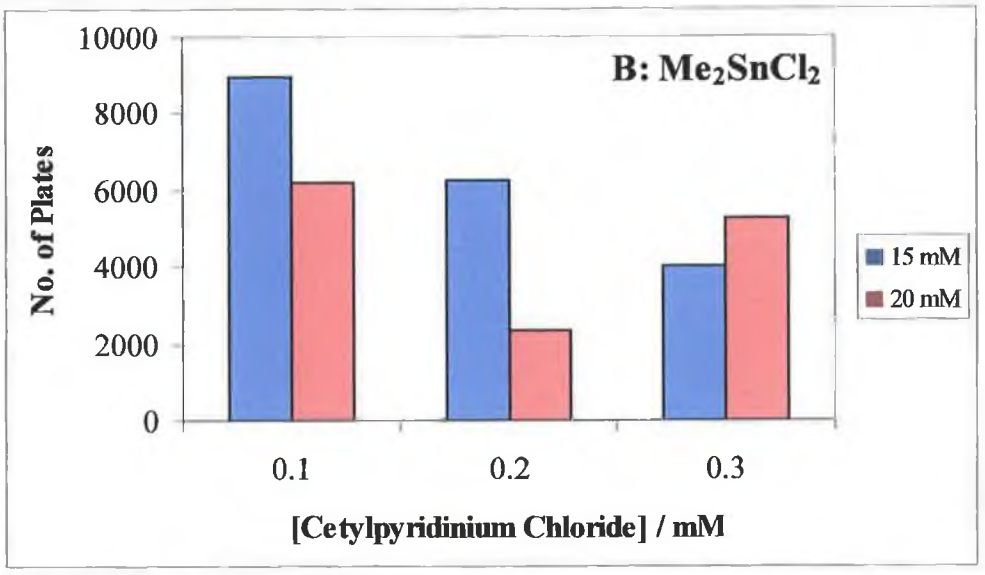


Fig [2.15]. Electropherograms of a 1000 ppm standard mixture of $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl using a) 0.1 mM, b) 0.2 mM and c) 0.3 mM Cetylpyridinium chloride. Separations were carried out using a 15 mM phosphate buffer at pH 8.25.





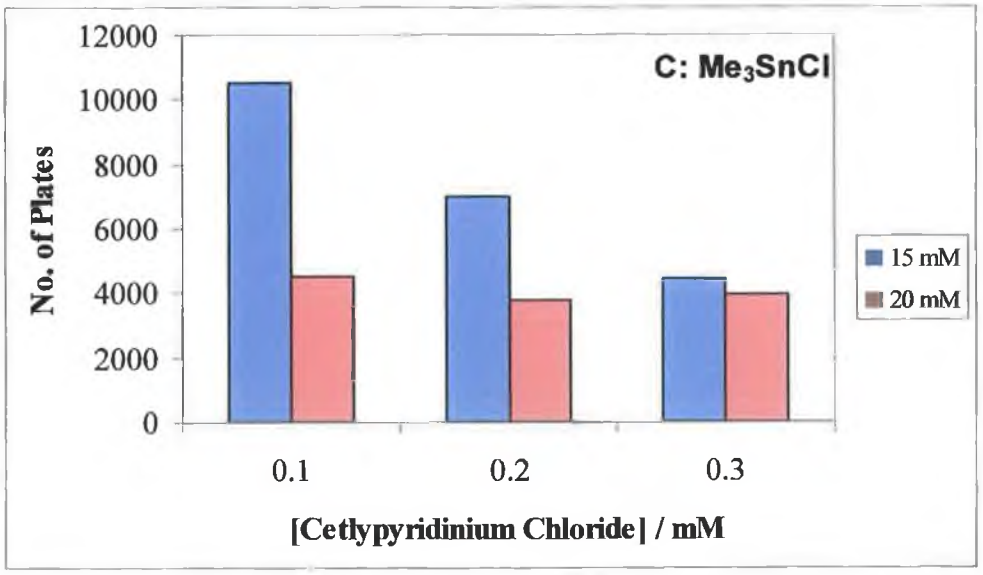


Fig. [2.16]. Comparison of calculated efficiency values for each tin species over a cetylpyridinium chloride concentration range of 0.1 mM to 0.3 mM, for both 15 mM and 20 mM phosphate buffer.

2.2.5.6. Optimisation of Separation Voltage

The electroosmotic flow rate as well as the velocity of a migrating ion, is proportional to the applied voltage used for separation. The conduction of electric current through an electrolytic solution results in the generation of heat because of frictional collisions between mobile ions and buffer molecules. Therefore the maximum voltage is restricted by the ability of the system to dissipate this Joule heat. The higher the applied voltage the more rapid the separation will occur.

By plotting the current against the applied voltage it is possible to determine the maximum applied voltage for a particular electrophoretic system. This is known as an Ohms Law plot. An ideal Ohms Law plot should yield a straight line. This is the case when the heat generated inside the capillary is adequately dissipated. At the highest voltages, the column cannot dissipate the heat generated by the applied power efficiently, and thermal breakdown may occur. Deviations from linearity are indicative of inadequate Joule heat dissipation. At the voltage where linearity is lost the heat dissipation capacity of the system has been exceeded. Operation on the linear portion of the curve will generally yield the highest number of theoretical plates.

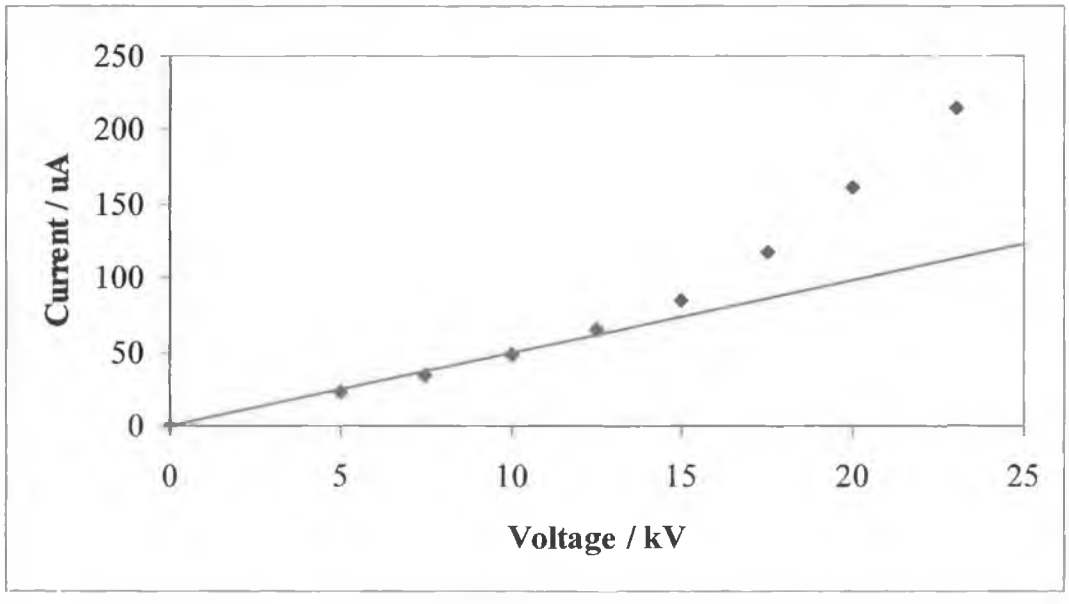


Fig. [2.17]. Ohms Law plot for 15 mM phosphate buffer system containing 0.2 mM cetylpyridinium chloride at pH 8.25.

As can be seen from Fig. [2.17] a deviation from linearity occurs at 15 kV. However at this voltage adequate separation of the methyltin chlorides results, and it was deemed unnecessary to further lower the voltage, as this would lead to longer analysis times. All further separations were thus carried out at 15kV.

2.2.5.7. Optimisation of Injection Time

In selecting an appropriate injection time the aim is to load the maximum amount of sample onto the capillary while not introducing significant band broadening which would lead to reduced efficiency capabilities. It is important to ensure that the sample injection method employed is capable of delivering the sample onto the column efficiently and reproducibly. In using hydrodynamic (pressure) injection a negative pressure (vacuum) is applied to the outlet end of the capillary for a brief period of time, causing sample to enter the end of the capillary. By increasing the length of time during which the pressure is applied then the sample volume is increased.

The influence of injection time on the peak areas of the methyltin chlorides can be seen in Fig [2.18]. Injection times in the range 1 to 10 seconds were investigated, and the variation in peak area for each methyltin was recorded. The increase in peak area is approximately linear for all three tin compounds, with the greatest increase in peak area apparent for Me_3SnCl. The increase in peak area was least for $MeSnCl_3$ as this compound does not absorb appreciably at 190 nm.

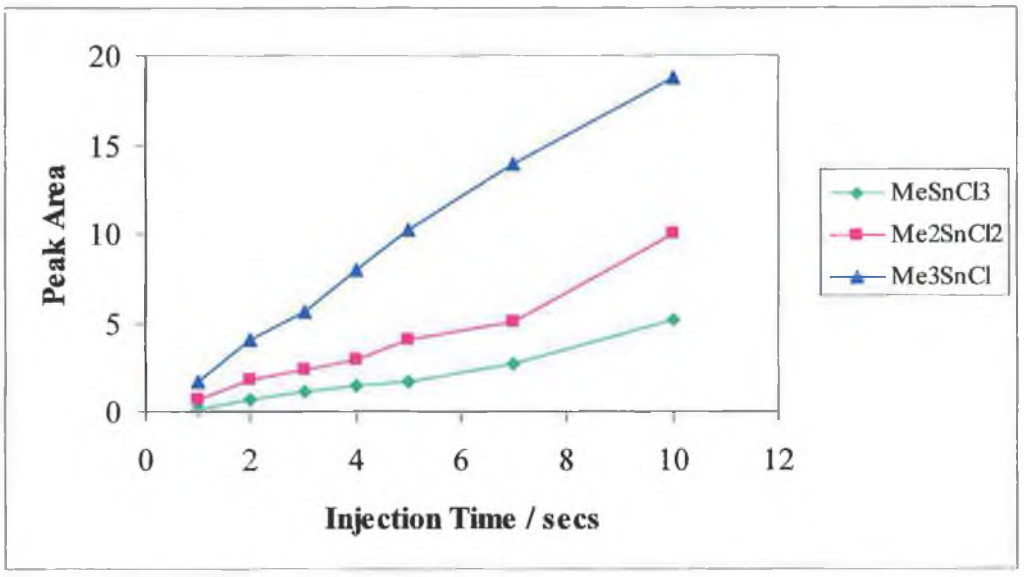


Fig. [2.18]. Variation in peak area for each methyltin compound with increasing injection time.

The effect of increasing injection times on the resolution of Me_2SnCl_2 and Me_3SnCl is illustrated in Table [2.4]. Increasing the injection time from 1 to 2 seconds gave an increase in resolution of approximately 70%. Further increase in injection time did not improve the resolution. As the injection time was increased further the peak areas obtained also increased and eventually they began to overlap at 5 seconds. Therefore the resolution of the two compounds decreased as injection time increased. The optimum injection time was selected to be 2 seconds as this gave maximum resolution between Me_2SnCl_2 and Me_3SnCl. As expected, increasing the length of the injection plug results in the deterioration in the efficiency of the separation. The calculated theoretical plate numbers for each peak at increasing injection times are compared in Fig. [2.19]. For all compounds there is a marked decrease in efficiency between 1 and 5 seconds. The high efficiencies obtained at lower injection times have to be compromised due to the small peak areas that result at these injection times. An injection time of 2 seconds gave reasonable efficiency and adequate peak area.

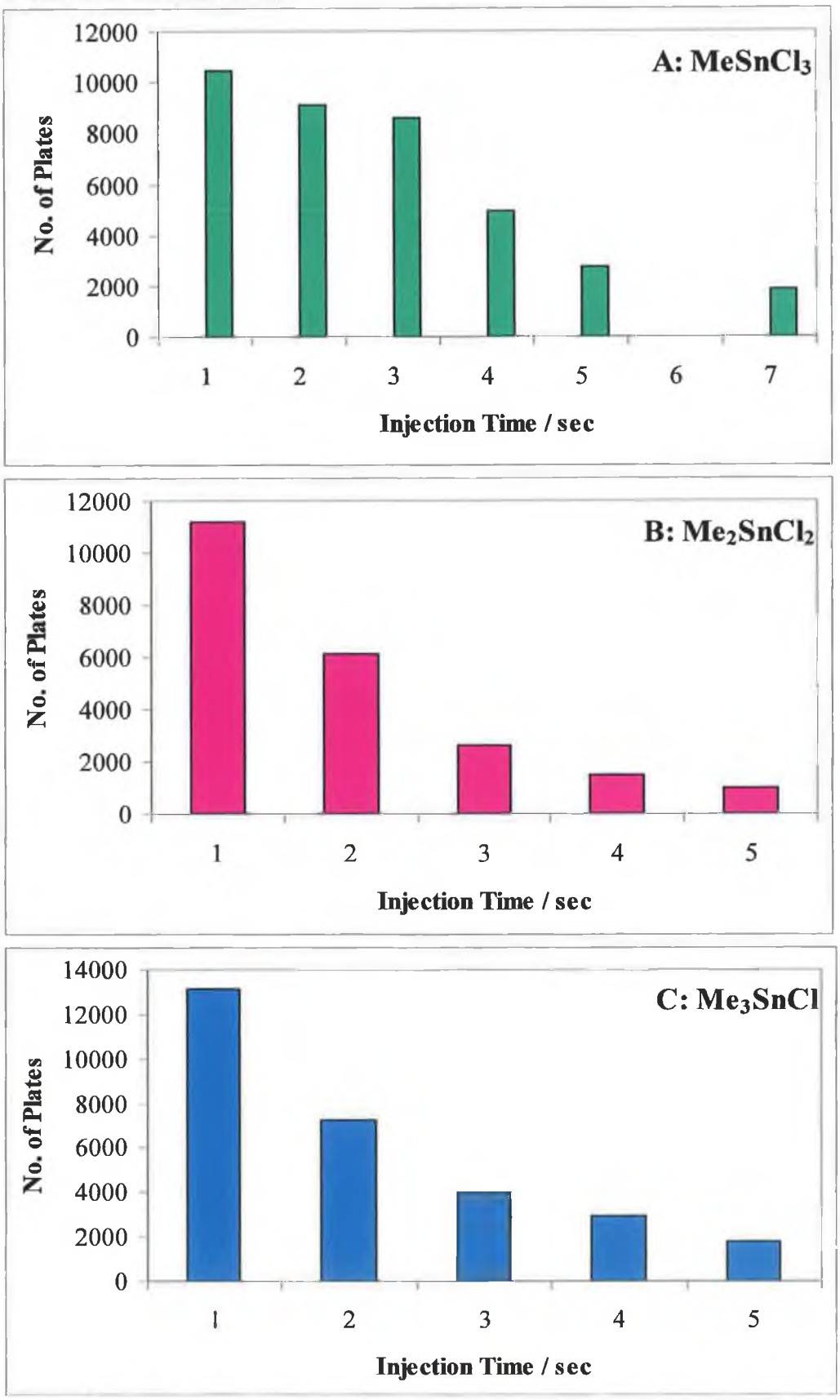


Fig. [2.19]. Variation in calculated efficiency values for each tin species with increasing injection times.

Inj. Time / sec	Resolution
1	2.109
2	3.047
3	1.885
4	1.111
5	1.001

Table [2.4]. Effect of increasing injection time on the resolution of Me_2SnCl_2 and Me_3SnCl.

2.2.5.8. Optimisation of Capillary and Dimensions

In selecting the capillary dimensions several factors must be taken into consideration. Firstly, use of a longer capillary gives improved resolution but also increases migration times and secondly, use of a wider bore capillary leads to a decrease in migration time and increased sensitivity but internal heating problems may occur. In the separation of Me_3SnCl, Me_2SnCl_2 and $MeSnCl_3$, it was decided to use a 100 μM capillary, as this gives reduced migration times although Joule heating was found to occur, as depicted in the Ohms Law plot in Fig.[2.17]. The capillary length was chosen to be 57 cm, and this length was used throughout all optimisation experiments.

2.2.5.9. Optimum Operating Conditions

Temperature	25°C
Buffer pH	8.25
Buffer Concentration	15 mM
Separation Voltage	-15 kV
Injection Time	2 sec
Cetylpyridinium Chloride Concentration	0.2 mM
Column dimensions	100 μm I.D. x 57 cm
Wavelength	190 nm

Table [2.5]. Optimum operating conditions for the separation of $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl.

2.2.6. Validation of Method

Procedure validation took the form of intra- and inter-day assays. The separations were carried out using the conditions listed in Table [2.5]. A plot of the calibration curves obtained for $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl is shown in Fig. [2.20].

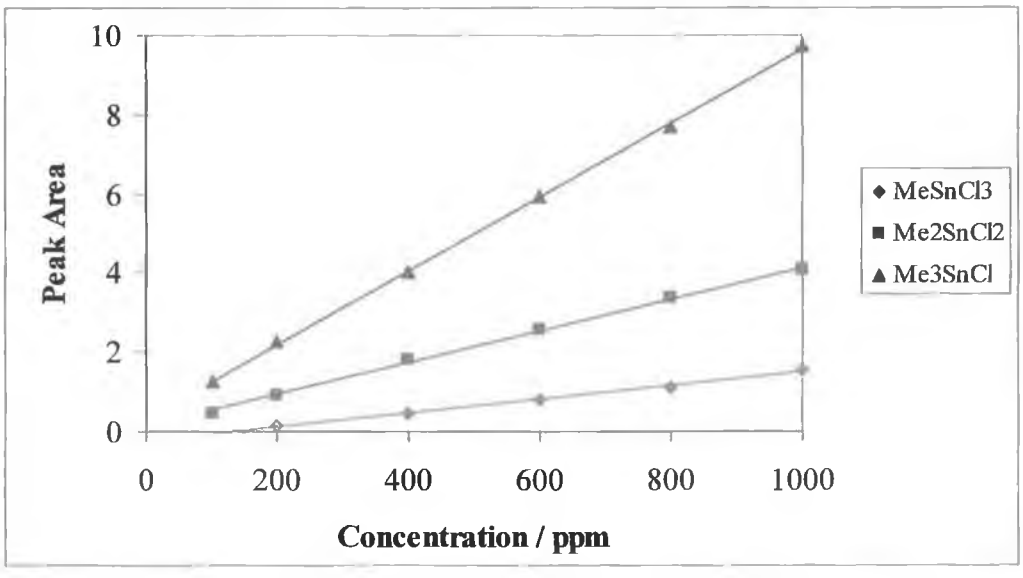


Fig. [2.20]. Calibration graphs of peak area versus concentration for $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl.

All three compounds remain linear over the concentration range examined, with R^2 values consistently greater than 0.996. These values are listed in Table [2.6]. The limit of detection of each species was not examined, but sample stacking could be used to apply the method below the concentration range examined as an alternative method to increase sensitivity.

Compound	R^2
$MeSnCl_3$	0.9968
Me_2SnCl_2	0.9986
Me_3SnCl	0.9996

Table [2.6]. Linear regression values for the calibration curves for $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl.

Statistical validation of the separation of $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl gave within-day variability of less than 3%, which is extremely low. Between day variability was found to be higher but still legitimate for a valid separation. The least variability was found for Me_3SnCl, for which coefficients of variation of 2.10% and 2.41% for within-day and between-day respectively were achieved. Greater fluctuations in the migration time from day to day for Me_2SnCl_2 resulted in higher percentage variability recordings. The values obtained for $MeSnCl_3$ were comparatively higher than those obtained for either of the other two species analyzed.

Compound	Within-day variability (n=6)	Between-day variability (n=6)
$MeSnCl_3$	3.23%	3.42%
Me_2SnCl_2	2.12%	4.51%
Me_3SnCl	2.10%	2.41%

Table [2.7]. Statistical validation for the separation of $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl.

As a result of these findings the separation of $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl is possible using the method described. In terms of linearity and reproducibility the method was found to be valid with R^2 values of greater than 0.99, and coefficients of variability found to be less than 5% in all cases.

2.3. Conclusions

The viability of capillary electrophoresis in the separation of the methyltin chlorides has been demonstrated. The use of the flow modifying agent cetylpyridinium chloride was also established. Previously such cationic surfactants as cetyltrimethyl ammomium bromide (CTAB) have been used, but these led to reproducibility problems. No such problems were found using cetylpyridinium chloride, with coefficients of variability found in the range 2.1-3.2% for within-day analysis, and in the range 2.4-4.5% for between-day analysis. The high efficiency, satisfactory linearity and acceptable reproducibility values determine this method to be feasible in the separation of $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl.

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3. UV Photoassisted Degradation of Methyltin Chlorides in Deuterated Solvents and Investigation of the Transmethylation Reaction of Tetramethyltin with Mercury (II) Chloride with Qualitative Analysis by Nuclear Magnetic Resonance Spectroscopy

3.1. Introduction

In Chapter 1 the various methods of introduction of organotins into the environment were discussed. These consisted primarily of direct introduction through their use as PVC stabilizers and also via agricultural applications. However, it is their fate once they have entered the environment which is of paramount importance. It is clear that a number of environmental interactions occur, which lead to the formation of new organometallic compounds.

The degradation of organotins on UV exposure has been studied and been shown to lead to the sequential removal of organic groups to form less toxic compounds by Blunden et al.[1,2] The nature of the solvents in which these studies have been performed has been shown to have an effect on the nature of the products of the reactions. It is our aim to further study the UV degradation of tetramethyltin and the methyltin chlorides in various solvents in an attempt to understand the mechanism of the reaction.

Organotins in the environment are subject to various other degradation reactions, besides UV degradation. One such type of reaction is chemical cleavage, by either nucleophilic or electrophilic reagents. Transmethylation reactions are commonplace, and trimethyltin species have been shown to transmethylate Pd, and Hg cations, leading to the formation of a dimethyltin species and a monomethyl derivative. These reactions, however, may lead to the formation of more toxic products.

It had previously been established that subjecting methyltin species to UV irradiation could lead to the successive demethylation of these species to form less toxic compounds; the aim here was to determine the degradation pathway and to find the mechanism of the cleavage of the methyl group. It was also clear that a transmethylation reaction was likely to occur between tetramethyltin and mercury (II) chloride, but the ultimate aim was to establish the mechanism of the methyl transfer. One method of determining the mechanism of methyl cleavage is the use of Laser flash photolysis.

3.1.1. UV Degradation

The study of the UV degradation of organotins in aqueous solutions has received considerable attention, and, results seem to suggest a progressive degradation of triorganotin species to di-, mono- and inorganic tin species. Ultraviolet breakdown is one of the most significant modes of degradation of organotins in the environment. The effect of UV light on the degradation of the methyltin chlorides was previously examined by Blunden[3]. He studied the UV degradation of the methyltin chlorides in both carbon tetrachloride and water, and analyzed the reaction by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR).

The photolytic degradation of relatively high concentrations (8.2 g/l) of trimethyltin in water appeared to proceed via the formation of dimethyltin directly to inorganic tin without the formation of monomethyltin. In contrast, photolysis of trimethyltin in carbon tetrachloride, appeared to follow a sequential demethylation to inorganic tin. The half-life of photolysis of trimethyltin in water was found to be 60 h, and the half-lives of photolysis of dimethyltin and monomethyltin in separate experiments in which they were the starting materials were estimated to be 300 h and >300 h respectively. It was suggested that if the same mechanism were operative at concentrations of methyltin species in the ng/l-µg/l range observed in the environment, then the corresponding half-lives of sunlight photolysis would be longer.

As this work provides only limited information on the UV degradation of the methyltin chlorides, it was decided to determine the degradation pathways of tetramethyltin and of trimethyltin chloride in such solvents as chloroform and acetonitrile. It was thought that the use of these solvents could provide a valuable insight into the mechanism of methyl loss/degradation of these tin compounds.

3.1.2. Transmethylation Reactions

Transmethylation reactions between organometals and metal ions in aqueous solutions in biotic and abiotic systems, with and without the presence of sediments have been investigated[4,5]. Such reactions may have significant consequences for the environmental formation and distribution of organometals and the study of these transmethylation reactions is important therefore, in predicting the fate of organometals in the environment.

Transmethylation between organometallic species and aquatic metal ions has been reported to occur in water between Me_3Sn^+ and Hg(II) with Hg(II) as the end acceptor for the methyl group.

$$Me_3Sn^+ \quad + \quad Hg^{2+} \rightarrow \quad Me_2Sn^{2+} \quad + \quad MeHg^+$$

Very little is known about the methylation process involved in the methylation of Hg(II) by methyltin. Howell et al.[6] studied the methylation of Hg(II) by methyltin species, including tetramethyltin, in 0.59 mol dm^{-3} NaCl using ^{199}Hg and ^{119}Sn NMR and polarography to detect reactants and products. NMR confirmed methyl transfer from Me_3Sn^+ to Hg(II) by identifying the Me_2Sn^{2+} and MeHgCl products. In addition polarography demonstrated approx. 85% methyl transfer from Me_3Sn^+ to Hg(II) in two days. Bellama and co-workers[7] measured second-order rate constants for the methylation of Hg(II) by Me_3Sn^+ in different media containing sodium chloride and sodium perchlorate ($NaClO_4$). Cerrati et al.[8] studied the kinetics of the methylation of Hg(II) by Me_3Sn^+, Me_2Sn^{2+} and $MeSn^{3+}$ at low concentrations in 100% seawater and 50% seawater at pH values ranging from 4.5 to 8.0. They found that $MeSn^{3+}$ was the fastest methyl donor and that reaction rates increased as pH increased.

In an attempt to determine the fate of organotin compounds in the environment we have examined the possibility of methyl group transfer from tetramethyltin to mercury(II) chloride and attempted to identify the products of the reaction.

3.1.3. Flash Laser Photolysis

Flash photolysis is a method used for the initiation and study of primary photochemical processes. Light absorption occurs very rapidly (10^{-14} s) but the decay processes subsequent to this absorption cover a much wider range of times from picoseconds to seconds.

When a sudden flash of high intensity light is absorbed by a reactant, a relatively high concentration of excited molecules and/or free radicals are formed, and transient species can be observed by optical absorption or emission spectroscopy. The flash spectroscopy technique involves measurement of a complete absorption spectrum in a whole range of wavelengths recorded at a preset time after the photolysis flash. Flash spectroscopy supplies data on the extent of photochemical reactions as well as spectra of transient intermediates formed after light absorption and data on lifetimes and kinetics of transient intermediates. Flash spectroscopy is used mainly in the study of absorption spectra of simple free radicals in the gas and liquid phases.

The selection of solvent is very important if photochemical reactions are studied in solution. Any solvent used for the study of a photochemical reaction should be spectrally pure and not absorb the incident light. The solvent should also be photochemically stable and should not participate in primary or secondary reactions. Solvent radicals formed from photolysis of the solvent may react with reagents, completely changing the mechanism and kinetics of the observed reactions. Only a few solvents such as paraffinic hydrocarbons and alcohols are unreactive photochemically when irradiated in the 200-700 nm range. Chloroform and tetrahydrofuran are photolysed in this region producing reactive free radicals.

Laser flash photolysis of tetramethyltin in a number of solvents was used to determine if the transmethylation reaction proceeds via the free radical pathway suggested elsewhere[9]. In the Laser flash photolysis experiment, absorbance changes were monitored. Laser

photolysis produced a transient species, whose absorbance was recorded as a function of time.

Initially, I_0, the intensity of monitoring light being transmitted through the solution before the laser flash, was measured. The laser was then fired and the beam passed through the power meter, triggering the oscilloscope, and hit the sample cuvette, producing a transient species. The monitoring beam traversed the region of the cuvette where the laser passed. The oscilloscope recorded values of I_t, the change in voltage with time from the photomultiplier output, corresponding to a change in absorbance with time, at the monitoring wavelength. The trace was stored on an oscilloscope.

Thus, each trace reveals the difference in absorbance at that particular wavelength between the parent compound and whatever intermediate species or reactants were created by the flash.

3.2. Experimental

3.2.1. Reagents

Tetramethyltin was synthesized in the laboratory, and trimethyltin chloride, dimethyltin dichloride, methyltin trichloride and mercury(II) chloride were purchased from Sigma Aldrich. The deuterated solvents, chloroform-d_3, acetonitrile-d_3, dimethylsulfoxide-d_6 (DMSO-d_6), D_2O, and spectroscopic grade solvents chloroform, tetrahydrofuran (THF) and cyclohexane, were also obtained from Sigma Aldrich.

Initially, tetramethyltin was synthesized in a Grignard reaction, from tin (II) chloride and methyl magnesium iodide, using diethylether as solvent[10]. The reaction proceeded quite readily, and isolation of the product and subsequent analysis by ^{1}H NMR spectroscopy revealed a chemical shift for tetramethyltin in chloroform-d_3 of 0.05 ppm. This is consistent with that obtained for an authentic sample and with literature values[11].

3.2.2. Instrumentation

UV irradiation was provided by an Oriel Scientific 100 W supply utilising a mercury arc lamp, and emitting wavelengths above 180 nm. The solutions being irradiated were contained in stoppered quartz NMR tubes – 5 mm – (Sigma Aldrich) which were positioned at 15 cm from the source so as to eliminate any possible thermal effects.

The laser used was a Spectron Laser which contains a Nd:YAG (neodymium-doped yttrium aluminium garnet) crystal. The emitted radiation operates at a fundamental frequency of 1064 nm but conversion to second, third or higher harmonics allowed radiation of other wavelengths to be produced. The selected wavelength was 266 nm. The laser was directed into the sample cuvette by a system of prisms. A power meter was placed before the cuvette to trigger the oscilloscope. The UV-Vis monitoring light source was a Xenon arc lamp used at right angles to the laser beam. A UV filter was placed between the monitoring source and the sample to prevent photolysis of the sample by the

monitoring beam. T he beam passes through the sample cell and is focussed via a circular quartz lens onto the slit of a monochromator. The light detector was a five stage photomultiplier, whose output was connected via a variable-load resistor to the input channel of the oscilloscope. The oscilloscope was in turn interfaced to an Olivetti 286 personal computer.

3.2.3. Spectroscopic Techniques

The proton nuclear magnetic resonance spectroscopic experiments presented here were carried out using a Bruker AC400 spectrometer, equipped with a broad band multinuclear probe and operating at 400 MHz. All the solvents used were deuterated, and contained TMS to which all chemical shifts were related. The ^{1}H NMR spectra were interpreted with the aid of the 1D WIN-NMR program. Products were identified by comparison with authentic samples.

The UV-Vis spectra experiments were carried out on a Shimadzu UV-240 spectrophotometer interfaced with a PC equipped with a Shimadzu UVPC processing program.

3.2.4. Preparation of solutions

3.2.4.1. UV degradation

Approximately 0.1 M solutions of tetramethyltin or trimethyltin chloride were prepared in either chloroform-d_3 or in acetonitrile-d_3. These solutions were placed in quartz NMR tubes and stoppered. The samples were then placed at 15 cm in front of the UV source and irradiated. Proton NMR analysis was performed at regular intervals, and the samples were returned to their position before the lamp. ^{1}H NMR analysis has the advantage of being non-destructive and so the sample remained intact throughout the duration of the experiment. Analysis was discontinued after approx. 100 hours irradiation.

3.2.4.2. Transmethylation Reactions

Each transmethylation reaction was performed in a 5 mm NMR tube. Into each tube was placed approx. 1 ml deuterated solvent (chloroform-d_3), along with the relevant concentrations of tetramethyltin and mercury(II) chloride. ^{1}H NMR analysis was performed on mixing, and at regular time intervals thereafter to determine the time necessary for the reaction to reach equilibrium.

Concentration study

Experiment	Me_4Sn	$HgCl_2$
i)	0.1 M	0.1 M
ii)	0.2 M	0.2 M

Mole ratio study

Experiment		Me_4Sn	$HgCl_2$
i)	1:1	0.2 M	0.2 M
ii)	1:2	0.2 M	0.4 M
iii)	1:3	0.2 M	0.6 M
iv)	1:4	0.2 M	0.8 M

3.2.4.3. Laser flash photolysis

Tetramethyltin samples with an optical density of ~ 2.0 were prepared in degassable cells using various spectroscopic grade solvents, such as chloroform, THF and cyclohexane. The sample was degassed under reduced pressure, using an Ultra High Vacuum and stored under Argon. An initial UV spectrum was recorded. The laser wavelength was set to record at 266 nm and the sample scanned from 350 to 500 nm. All results were recorded on the oscilloscope and on the PC.

3.3. Results

3.3.1. UV Degradation

3.3.1.1. UV Irradiation of Tetramethyltin in Chloroform-d_3

Experimental conditions were set up to allow the qualitative analysis of the products of the UV degradation of tetramethyltin in deuterated chloroform. It was expected that there would be a successive elimination of methyl groups from the tetramethyltin leading to the formation of trimethyltin chloride, dimethyltin dichloride, monomethyltin trichloride and finally inorganic tin. This was somewhat the case found experimentally. The removal of the initial methyl group to form trimethyltin chloride was observed. The formation of trimethyltin chloride was observed, in small quantities after 4 h. Dimethyltin dichloride was then observed as a product of the reaction after approx. 32 h. No further degradation to monomethyltin trichloride was observed.

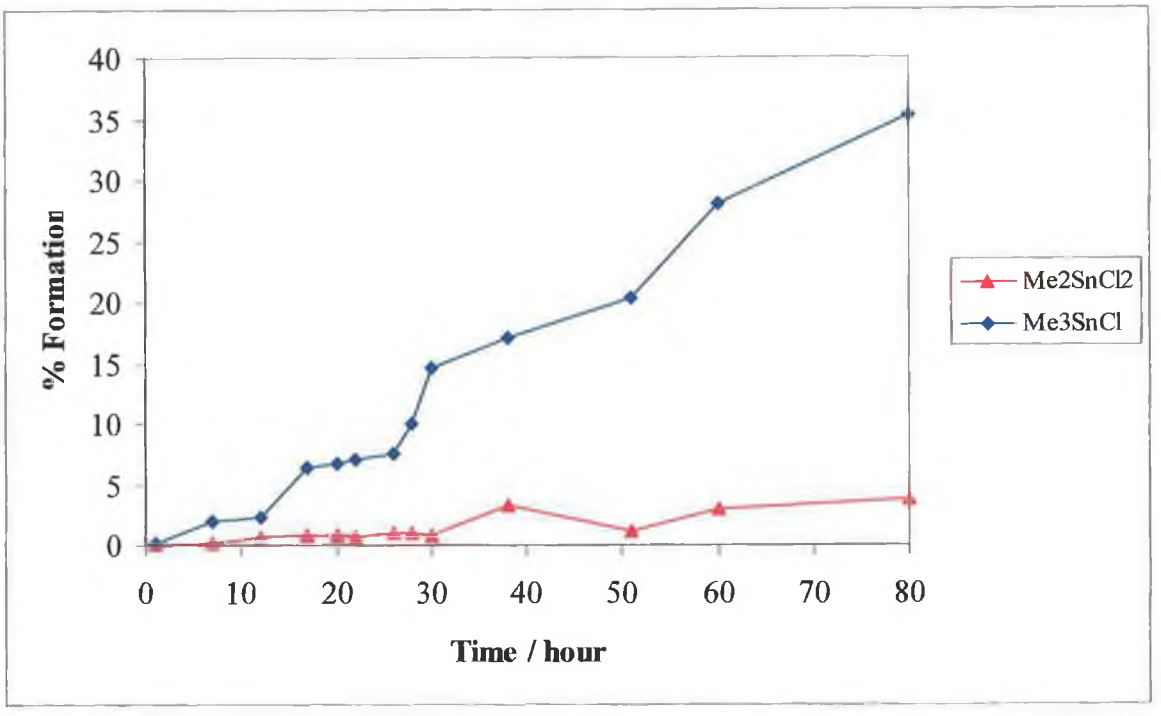

Fig. [3.1]: Formation of Me_3SnCl and Me_2SnCl_2 as degradation products in the UV degradation of tetramethyltin.

When the solutions were irradiated in the quartz NMR tubes and the samples then taken for NMR analysis, no species other than the organotin compounds were detected. During the course of irradiation, the sample volume decreased, and on occasion it was necessary to add further solvent to the tube. It was thought that the formation of methane could be a possible explanation for this. However, methane was not detected with NMR analysis. Blunden[3] also noted the formation of methane in his UV degradation experiments. He irradiated a 0.05 M solution of Me_3SnCl in carbon tetrachloride, which was in a sealed silica NMR tube which was filled virtually to the top. NMR analysis revealed that the major non-tin containing compound was methane ($\delta(^1H)$ 0.23 ppm, Lit 0.23 ppm)[12]. He found that the formation of methane accounted for approximately 50-60% of the methyl groups cleaved from the tin atoms. Significantly smaller amounts (each accounting for about 15% of the methyl groups cleaved) of both methyl chloride ($\delta(^1H)$ 3.01 ppm, Lit 3.06 ppm[12]) and ethane ($\delta(^1H)$ 0.8 ppm, Lit 0.86 ppm[12]) were observed by Blunden in the same experiment.

3.3.1.2. UV Irradiation of Trimethyltin chloride in Chloroform-d_3

The samples here were prepared in the same manner as was used for the UV degradation of tetramethyltin. Analysis was again performed by ^{1}H-NMR spectroscopy. The formation of dimethyltin dichloride was observed, as expected, after 4 hours. No monomethyltin trichloride species was determined.

These results are therefore in agreement with Blunden, in that no methyltin species was observed. It is possible an inorganic tin species was formed but that it would not be detected using ^{1}H-NMR spectroseopy.

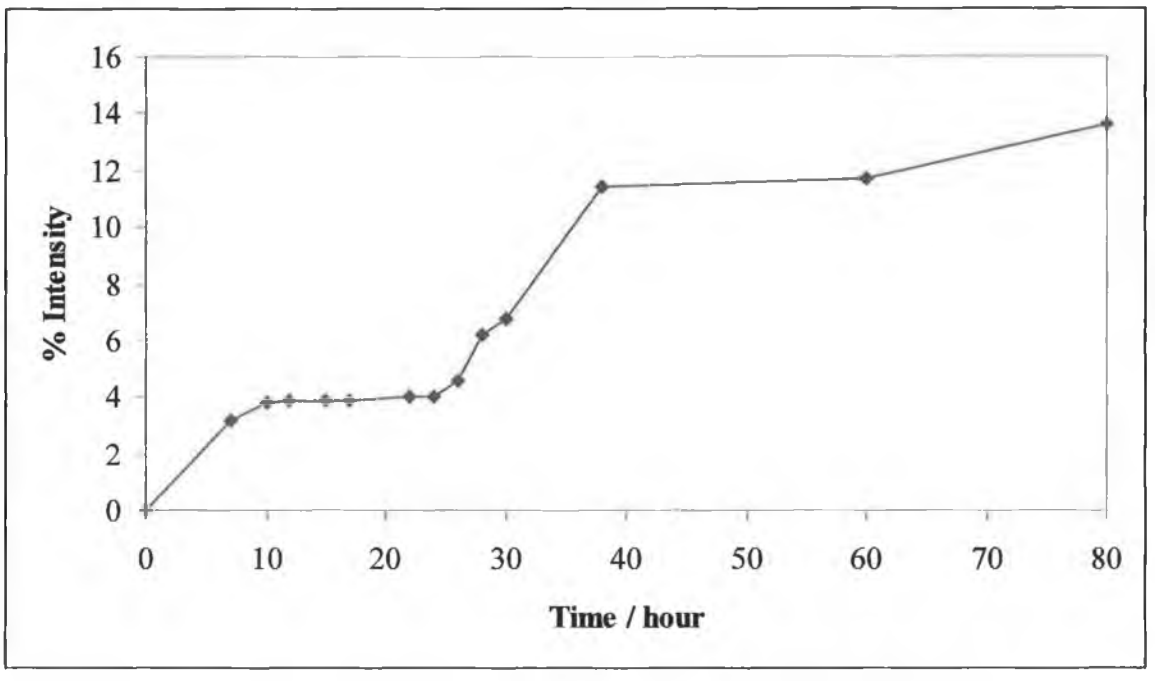

Fig. [3.2]: Formation of Me_2SnCl_2 as a product of the UV degradation of Me_3SnCl.

3.3.1.3. UV Irradiation of Trimethyltin chloride in Acetonitrile-d_3

Here the preparation of samples was the same as before with the exception that deuterated acetonitrile was used as solvent instead of chloroform-d_3. Following the results of the previous experiments it would be expected that again the sequential loss of methyl groups would be observed – at least one methyl group would be expected to be lost resulting in the formation of dimethyltin dichloride. However, this was not the case found experimentally. No dimethyltin dichloride was observed throughout the duration of irradiation (>100 hours). No other products were observed.

3.3.2. Transmethylation Reactions

It is evident from examination of the NMR spectra of the transmethylation reactions between tetramethyltin and mercury(II) chloride that species other than tetramethyltin are present. These products of the transmethylation reaction were identified by comparison of their chemical shifts with those of authentic samples. The results obtained indicate that a methyl group was transferred from tetramethyltin to mercury(II) chloride to form trimethyltin chloride and methylmercury chloride. The amount of methylmercury chloride formed, and therefore the extent of reaction, was increased with an increase in the initial concentrations of the reactants used.

From the concentration experiments performed it was found that the reaction of tetramethyltin with mercury(II) chloride resulted in the formation of trimethyltin chloride and methylmercury chloride as products of the reaction. Increasing the concentrations of both reactants from 0.1 M to 0.2 M led to increased formation of Me_3SnCl and MeHgCl as products of the transmethylation reaction, and reduced levels of tetramethyltin remaining after the reaction, as shown in Table [3.1].

	Reactant Concentration	
	0.1 M	0.2 M
Me_4Sn	100	20
Me_3SnCl	70	100
MeHgCl	30	40

Table [3.1]: Relationship between Tetramethyltin and Mercuric Chloride Concentrations on Transmethylation. % Intensity of the Products of Reaction

Upon varying the relative mole ratio of Me_4Sn:$HgCl_2$ from 1:1 to 1:2, 1:3 and 1:4, it was clear that the transmethylation reaction proceeded to an even greater extent at the higher $HgCl_2$ concentrations. As such, at a 1:1 mole ratio of Me_4Sn:$HgCl_2$, some of the initial Me_4Sn remained after the transmethylation reaction, and Me_3SnCl and MeHgCl are the only products of the reaction. Increasing the reaction ratio to 1: 2, led to trimethyltin chloride being formed as the major product of the reaction, but in this instance, dimethyltin dichloride is also formed, as a product of the reaction. This was also the case for a 1:3 ratio, where the levels of Me_2SnCl_2 formed are higher than at a 1:2 ratio. At a Me_4Sn:$HgCl_2$ mole ratio of 1:4, although trimethyltin chloride is formed as a product of the transmethylation reaction for tetramethyltin and mercury(II) chloride, dimethyltin chloride is the major tin containing product of the reaction.

From Table [3.2] it can be seen that as the levels of $HgCl_2$ were raised there was a decrease in the level of Me_4Sn detected. Increasing the mercury (II) chloride concentration further led to a reduction in the $MeSnCl_3$ levels detected. The formation of dimethyltin dichloride as a product of the transmethylation reaction of tetramethyltin with mercury(II) chloride was observed for all reactions with a mole ratio of Me_4Sn:$HgCl_2$ greater than 1:1, and throughout, the increases in levels of both Me_2SnCl_2 and MeHgCl were apparent. It was thought therefore that the general scheme for the transmethylation reaction was:

$$Me_4Sn + HgCl_2 \rightarrow Me_3SnCl + Me_2SnCl_2 + MeHgCl$$

	Mercury (II) Chloride Concentration			
	1:1	1:2	1:3	1:4
Me_4Sn	20	—	—	—
Me_3SnCl	100	100	100	20
Me_2SnCl_2	—	5	20	100
MeHgCl	40	70	60	80

Table [3.2]: Relationship Between The Relative Concentrations of Tetramethyltin to $HgCl_2$ Concentration. % Intensity of Products of Reaction, Using 0.2 M Me_4Sn.

3.3.3. Laser Flash Photolysis

Table [3.3] shows the results of the Laser flash photolysis of tetramethyltin in a number of solvents. It can be seen that transient formation was detected in all solvents in the 420-440 nm region.

Solvent	K_{obs} range /s	Transient Formation (nm)
Cyclohexane	20,000 - 30,000	440
Chloroform	170,000 - 210,000	420
THF	120,000 - 220,000	420-440

Table [3.3]: Photolysis of Tetramethyltin in Various Solvents.

As mentioned previously, the effect of the solvent used must be considered. An alkane solvent, such as cyclohexane does not itself undergo photolysis in the UV region examined to form reactive radicals, and therefore it allows recombination of the species much faster than other solvents. As a result cyclohexane would be expected to show a much shorter transient lifetime (k_{obs}). Solvents such as THF and chloroform are photolysed in this UV region producing reactive free radicals which would be expected to

temporarily hold on to any radical species formed from the photolysis of tetramethyltin, giving a larger transient lifetime. The results obtained therefore seem to correlate with the hypothesis for a free radical mechanism for the removal of the methyl group from tetramethyltin.

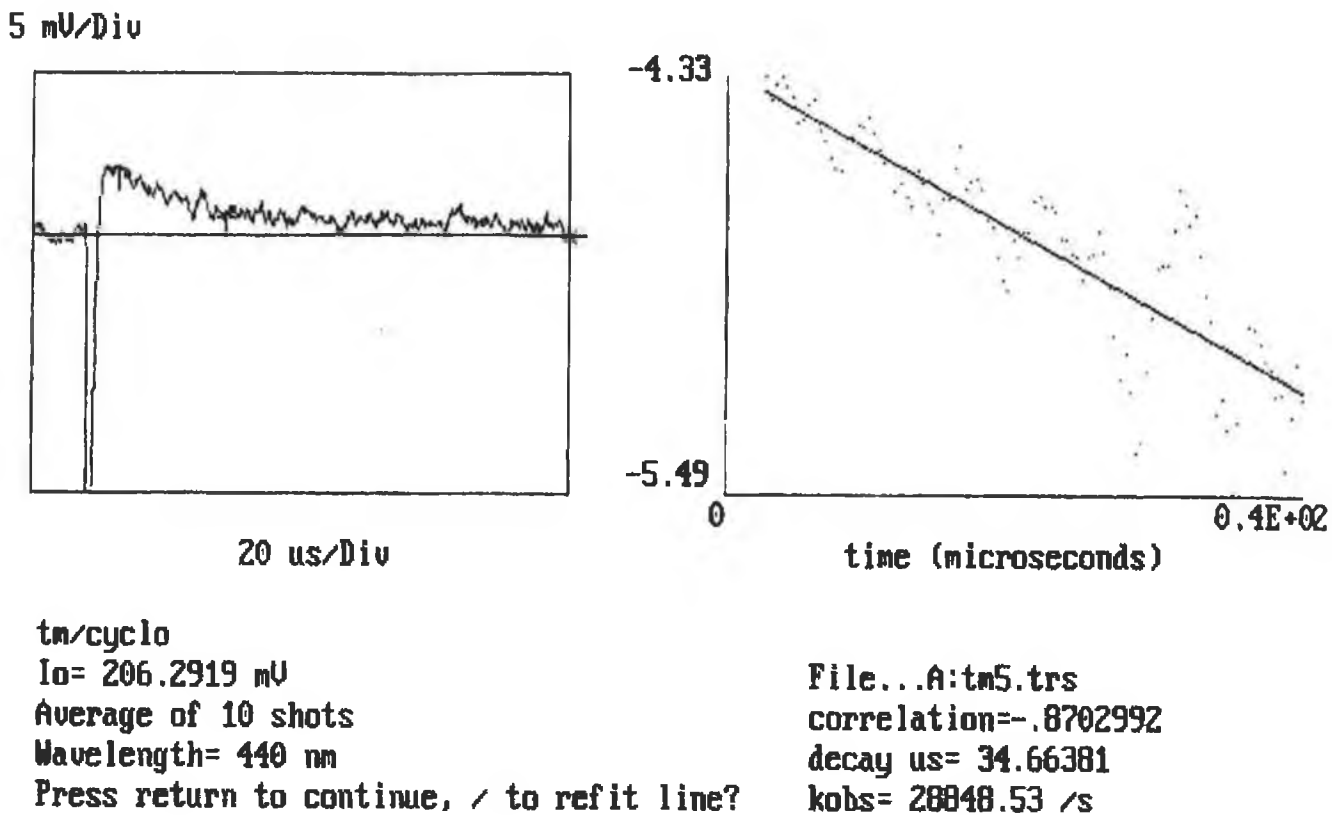

Fig. [3.3]: Laser flash photolysis of tetramethyltin performed in cyclohexane

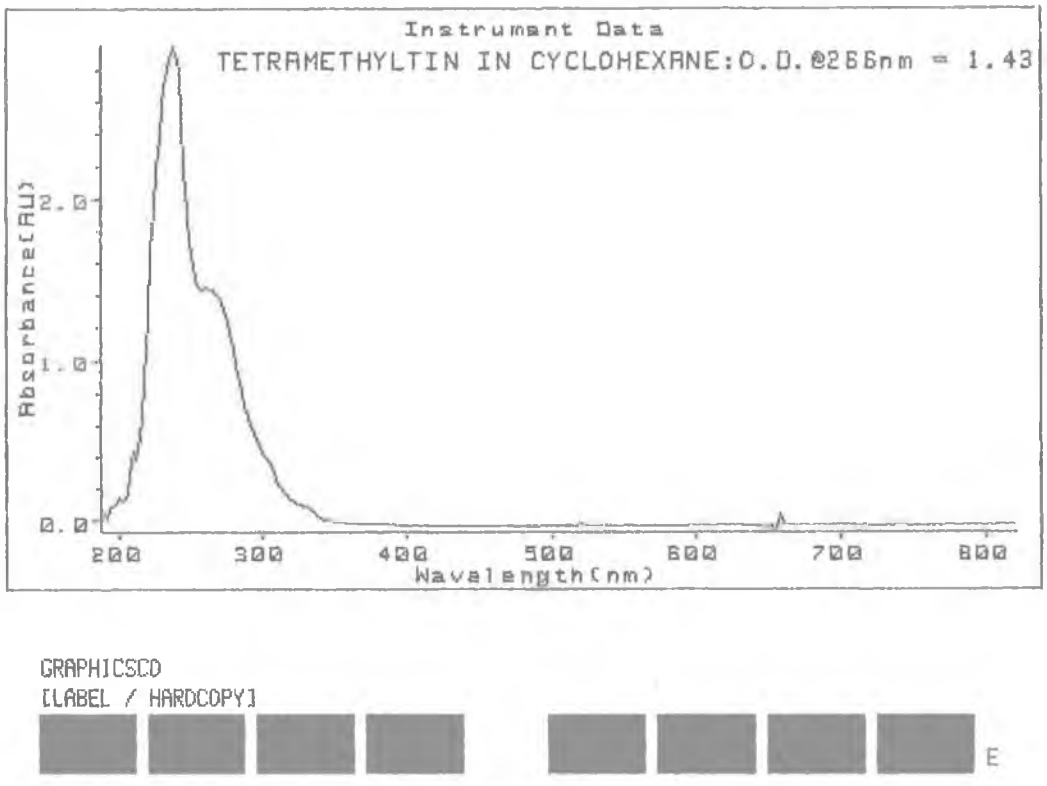

Fig. [3.4]: UV spectrum of tetramethyltin in cyclohexane prior to Laser flash photolysis experiment.

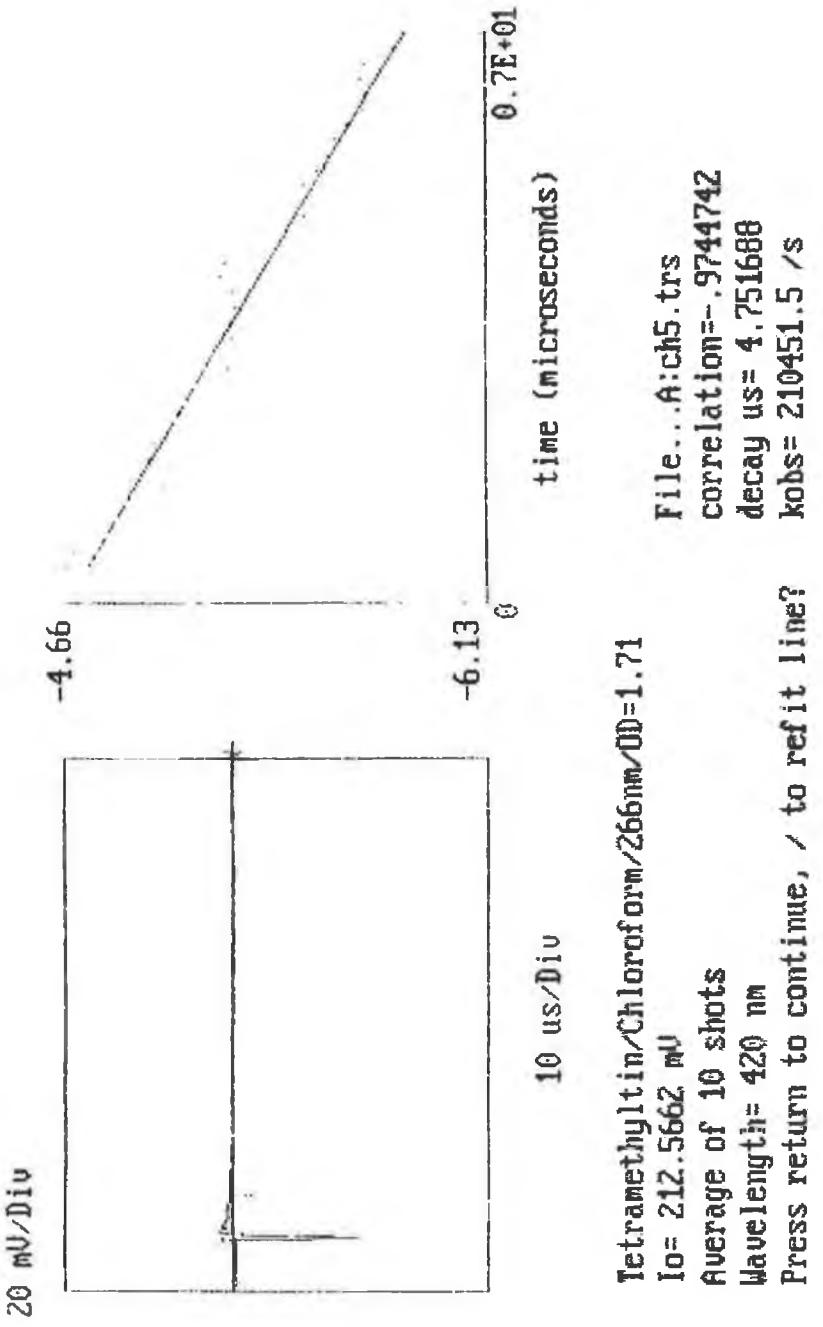

Fig. [3.5]: Laser flash photolysis of tetramethyltin performed in chloroform

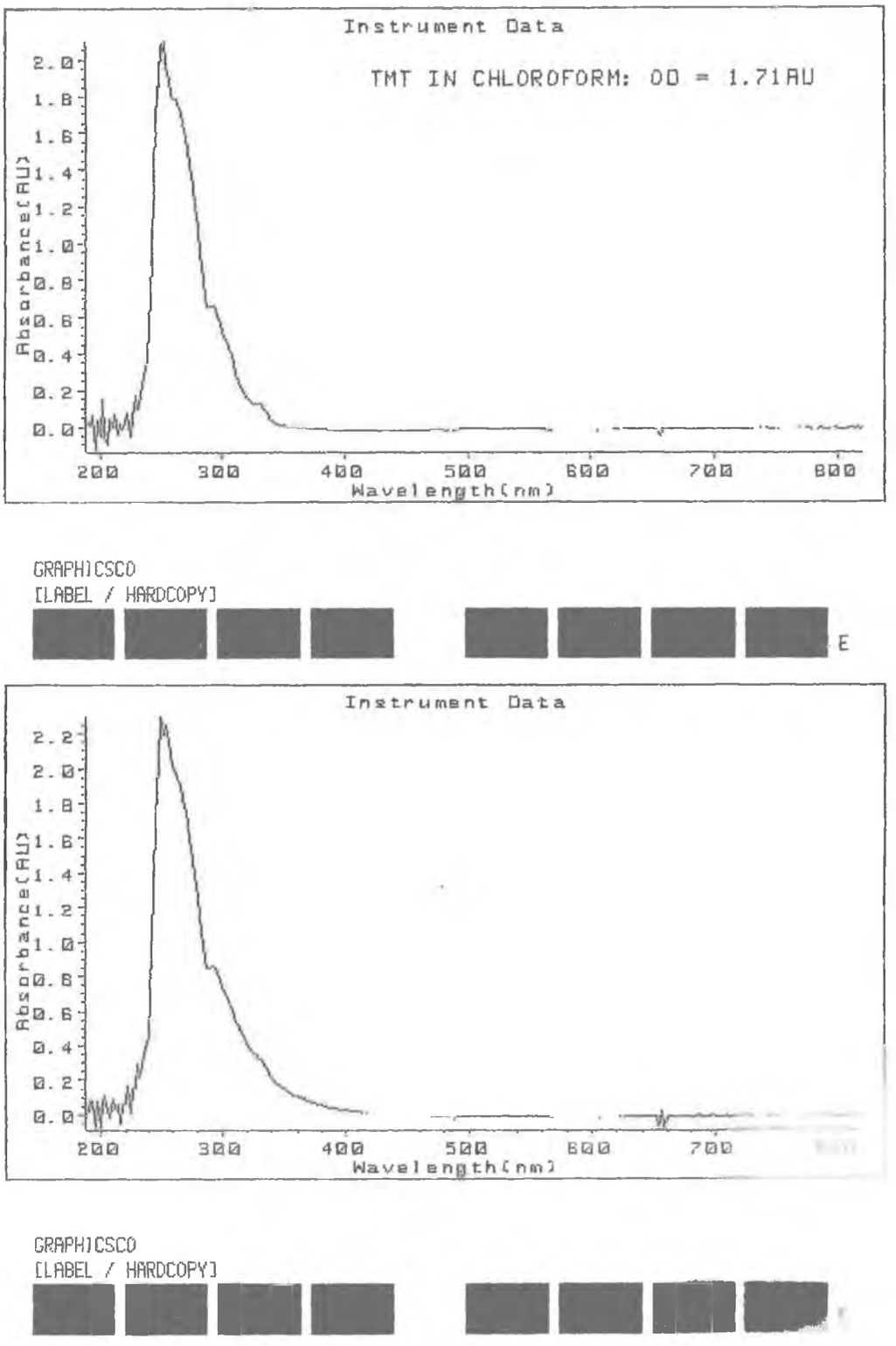

Fig. [3.6]: UV spectrum of tetramethyltin in chloroform prior to and after Laser flash photolysis experiment.

3.4. Discussion

The UV degradation of tetramethyltin and trimethyltin chloride in deuterated chloroform, under laboratory conditions, was shown to lead to the formation of trimethyltin chloride and dimethyltin dichloride as degradation products in the case of tetramethyltin and to the formation of dimethyltin dichloride as the sole degradation product in the UV degradation of trimethyltin chloride. These results appear to be in agreement with those found by Blunden[3]. He also observed the loss of a methyl group in the UV degradation of methyltins in carbon tetrachloride and in water. However, monomethyltin trichloride was not detected in his studies, but rather an inorganic tin species was found to be the final degradation product. During our experiments of the UV degradation of tetramethyltin and trimethyltin chloride in chloroform-d_3, no monomethyl derivative was found. When a similar UV degradation experiment of trimethyltin chloride was performed in deuterated acetonitrile it appeared that the trimethyltin chloride did not undergo a degradation reaction and no degradation products were detected.

It was thought that the reaction of the tin species with light resulted in the formation of a radical methyltin species and a methyl radical species. This radical methyltin species may then combine with solvent molecules, which are split by the intensity of the UV radiation to form the degradation products. From these observations, the following reaction mechanism for the degradation process was proposed, (Scheme [3.1]).

$$Me_4Sn \xrightarrow{h\upsilon} Me_3Sn^{\bullet} + Me^{\bullet}$$

$$CDCl_3 \xrightarrow{h\upsilon} CDCl_2^{\bullet} + Cl^{\bullet}$$

$$Me_3Sn^{\bullet} + Me^{\bullet} \longrightarrow Me_4Sn$$

$$Me_3Sn^{\bullet} + Cl^{\bullet} \longrightarrow Me_3SnCl$$

Overall:

$$Me_4Sn \xrightarrow{h\upsilon} Me_3Sn^{\bullet} + Me^{\bullet} \xrightarrow{CDCl_3} Me_3SnCl + Me^{\bullet} + CDCl_2^{\bullet}$$

Scheme [3.1]: UV Irradiation of tetramethyltin in Chloroform-d_3.

As Me_2SnCl_2 is also formed as a degradation product in the UV degradation of tetramethyltin, the following mechanism is proposed:

$Me_4Sn \rightarrow Me^{\bullet} + Me_3Sn^{\bullet}$

$Me_3Sn^{\bullet} \rightarrow Me^{\bullet} + Me_2Sn:$

The degradation seemed to occur by a stepwise reaction;

$$Me_4Sn \rightarrow Me_3SnCl \rightarrow Me_2SnCl_2$$

Possible side reactions in the UV degradation of tetramethyltin and trimethyltin chloride in deuterated chloroform may include:

$CH_3^{\bullet} + Cl_2^{\bullet} \longrightarrow CH_3CDCl_2$

$CH_3^{\bullet} + CDCl_3 \longrightarrow CH_3Cl + CDCl_2^{\bullet}$

$CH_3^{\bullet} + D^{\bullet} \longrightarrow CH_3D$

$2CDCl_2\bullet \rightarrow C_2D_2Cl_4$

$2Me\bullet \rightarrow C_2H_6$

The possible formation of methane as a degradation product was thought to occur by hydrogen abstraction by a methyl group from an organotin molecule, e.g.:

$$Me\bullet + Me_4Sn \rightarrow \begin{matrix} Me_3Sn \\ | \\ CH_2\bullet \end{matrix} + CH_4$$

No features were seen in any of the NMR spectra to account for the products formed from the organotin radical produced after abstraction of a hydrogen atom. This species

may react with the solvent or possibly dimerise, but in either case, the compound produced must itself undergo fairly rapid breakdown, and thus escape detection.
It should be noted that none of these products were detected using ^{1}H-NMR in these UV experiments.

The ultraviolet degradation of trimethyltin chloride gave further insight into the reaction mechanism. The solvent used had a marked affect on the reaction. Reaction in chloroform, as in Scheme [3.2], led to dimethyltin dichloride as the sole degradation product. However, reaction in acetonitrile did not lead to the formation of dimethyltin dichloride (Scheme [3.3]). No other products were observed. This result is significant as it suggests the cleavage of the tin-carbon bond leads to the formation of radical species, which when chloroform is used leads to the formation of products:

$$Me_3SnCl \xrightarrow{h\upsilon} Me_3Sn^{\bullet} + Cl^{\bullet} \xrightarrow{CDCl_3} Me_2SnCl_2 + CH_3^{\bullet} + CDCl_2^{\bullet}$$

Scheme [3.2]: UV Degradation of Trimethyltin Chloride in Chloroform-d_3.

whereas, when deuterated acetonitrile is used as solvent, it was thought that recombination occurred and the suggested route is:

$$Me_3SnCl \xrightarrow{h\upsilon} Me_3Sn^{\bullet} + Cl^{\bullet} \xrightarrow{CD_3CN} Me_3SnCl$$

Scheme [3.3]: UV degradation of trimethyltin chloride in acetonitrile-d_3.
The intensity of irradiation and the wavelengths used for irradiation must also be considered. It is possible that wavelengths below 235nm were used which lead to carbon-chloride bond cleavage in the solvent, and therefore a percentage of the organotin chlorides may be degrading because of direct substitution of the methyl groups by chlorine radicals from the solvent:

$$Me_3SnCl + Cl^{\bullet} \rightarrow Me_2SnCl_2 + Me^{\bullet}$$

Quantitative analysis with a low limit of detection is required to determine the presence of other species such as methyltin trichloride, which may be present at trace levels, and was not detected by ^{1}H NMR analysis. The levels of methyltin trichloride would be expected to be quite low, as the stepwise reaction has resulted in low levels of dimethyltin dichloride. Further experiments, looking at the UV degradation of dimethyltin dichloride and methyltin trichloride, are required to determine the exact degradation process.

Transmethylation reactions between organometallic species and aquatic metal ions has been reported to occur in water between Me_3Sn^+ and Hg(II), with Hg(II) as the end acceptor for the methyl group. Such reactions may have significant consequences for the environmental formation and distribution of organometals. Brinckman[13] reported that Me_3Sn^+ reacts with mercury (II) chloride in a second order process according to the equation:

$$(CH_3)_3Sn+ \quad + \quad Hg^{2+} \quad \rightarrow \quad (CH_3)_2Sn^{2+} \quad + \quad CH_3Hg^+$$

Under the experimental conditions examined by Brinckman, the $(CH_3)_2Sn^{2+}$ product was found not to react with mercury(II) chloride.

Although it was known that a transmethylation reaction could occur between a methylated tin species and mercury(II) chloride, it was not clear if this reaction would occur for each of the methyltin chlorides, and to what extent the relative concentrations of reactants affected the nature of products formed. Therefore, as a preliminary study, it was decided to perform the transmethylation reaction of tetramethyltin with mercury(II) chloride and to determine the products of the reaction. The effect of increasing the concentration of one reactant relative to the other was subsequently examined. From Brinckman's studies it was expected that a transmethylation reaction would occur between tetramethyltin and mercury (II) chloride leading to the formation of trimethyltin chloride and methylmercury chloride as products. No further reactions were expected to occur, i.e. it was not thought likely that the trimethyltin product would itself undergo a

transmethylation reaction with the mercury(II) chloride to form dimethyltin dichloride and methylmercury chloride.

^{1}H NMR was employed to analyse the various transmethylation reactions as they proceeded. Using NMR numerous species may be verified simultaneously and the relative concentrations of each species may be determined as a function of time. The overall aim of this work was to determine firstly if a transmethylation reaction occurred between tetramethyltin and mercury(II) chloride and secondly, to establish the mechanism of this methyl transfer. As such, qualitative analysis using NMR was sufficient in the early stages of investigation.

By performing the transmethylation reaction of tetramethyltin and mercury(II) chloride it was found that trimethyltin chloride and methylmercury chloride were formed as products of the reaction. Increasing the concentrations of both reactants led to greater relative levels of products being formed with less of the reactants remaining on analysis. this suggests that the reaction is more likely to go to completion (i.e. all reactants being used up) at higher concentrations of tetramethyltin and mercury(II) chloride.

From these results, it was decided to vary the concentration of one reactant relative to the other and determine the effect and consequences on the products of the reaction. Therefore the concentration of $HgCl_2$ reacted was increased to twice, three and finally four times that of tetramethyltin. The effect of these actions was quite remarkable. Increasing to a mole ratio of Me_4Sn:$HgCl_2$ of 1:2 led to the formation of dimethyltin dichloride as a product of the reaction. This was quite a significant result as it suggests either

i. Two methyl groups are removed from the tetramethyltin simultaneously, or
ii. The trimethyltin chloride product formed on the reaction of tetramethyltin with mercury(II) chloride itself reacts with the excess mercury (II) chloride to form dimethyltin dichloride.

Increasing the mole ratios further to 1:3 and 1:4 led to a significant increase in the levels of dimethyltin dichloride being formed with a concurrent decrease in the levels of trimethyltin chloride detected. In all cases an increase in the levels of methylmercury chloride was found. This would therefore suggest that it is a case of the trimethyltin chloride reacting with the mercury (II) chloride to form dimethyltin dichloride and methylmercury chloride. This result seems to differ from the results found by Brinckman[13]. He indicated that a secondary reaction would not occur involving the products of the transmethylation reaction of trimethyltin chloride and mercury (II) chloride.

Experimentally, at mole ratios of $Me_4Sn:HgCl_2$ of 1:2 or greater the transmethylation reaction was thought to proceed according to the following equations:

$$Me_4Sn + HgCl_2 \rightarrow Me_3SnCl + MeHgCl$$
$$Me_3SnCl + HgCl_2 \rightarrow Me_2SnCl_2 + MeHgCl$$

This series of experiments have shown that the transformation of methyl groups from one species to another is possible for those species used in our experiments. It has been demonstrated that a transmethylation reaction occurs between tetramethyltin and mercury(II) chloride resulting in the formation of trimethyltin chloride and methylmercury chloride when the reaction is performed at a 1:1 mole ratio of $Me_4Sn:HgCl_2$. At higher mole ratios, dimethyltin dichloride is also formed as a product of the reaction. The amount of transmethylation may depend on conditions such as temperature and light intensity, which has not been investigated. However, there does seem to be a functional relationship between the concentration of the relevant species. The mechanism of the methyl transfer has not, as yet, been ascertained.

In the UV degradation of tetramethyltin in chloroform-d_3, it was thought that the loss of the methyl group to form trimethyltin chloride was the result of a free radical mechanism. It was therefore thought that the methyl transfer in the transmethylation reaction of

tetramethyltin and mercury(II) chloride would also be by a free radical mechanism. One way to investigate this is by use of Laser flash photolysis.

In the flash laser photolytic experiments carried out in this work a transient species was formed which absorbed radiation at 420-440 nm. The time taken for this transient to decay back to the parent compound was recorded and termed its lifetime. The effect of the solvents used on transient formation was then examined. Initially spectroscopic grade cyclohexane was used as solvent. Cyclohexane is known not to be affected by radiation at the wavelengths examined. The lifetime of the transient produced was recorded. Other solvents such as tetrahydrofuran and chloroform are themselves photolysed in this region and form reactive radicals which may then combine with the new transient species, thus the time taken for the transient to return to its previous state will be longer, thus giving a longer lifetime result.

These results indicate that the photochemical loss of a methyl group from tetramethyltin would occur via the formation of a free radical. This applies to the UV degradation of tetramethyltin and also to the transmethylation reaction with mercury (II) chloride. Both of these processes, it would appear, happen via the loss of a methyl group from the original methyltin species.

3.5. References

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4. The Behaviour of the Methyltin Chlorides in the Presence of Inorganic Mercury (II): Transalkylation of Mercury Species and their analysis by CZE

4.1. Introduction

The existence of organometallic species in the environment has been attributed to a number of factors including the decay of species by dealkylation thus leading to the formation of other organometallic species. Chemical decay of various species independently of bacterial activity may also occur. Another possibility is their direct entry into the environment by man i.e. through agricultural applications etc. Organometallic compounds may be formed in the environment via the process of biomethylation. All of these methods of introduction have been discussed in chapter 1. The fate of organometallic compounds in the environment is a major concern, and therefore speciation of trace elements is important because of its impact on the environment. Information on speciation is vital in ecotoxicological studies of the natural production of organic forms of elements in the environment, of the fate of organometallic pollutants and of transformations of organometallics via biotic processes in biogeochemical pathways.

However, the behaviour of each of these organometallic compounds with each other is not greatly understood, and it is thought that the abiotic combination of some organometallic species already present in the environment with other metallic species could lead to the formation of new organometallic compounds. These transmethylation reactions are known for some organometallic species.

Transmethylation reactions between organometallic species and aquatic metal ions has been reported to occur in water between Me_3Sn^+ and Hg(II) with Hg(II) as the end acceptor for the methyl group.

In principle these transmethylation reactions may involve the transfer of a carbonium ion, alkylation by a carbanion or a radical reaction. A partial clarification of this type of formation of organometallic species is the aim of our experiments. In an attempt to probe the pathways and fate of organometallic species in the environment the feasibility of

methyl transfer from the methyl derivatives of tin to inorganic mercury have been examined in this study. Therefore, solutions of trimethyltin chloride, dimethyltin dichloride and methyltin trichloride were mixed with inorganic mercury (II). The solutions were analyzed for the nature and quantity of all organometallic compounds present, for the duration of the transmethylation reaction.

The analysis of these organometallic compounds was performed using the capillary electrophoresis system developed for the analysis of methyltin compounds, which was described in Chapter 2. This system was found to be applicable in the analysis of inorganic mercury compounds and of methylmercury compounds.

The detection and quantification of the products of the transmethylation reaction of the various methyltin compounds with inorganic mercury was the primary aim of this work. Subsequent to this, the determination of the order of the reaction, and the rate constants for the transmethylation reaction of each methyltin compound with inorganic mercury were important. If possible an exploration of the mechanism of the reaction would also be effected.

The first study in the area of transmethylation reactions was performed in 1974 by Jewett and Brinckman[1]. They found that, in water, the demethylation of Me_3Sn^+ was a facile process resulting in the formation of $MeHg^+$ from Hg(II). They then extended the scope of their study to include other potential methylators and acceptors. They reported a second order process for the transmethylation reaction.

Chau et al.[2] also studied the transmethylation reactions of organometals and metal ions in aqueous solutions in biotic and abiotic systems, with and without sediment. They found that alkyllead compounds can transfer their alkyl groups to Sn(II) and Sn(IV) ions to form various methyltin compounds in biotic and abiotic systems. The presence of sediment enhanced the transmethylation reactions. It was found that methyltin compounds did not transfer their methyl groups to Pb(II). Methylarsenic acids were

found to transfer their methyl groups to Sn(II) and Sn(IV) in an abiotic system, but not in a biotic system containing sediment.

Cerrati et al.[3] have studied the kinetics of model reactions for the abiotic methylation of mercury (II) by mono-, di-, and tri-methyltin in sea water. Their kinetic studies of reactions between mercury (II) and methyltin compounds under pseudo first order conditions in seawater show that relative rates of methylmercury formation under the same conditions were: monomethyltin>trimethyltin>dimethyltin.

In a recent study, Rosenkranz et al.[4] have shown that four different mercury species were formed by the abiotic reaction of inorganic mercury with different organolead and organoarsenic compounds. They found that the transfer of one or two alkyl groups to inorganic mercury was possible under specified conditions of buffer and pH. The rate of the transalkylation reaction was found to be dependent on the pH.

The transfer of methyl groups from one species to another has therefore been shown to occur under various experimental conditions. However, few researchers have reported kinetics data for the reaction. Bellama et al.[5] measured second order rate constants of approx. 13-37 $dm^3\ mol^{-1}\ h^{-1}$ for methylation of Hg(II) by Me_3Sn^+ in different media containing sodium chloride (NaCl) and sodium perchlorate ($NaClO_4$), but not in seawater.

Howell et al.[6] studied the methylation of Hg(II) by Methyltin compounds in solution, to which NaCl had been added. Me_2Sn^{2+} and MeHgCl were identified as products of the reaction. About 85% methyl transfer from Me_3Sn^+ to Hg(II) in two days was reported. The authors did not report any kinetics data.

Thus, it was deemed necessary to perform a complete study of the transmethylation reaction of three methyltin compounds with $HgCl_2$. This comprised reacting known species and identification of the products of the reaction. A study of the effect of pH on the reaction was also necessary, as the pH has been shown to affect the number of methyl

groups transferred in the reaction, and thus the products formed. A careful study of the kinetics of the methylation of Hg(II) by Me_3SnCl, Me_2SnCl_2 and $MeSnCl_3$ was also necessary. These model reactions were carried out in buffer solution without the addition of NaCl to the reaction mixture. Further studies in which the methyltin species was bound to a solid support were also carried out and these shall be discussed in Chapter 5. An attempt was endeavoured to determine the mechanism of the transmethylation reaction for each species investigated.

In order to determine the rate constants for the reactions being studied it was first necessary to determine the order of the reaction.

For a first order reaction $A \rightarrow P$

$$-da/dt = ka$$

Integrating gives

$$Ln(A_o/A) = kt$$

where Ao is the concentration of A at time t=0. This equation is valid for like reactants (A+A), or for stoichiometrically equivalent reactants (A+B) with equal initial concentrations.

Therefore, for a first order reaction a simple plot of Ln (A_o/A) vs. t, which yields a straight line of slope k, is sufficient to determine the order of the reaction.

When the initial molar concentrations are not the same , or when the reactants are not stoichiometrically equivalent (A+2B), more complex expressions are required.

$$A + B \rightarrow Products$$

$$da/dt = -kab$$

Integrating gives

$$Kt = 1/(B_0-A_0)\ln(A_0B/\ B_0A)$$

$$2A + B \rightarrow \text{Products}$$

$$da/dt = -ka^2b$$

Integrating gives

$$Kt = 2/(2B_0-A_0)(1/A - 1/A_0) = \text{etc}$$

If reactant or product concentration are measured as a function of time, the data should fit one of these expressions, provided the order is integral.

If it is second order we plot $(1/A-1/A_0)$ against time and expect to get a straight line, etc.

It is important to follow the reaction for a time which is long enough to make sure which line is straight, and which is curved, especially bearing in mind that experimental scatter of data usually produces cases that are not very clear cut. A reaction that is followed to 90% completion usually gives one unambiguous linear plot.

However if the reaction is not first order then the situation is much more complicated. A common way of simplifying the situation is to manipulate the reaction conditions so that pseudo-first-order kinetics are followed.

The half-life method is another method which may be used to determine the order of a reaction. The half-life of a reaction $t_{1/2}$ is the time the reaction takes to reach half completion, i.e. for 50% of the reactant(s) to be consumed.

For a first order reaction

$$t_{1/2} = (1/k)\ln 2,$$

and the half-life is independent of concentration. $t_{1/2}$ may be evaluated from a single kinetic run, where the times taken for the concentration to fall from A_0 to $A_0/2$ ($t_{1/2}$), from A_0 to $A_0/4$ ($t_{3/4}$),etc. are measured. Since the half-life is independent of initial

concentration, the time taken for the reactants concentration to fall from A_0 to $A_0/2$ ($t_{1/2}$) is equal to the time taken to fall from $A_0/2$ to $A_0/4$ ($t_{3/4}$- $t_{1/2}$), etc.

For a second order reaction,

$$t_{1/2} = 1/kA_0,$$

and the half life increases linearly with the reciprocal of the initial concentration. Thus for a single kinetic run, $t_{1/2}/(t_{3/4}- t_{1/2})$ is equal to 0.5, in contrast to the behaviour shown for a first order reaction.

All methylation reactions were run under conditions of pseudo-first-order kinetics with a minimum of a 2-fold molar excess of $HgCl_2$ over the methyltin species. This means that all the reagents except one, is present in such great excess that its concentration does not change significantly during the course of the reaction – in this case $HgCl_2$. From the data obtained from each transmethylation reaction decay plots were prepared, and plots to determine *k*, the pseudo-first-order rate constant, were also prepared. From the constant *k*, it is then possible to determine k_2 the second order rate constant for the reaction. A comparison of these k_2 values was made, firstly with other transmethylation reactions of the same methyltin compound, in which either the initial methyltin concentration was altered or in which the initial mercury (II) chloride concentration was altered. Secondly, a comparison of the second-order-rate constants of the various methyltin species was examined in order to reveal the order in which the methyl group is most easily removed.

4.2. Experimental

4.2.1. Instrumentation

4.2.1.1. CZE Apparatus and Reagents

The CZE apparatus used in all these experiments was as described in Chapter 2. The optimum operating conditions are outlined in Table 4.1, and were used throughout. The rinsing procedure used was 0.5 min high pressure wash with NaOH, followed by 1 min wash with runbuffer prior to each run. Mono-, di- and trimethyltin chlorides were obtained from Sigma Aldrich. Mercury (II) chloride and dimethylmercury were also obtained from Sigma Aldrich. All stock solutions were prepared in Milli-Q water, and reaction mixtures prepared in phosphate buffer solution. The phosphate buffer used was as described in Chapter 2.

Optimum conditions for CZE analysis

Temperature	25°C
Buffer pH	8.25
Buffer Concentration	15 mM
Separation Voltage	-15 kV
Injection Time	2 sec
Cetylpyridinium Chloride	0.2 mM
Column dimensions	100 μm i.d. x 57 cm
Wavelength	190 nm

Table [4.1]: Optimum CZE operating conditions for the separation of $MeSnCl_3$, Me_2SnCl_2 and Me_3SnCl.

4.2.2. Formation of Solutions

All stock methyltin solutions were prepared in Milli-Q water. Initially 5 x 10^{-2} M stock standard solutions of trimethyltin chloride, dimethyltin dichloride and methyltin trichloride were prepared. From each of these solutions, a series of dilutions was performed to obtain the final working range of standard solutions (0.5 x 10^{-3} -5 x 10^{-3} M). Reasonably high reactant concentrations were used in all experiments to demonstrate the occurrence of the transmethylation reaction and to aid in the analysis and identification of both reactants and products in their analysis by CZE. Fresh buffer solutions were prepared daily.

In the case of trimethyltin chloride, for each concentration of trimethyltin chloride three different experiments were performed with different concentrations of inorganic mercury. These corresponded to a 1:2, 1:3 and 1:4 mole concentration ratio of Me_3SnCl:$HgCl_2$.

From the 5 x 10^{-2} M stock solution, dilutions were made to prepare further stock solutions of 4.5 x 10^{-3}, 9 x 10^{-3}, 1.8 x 10^{-2}, 2.7 x 10^{-2}, 3.6 x 10^{-2} and 4.5 x 10^{-2} M. 0.5 ml of each stock was placed in a vial and each solution diluted to 4.5 ml with runbuffer, to give final concentrations in the range 0.5 - 5 x 10^{-3} M Me_3SnCl.

Accordingly, each solution was reacted with the appropriate concentration of mercury chloride, to give 1:2, 1:3 and 1:4 mole ratios, i.e. a 0.5 x 10^{-3} M solution of Me_3SnCl was reacted with 1.0 x 10^{-3} M, 1.5 x 10^{-3} M and 2.0 x 10^{-3} M solutions of $HgCl_2$ and so on. All solutions were analyzed immediately on mixing and analysis was performed directly on the reaction mixture, i.e. no sampling was necessary. Since the sample volumes in CZE are so small, the final solution was deemed to be of the same overall composition as the original sample. i.e. the volumes removed from the bulk were so small as to be insignificant.

(A) Me_3SnCl

Me_3SnCl / 10^{-3} M	$HgCl_2$/ 10^{-3} M		
	1:2	**1:3**	**1:4**
0.5	1.0	1.5	2.0
1.0	2.0	3.0	4.0
2.0	4.0	6.0	8.0
3.0	6.0	9.0	12.0
4.0	8.0	12.0	16.0
5.0	10.0	15.0	20.0

(B) Me_2SnCl_2

Me_2SnCl_2/ 10^{-3} M	$HgCl_2$ / 10^{-3} M		
	1:2	**1:3**	**1:4**
0.45	0.9	1.35	1.8
0.9	1.8	2.7	3.6
1.8	3.6	5.4	7.2
2.7	5.4	8.1	10.8
3.6	7.2	10.8	14.4
4.5	9.0	13.5	18.0

(C) $MeSnCl_3$

$MeSnCl_3$/ 10^{-3} M	$HgCl_2$ / 10^{-3} M		
	1:2	**1:3**	**1:4**
0.4165	0.833	1.25	1.66
0.833	1.66	2.5	3.33
1.66	3.33	5.0	6.66
2.5	5.0	7.5	10.0
3.33	6.66	10.0	13.3
4.165	8.33	12.5	16.6

Table [4.2]: Solutions prepared for the transmethylation reaction of (A) Me_3SnCl, (B) Me_2SnCl_2 and (C) $MeSnCl_3$ with $HgCl_2$.

4.3. Results

4.3.1. Transformation of methyl groups from Trimethyltin species

Experimental conditions were designed to mechanistically follow these successive transformations, which occur when Me_3SnCl is reacted with $HgCl_2$. The initial concentrations of all reactants were recorded and their concentrations at various time intervals was monitored. The formation of products was also recorded and their concentrations monitored with time.

The results of the transalkylation reaction of trimethyltin chloride with inorganic mercury are shown in the time decay plot in Fig. [4.1]. The % transformed was calculated by recording the initial $HgCl_2$ peak area and the peak area which remained after a specified time period had elapsed. These were the initial and final peak area values, respectively.

$$\% \text{ transformed} = \frac{(\text{initial peak area} - \text{final peak area})}{\text{initial peak area}} \times 100$$

Figs. [4.1] (a), (b) and (c) show the % transformation of $HgCl_2$ in the transmethylation reaction of Me_3SnCl with $HgCl_2$. Fig. [4.1] (a) shows the % transformed for a 1:2 mole ratio of Me_3SnCl:$HgCl_2$ at various Me_3SnCl concentrations. The % transformed stabilized at ~ 80% and increasing the Me_3SnCl concentration beyond $2x10^{-3}$ M did not lead to any further increase in the % transformed.

Similarly, Fig. [4.1] (b) shows the % transformation for a 1:3 mole ratio at various Me_3SnCl concentrations. In this instance the % transformed also increases to ~ 80% and again an increase in Me_3SnCl concentration beyond $2x10^{-3}$ M leads to no further increase in the % transformed.

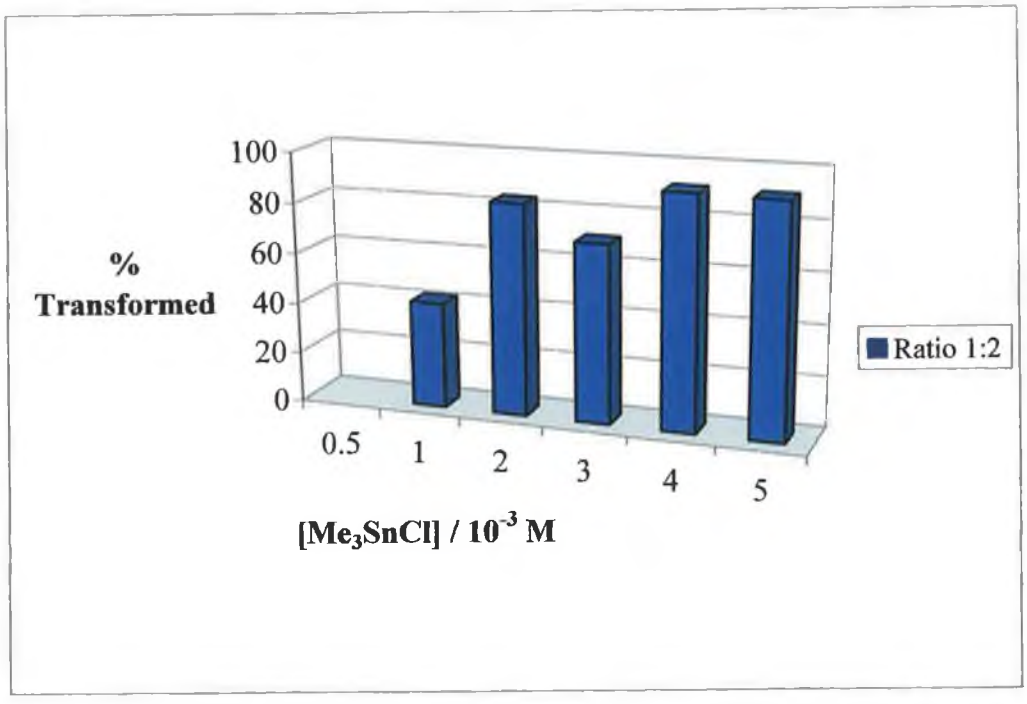


Fig. [4.1] (a): Variation in % $HgCl_2$ transformed for trimethyltin chloride at a $Me_3SnCl:HgCl_2$ ratio of 1:2.

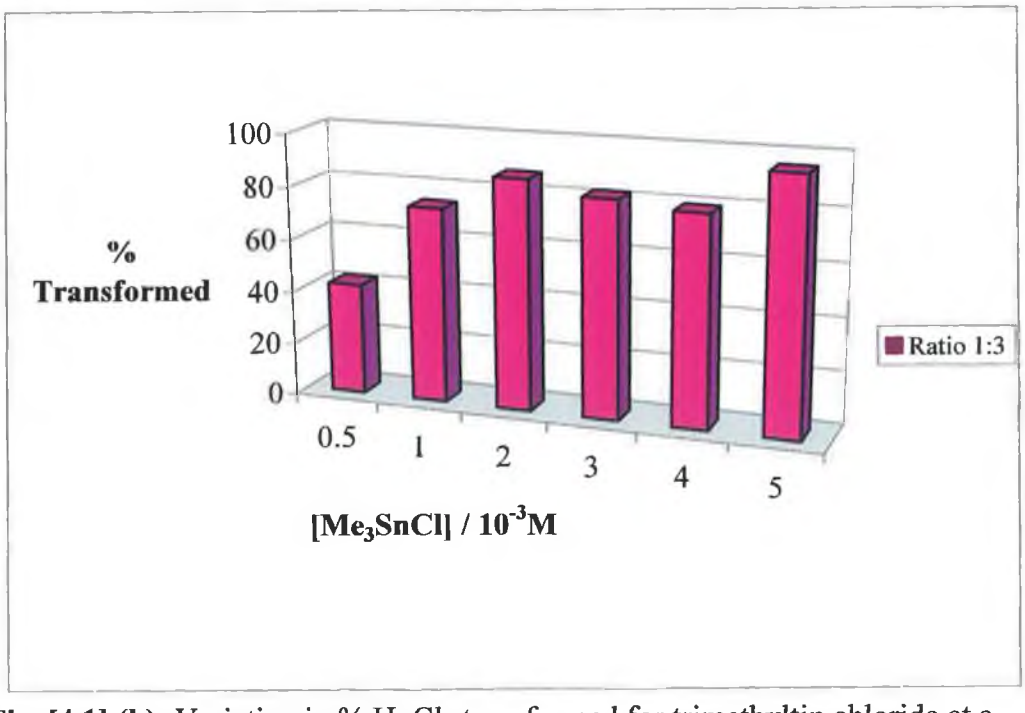


Fig. [4.1] (b): Variation in % $HgCl_2$ transformed for trimethyltin chloride at a $Me_3SnCl:HgCl_2$ ratio of 1:3.

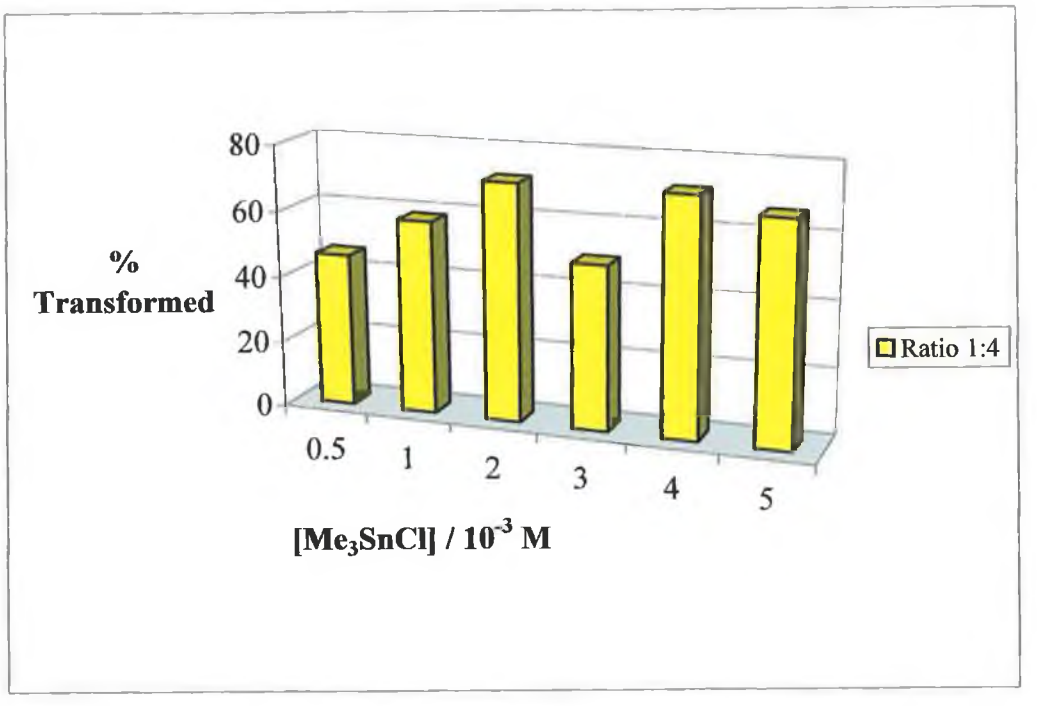


Fig. [4.1] (c): Variation in % $HgCl_2$ transformed for trimethyltin chloride at a Me_3SnCl:$HgCl_2$ ratio of 1:4.

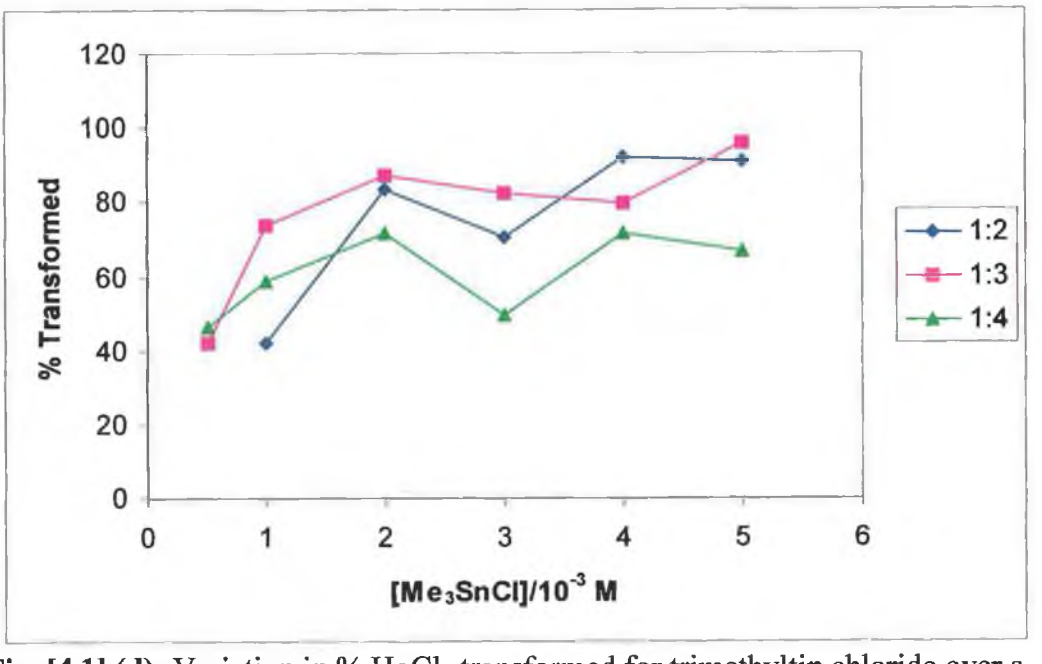


Fig. [4.1] (d): Variation in % $HgCl_2$ transformed for trimethyltin chloride over a Me_3SnCl concentration of 0.5 to 5 x 10^{-3} M at Me_3SnCl:$HgCl_2$ ratios of 1:2, 1:3 and 1:4.

Interestingly, for a 1:4 mole ratio of $Me_3SnCl:HgCl_2$ the % transformed shows a maximum of about 70%, as shown in Fig. [4.1] (c). Again, the maximum % transformation occurs at $2x10^{-3}$ M Me_3SnCl and beyond.

Fig. 4.1 (d) shows a graph which is a comparison of the three different mole ratios examined, illustrates the increase in % transformation up to $2x10^{-3}$ M and the subsequent plateaus seen. The % $HgCl_2$ transformed was greatest at the $Me_3SnCl:HgCl_2$ ratio of 1:3.

It is clear that transfer of at least one methyl group from the trimethyltin chloride is possible and happens under the conditions mentioned. The transfer of the methyl group leads to the formation of methylmercury chloride and dimethyltin dichloride as products. When the mercury salt is in "excess" then both trimethyltin chloride and dimethyltin dichloride transfer methyl groups to the inorganic mercury to form methylmercury chloride and methyltin trichloride.

Fig. [4.2] shows the individual electropherograms superimposed for a 1:3 mole ratio reaction of trimethyltin chloride and mercury chloride. It is clear that as time progresses there is a significant decrease in the levels of Me_3SnCl and $HgCl_2$ present in solution. There is also an apparent increase in the levels of dimethyltin dichloride and methylmercury chloride. A subsequent decrease in the concentrations of dimethyltin dichloride may also be noted which was possibly due to its further reaction with mercury chloride.

The dependence of the concentrations of reactants and products with time was also examined. A calibration curve was prepared for each of the reactants. No calibration curve was obtained for either methylmercury chloride or for dimethylmercury, both of which were suspected products (or intermediates) in the transmethylation reactions examined. The calibration curves obtained for trimethyltin chloride, dimethyltin dichloride, methyltin dichloride and mercury chloride are shown in Figs. [4.3] – [4.6].

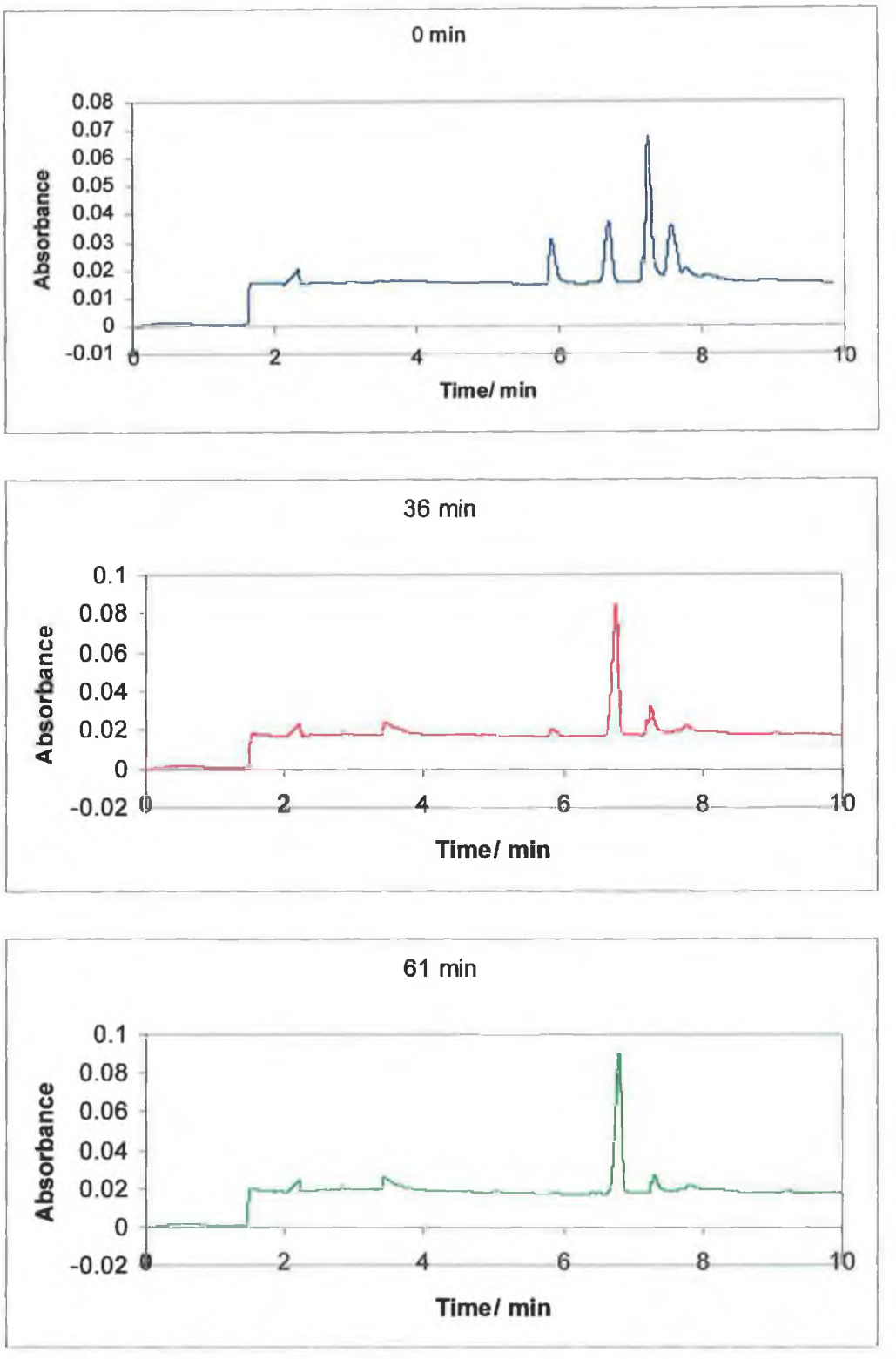


Fig. [4.2]: Electropherograms showing the transmethylation reaction of 3.0 x 10^{-3} M Me_3SnCl with a Me_3SnCl:$HgCl_2$ ratio of 1:3. The figure shows the levels of both reactants and products present in the system at (i) 0 min; (ii) 36 min and (iii) 61 min.

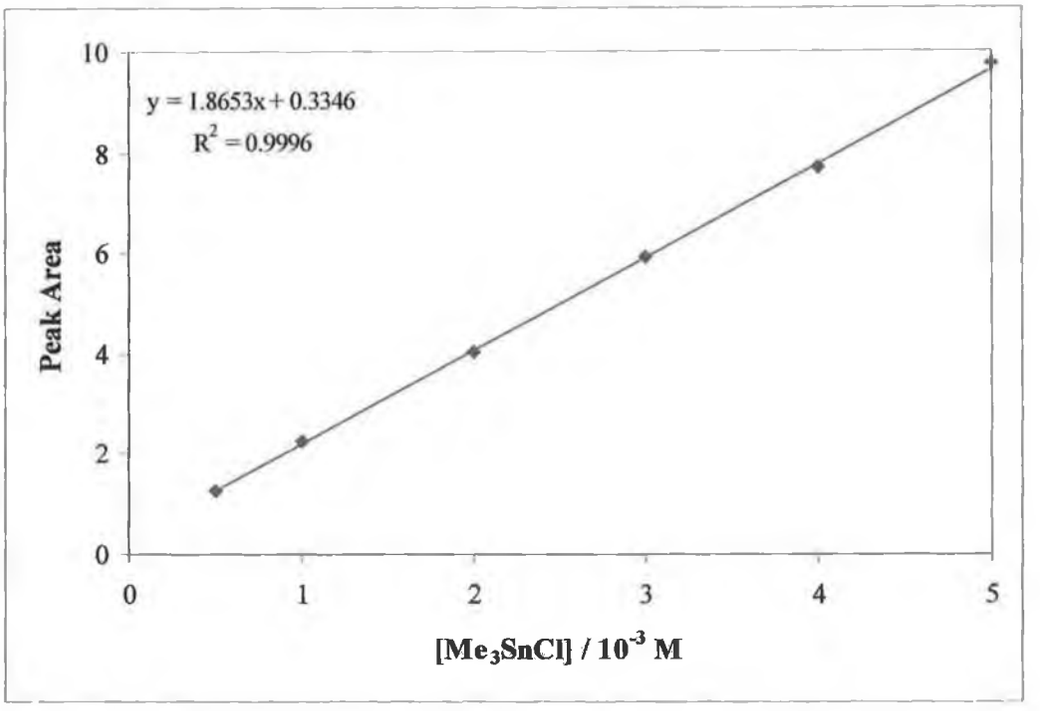


Fig. [4.3]: Trimethyltin chloride calibration curve.

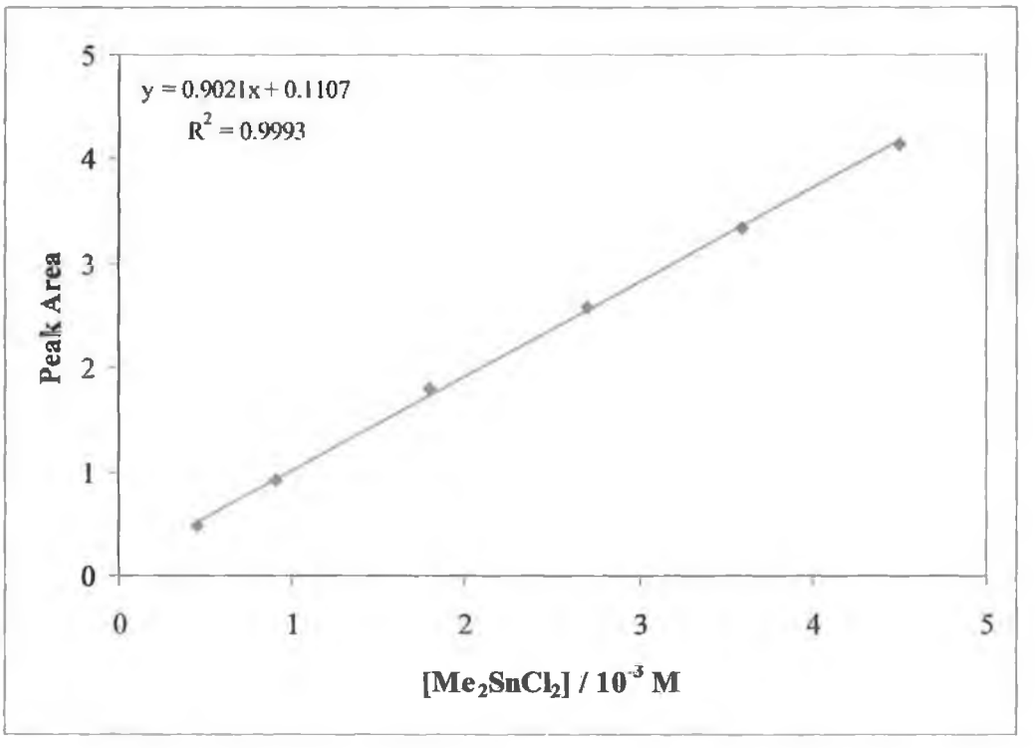


Fig. [4.4]: Dimethyltin dichloride calibration curve.

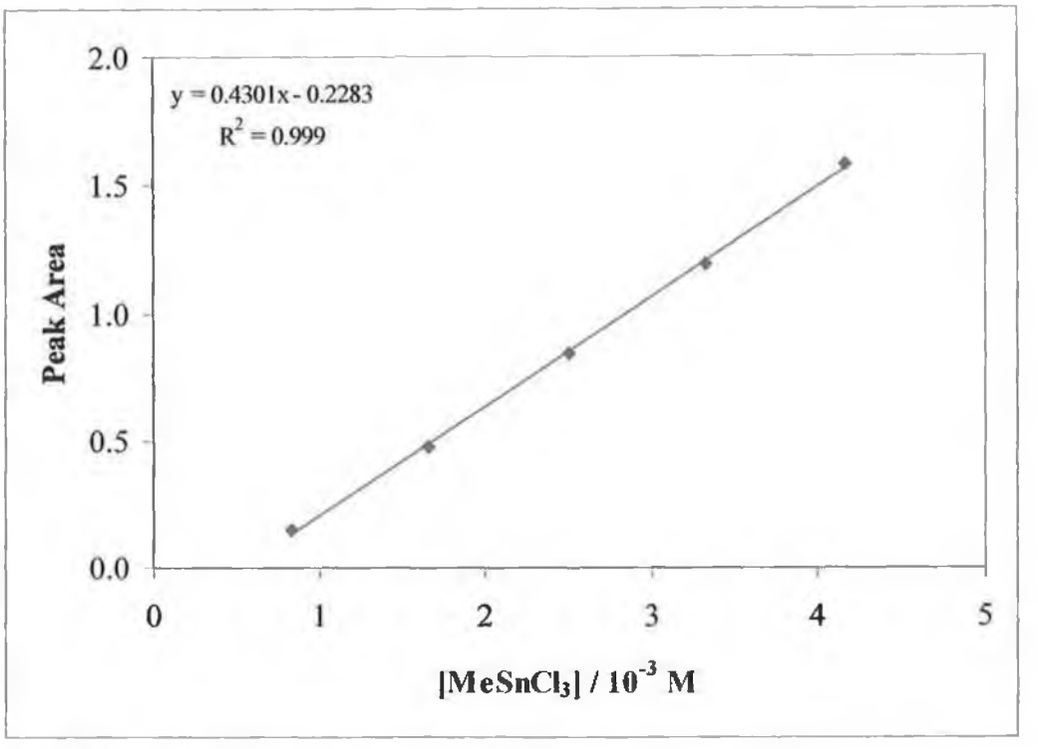


Fig. [4.5]: Methyltin trichloride calibration curve

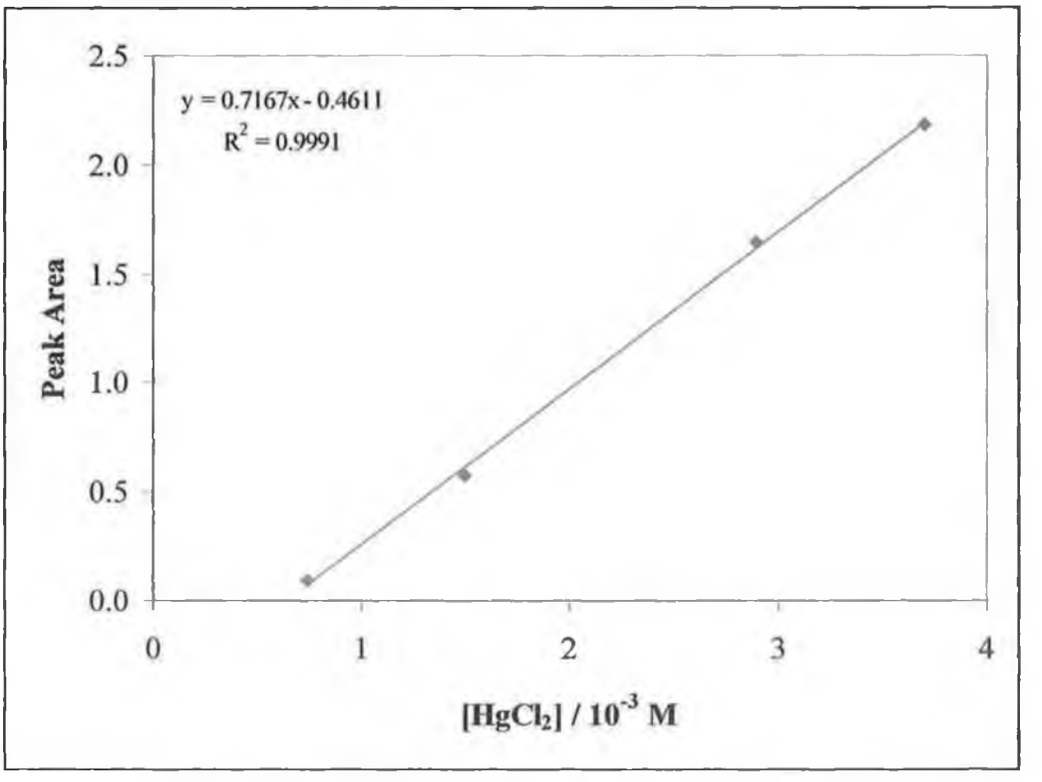


Fig. [4.6]: Mercury (II) chloride calibration curve

Each calibration curve was prepared from the analysis of each compound in triplicate. Average values were obtained for peak area, and these were plotted against concentration for each compound. The maximum signal was obtained for Me_3SnCl with the lowest signal being recorded for $MeSnCl_3$.

It can be seen from Figs. [4.7] – [4.9] that the transmethylation reaction proceeds very rapidly, with both reactants having been used up in the initial stages of the reaction. The rate at which dimethyltin dichloride is formed is dependent on the initial concentration of trimethyltin chloride.

Fig. [4.7] shows the time dependence curves for the transmethylation reaction of two concentrations of Me_3SnCl at a 1:2 mole ratio of $Me_3SnCl:HgCl_2$. In Fig. [4.7] (a) the decrease in both reactants, Me_3SnCl and $HgCl_2$, is apparent, as is the increase in concentrations of both Me_2SnCl_2 and, more notably, MeHgCl, the products of the reaction. However, looking at the reaction of 5×10^{-3} M Me_3SnCl with a 1:2 ratio of $HgCl_2$, it can be seen firstly that the rate of consumption of both reactants decrease more rapidly than was the case in Fig. [4.7] (a), the mercury chloride being totally consumed within 70 mins. Therefore no further increase in MeHgCl, the product of the reaction, was seen. Secondly, although Me_2SnCl_2 is formed as a product of the transmethylation reaction of Me_3SnCl and $HgCl_2$, the concentration is seen to initially increase, up to a maximum at 10 mins and subsequently decreases. Coincident with this decrease in Me_2SnCl_2 concentration is an increase in concentration of $MeSnCl_3$, suggesting that the dimethyltin dichloride formed during the reaction of Me_3SnCl with $HgCl_2$, is also reacting with the excess $HgCl_2$ to form $MeSnCl_3$ and MeHgCl.

For the 1×10^{-3} M reaction, the concentration of MeHgCl increases for the entire duration of the reaction whereas for the 5×10^{-3} M reaction the concentration levels off and is even seen to decrease after approx. 50 mins. Looking at both these figures, it is apparent that the higher the Me_3SnCl concentration the faster the reaction seems to proceed. A levelling off happens at ~ 20 mins.

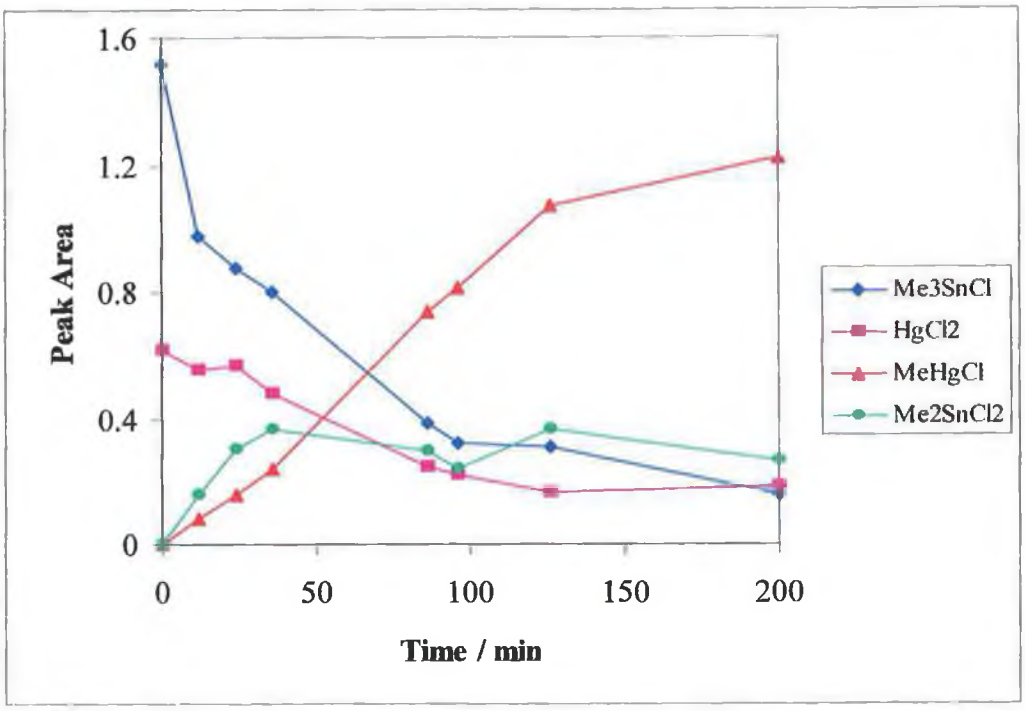


Fig. [4.7] (a): Time dependence curves for 1 x 10^{-3} M Me_3SnCl at a Me_3SnCl:$HgCl_2$ ratio of 1:2.

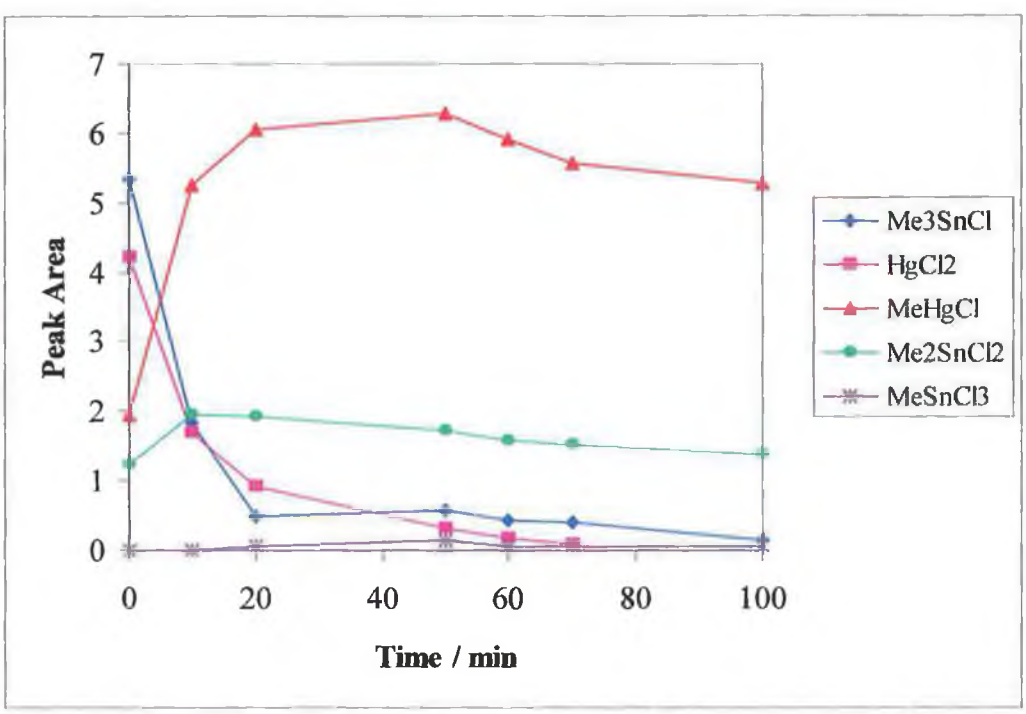


Fig. [4.7] (b): Time dependence curves for 5 x 10^{-3} M Me_3SnCl at a Me_3SnCl:$HgCl_2$ ratio of 1:2.

Fig. [4.8] shows the time dependence curves for the transmethylation reaction of two concentrations of Me_3SnCl at a 1:3 mole ratio of Me_3SnCl:$HgCl_2$. In Fig. [4.8] (a) the transmethylation reaction of a 1 x 10^{-3} M Me_3SnCl sample with 3 x 10^{-3} M $HgCl_2$ is shown. Again, as the reaction proceeds the levels of both reactants, Me_3SnCl and $HgCl_2$, decrease, and are totally consumed within 70 mins. As was the case for the transmethylation reaction at a 1:2 mole ratio, the levels of MeHgCl increase throughout the reaction period. Although significant levels of Me_2SnCl_2 are formed initially, these levels decrease down to quite low levels by the end of the reaction period. Significantly, as the levels of Me_2SnCl_2 decrease, the levels of $MeSnCl_3$ increase. Again, this was thought to be due to the concurrent reaction of Me_2SnCl_2 with $HgCl_2$, forming $MeSnCl_3$ and MeHgCl as products. A significant increase in MeHgCl concentration may also be seen. In Fig. [4.8] (b) the reaction of 5 x 10^{-3} M Me_3SnCl with 1.5 x 10^{-2} M $HgCl_2$ was monitored. This, again, corresponded to a 1:3 mole ratio of concentration of Me_3SnCl:$HgCl_2$. The levels of Me_3SnCl and $HgCl_2$ decrease significantly in the early stages of the reaction, with the $HgCl_2$ concentration depleted within ~60 mins. This indicates the reaction is complete. Again, Me_2SnCl_2 decreases as $MeSnCl_3$ increases.

This suggests a pathway is involved in the transmethylation reaction and only one methyl group is transferred at a time (and not two as suggested by some authors[4]). Due to this evidence for the formation of $MeSnCl_3$ by the reaction of Me_2SnCl_2 with $HgCl_2$, it became clear that the mechanistic pathway for the transmethylation reaction of Me_3SnCl with $HgCl_2$ involved a number of steps.

Therefore it was thought to be not a case of:

$$Me_3SnCl + HgCl_2 \rightarrow Me_2SnCl_2 + MeSnCl_3 + MeHgCl$$

but rather:

$$Me_3SnCl + HgCl_2 \rightarrow Me_2SnCl_2 + MeHgCl$$

$$Me_2SnCl_2 + HgCl_2 \rightarrow MeSnCl_3 + MeHgCl$$

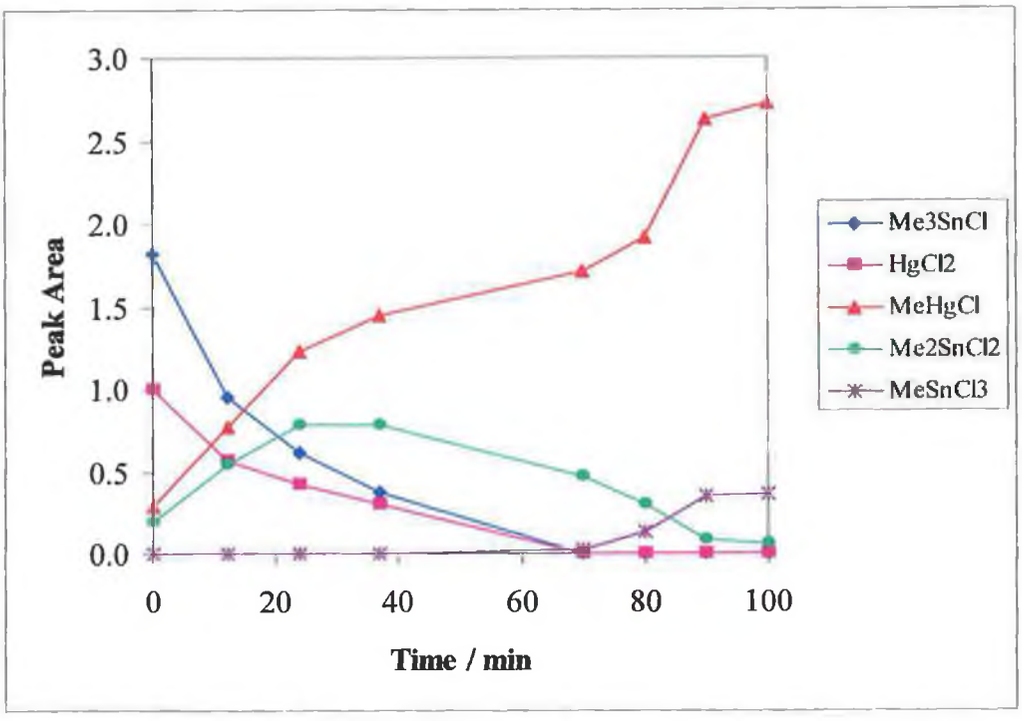


Fig. [4.8] (a): Time dependence curves for 1 x 10^{-3} M Me_3SnCl at a Me_3SnCl:$HgCl_2$ ratio of 1:3.

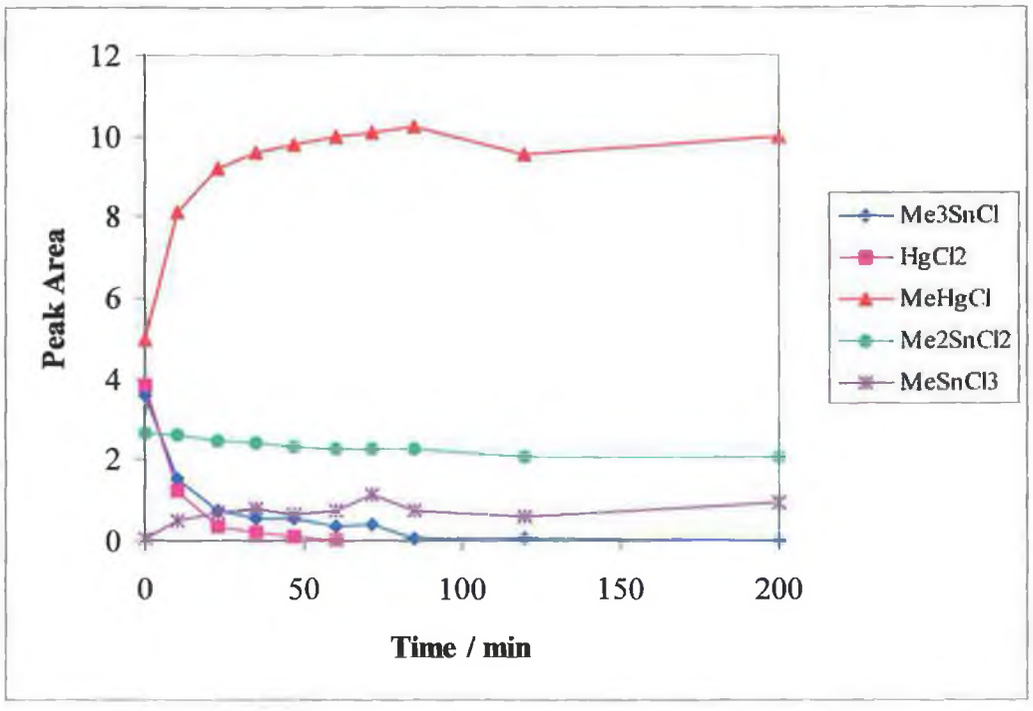


Fig. [4.8] (b): Time dependence curves for 5 x 10^{-3} M Me_3SnCl at a Me_3SnCl:$HgCl_2$ ratio of 1:3.

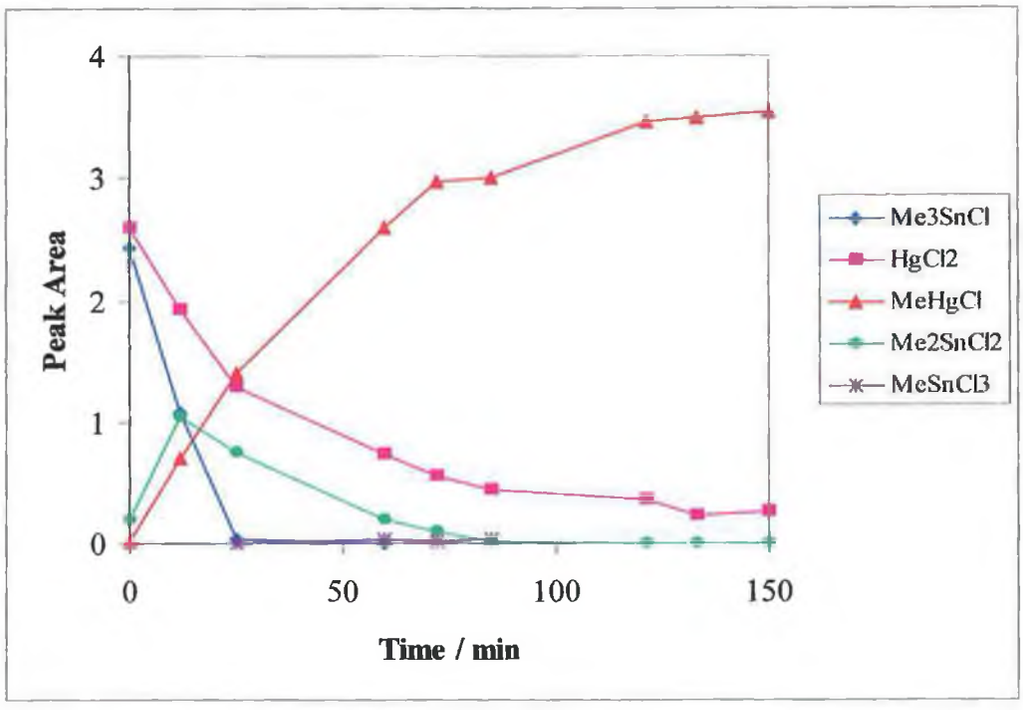


Fig. [4.9] (a): Time dependence curves for 1 x 10^{-3} M Me_3SnCl at a Me_3SnCl:$HgCl_2$ ratio of 1:4.

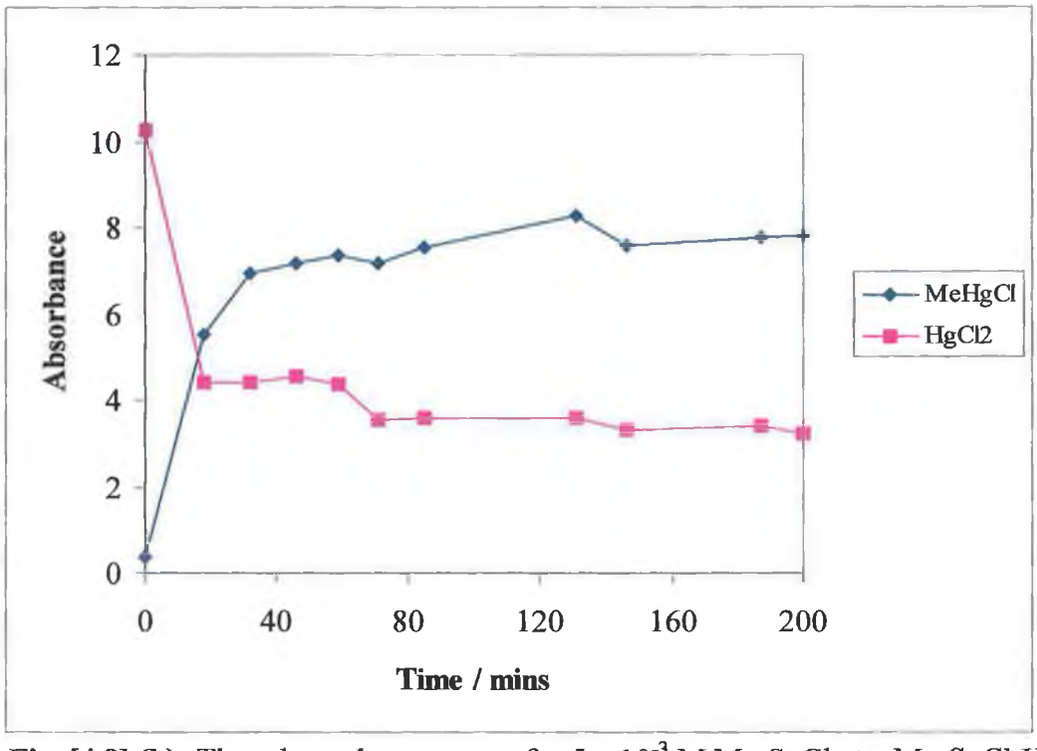


Fig. [4.9] (b): Time dependence curves for 5 x 10^{-3} M Me_3SnCl at a Me_3SnCl:$HgCl_2$ ratio of 1:4.

Fig [4.9] (a) shows a similar reaction but in this instance at a 1:4 mole ratio of $Me_3SnCl:HgCl_2$. Increasing the $HgCl_2$ concentration from 3×10^{-3} M to 4×10^{-3} M has the effect of apparently increasing the rate of the reaction. The levels of Me_3SnCl decrease very rapidly and has fully reacted within 25 mins. At this stage, the mercury chloride is still present in significant levels which means that the Me_2SnCl_2 produced can react with this $HgCl_2$ to form $MeSnCl_3$ and MeHgCl. The levels of MeHgCl continue to increase up to 120 mins and then level off. This happens at the same time as the Me_2SnCl_2 concentration decreases to zero. This suggests that any $MeSnCl_3$ formed does not undergo reaction with $HgCl_2$ to form MeHgCl and inorganic tin.

Table [4.3] shows the various transmethylation reactions attempted in this study, and also the concentrations of reactants which remained after 60 mins reaction time had elapsed. From Table [4.3] it is apparent that as the concentration of mercury chloride is increased the more rapid the reduction in Me_3SnCl concentration. As the concentration of Me_3SnCl is increased with respect to the $HgCl_2$ concentration the reaction proceeds at a greater rate.

$[Me_3SnCl]_0$	$Me_3SnCl:HgCl_2$	$[HgCl_2]_0$	$[Me_3SnCl]_{60\ mins}$	$[HgCl_2]_{60\ mins}$
0.5×10^{-3} M	1:2	1.0×10^{-3} M	0.127×10^{-3} M	1.22×10^{-3} M
	1:3	1.5×10^{-3} M	18.92×10^{-3} M	0.87×10^{-3} M
	1:4	2.0×10^{-3} M	-	1.07×10^{-3} M
1.0×10^{-3} M	1:2	2.0×10^{-3} M	0.14×10^{-3} M	1.16×10^{-3} M
	1:3	3.0×10^{-3} M	-	0.78×10^{-3} M
	1:4	4.0×10^{-3} M	-	1.66×10^{-3} M
2.0×10^{-3} M	1:2	4.0×10^{-3} M	0.01×10^{-3} M	0.66×10^{-3} M
	1:3	6.0×10^{-3} M	-	0.77×10^{-3} M
	1:4	8.0×10^{-3} M	-	2.27×10^{-3} M
3.0×10^{-3} M	1:2	6.0×10^{-3} M	-	1.78×10^{-3} M
	1:3	9.0×10^{-3} M	-	1.61×10^{-3} M
	1:4	1.2×10^{-2} M	-	6.05×10^{-3} M
4.0×10^{-3} M	1:2	8.0×10^{-3} M	-	0.66×10^{-3} M
	1:3	1.2×10^{-2} M	-	2.47×10^{-3} M
	1:4	1.6×10^{-2} M	-	4.55×10^{-3} M
5.0×10^{-3} M	1:2	1.0×10^{-2} M	-	0.89×10^{-3} M
	1:3	1.5×10^{-2} M	-	0.66×10^{-3} M
	1:4	2.0×10^{-2} M	-	6.64×10^{-3} M

Table [4.3]: Transmethylation reactions attempted in aqueous solution

As the concentration of $HgCl_2$ or Me_3SnCl is increased the faster the reduction in Me_3SnCl. At higher Me_3SnCl concentrations the consumption of Me_3SnCl is complete within 60 mins. The greater the initial concentration of $HgCl_2$, leads to greater levels of $HgCl_2$ present after 60 mins.

Fig. [4.10] shows a comparison of the decrease in $HgCl_2$ in solution with increasing time, as the initial concentration of Me_3SnCl is increased from 0.5×10^{-3} to 5×10^{-3} M.
Fig. [4.10] shows the effect of increasing the Me_3SnCl concentration on (a) the rate of decrease of $HgCl_2$ and (b) the rate of increase of MeHgCl, with increasing time. Each

reaction was carried out at the same ratio of $Me_3SnCl:HgCl_2$. In Fig. [4.10] (a) the reaction ratio of $Me_3SnCl:HgCl_2$ was maintained at 1:4 for each Me_3SnCl concentration. So, as the Me_3SnCl concentration is increased from 0.5×10^{-3} M to 5×10^{-3} M it can be seen that the levels of $HgCl_2$ remaining at the end of the reaction increase. This was as expected, as greater $HgCl_2$ levels were added to the mixture at higher Me_3SnCl concentrations, and was not all be used up in the reaction.

Fig. [4.10] (b) shows the rate of increase of MeHgCl with increasing reaction time. Here, the reaction ratio of $Me_3SnCl:HgCl_2$ was maintained at 1:3. Therefore, as the Me_3SnCl concentration increased, in the same concentration range as before, the levels of MeHgCl also increased. At lower Me_3SnCl concentrations however, the MeHgCl concentrations continue to increase throughout the reaction for higher concentrations ($>3.0 \times 10^{-3}$ M) the levels of MeHgCl initially increase dramatically and then level off. This levelling off occurs because of the total consumption of either the methyltin species or the mercury chloride at this stage of the reaction.

Fig. [4.11], shows the effect of increasing the $HgCl_2$ concentration on (a) the rate of decrease of Me_3SnCl and (b) the rate of increase of MeHgCl. Here, the concentration of Me_3SnCl remained constant for each experiment and the concentration of $HgCl_2$ was increased from a 1:2 to 1:3 to 1:4 mole ratio of $Me_3SnCl:HgCl_2$.

In Fig. [4.11] (a) the reaction of a 1×10^{-3} M Me_3SnCl with a 1:2, 1:3 and a 1:4 mole ratio of $Me_3SnCl:HgCl_2$ was examined. The effect of increasing the $HgCl_2$ concentration has a marked effect on the rate at which the Me_3SnCl is consumed in the transmethylation reaction. At the lowest $HgCl_2$ concentration (1:2 ratio), some Me_3SnCl remains present in the reaction mixture even after 200 min of reaction time has elapsed.

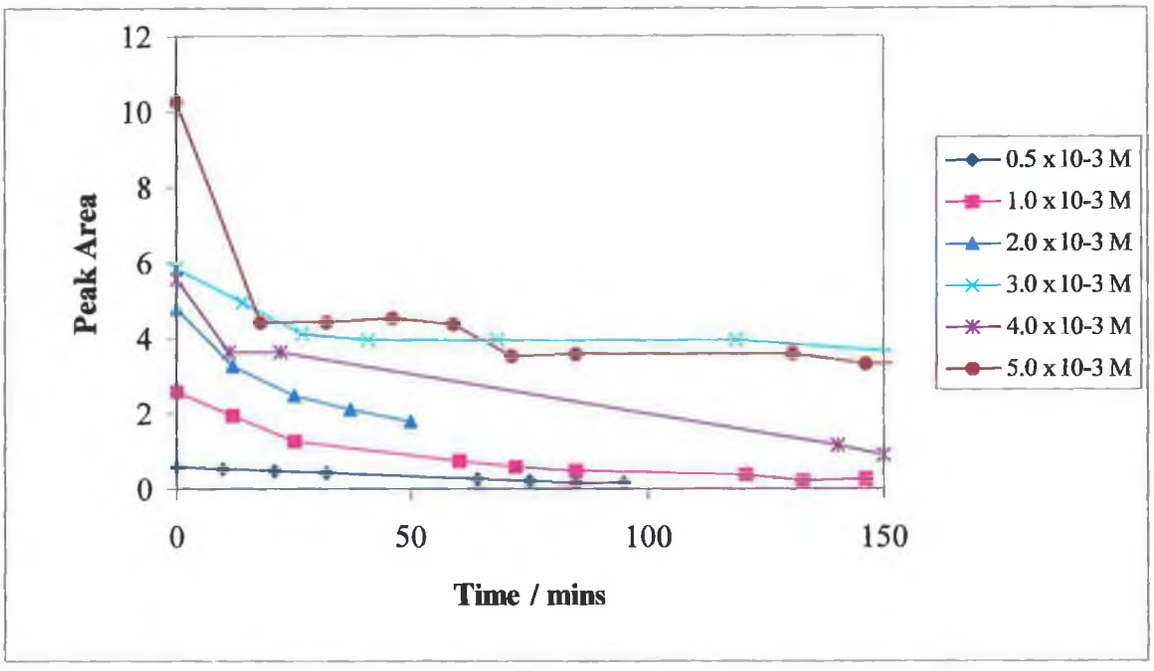


Fig. [4.10] (a): Comparison of the rate of consumption of $HgCl_2$ using trimethyltin chloride concentrations in the range 0.5 to 5.0 x 10^{-3} M, at a 1:4 ratio of Me_3SnCl:$HgCl_2$.

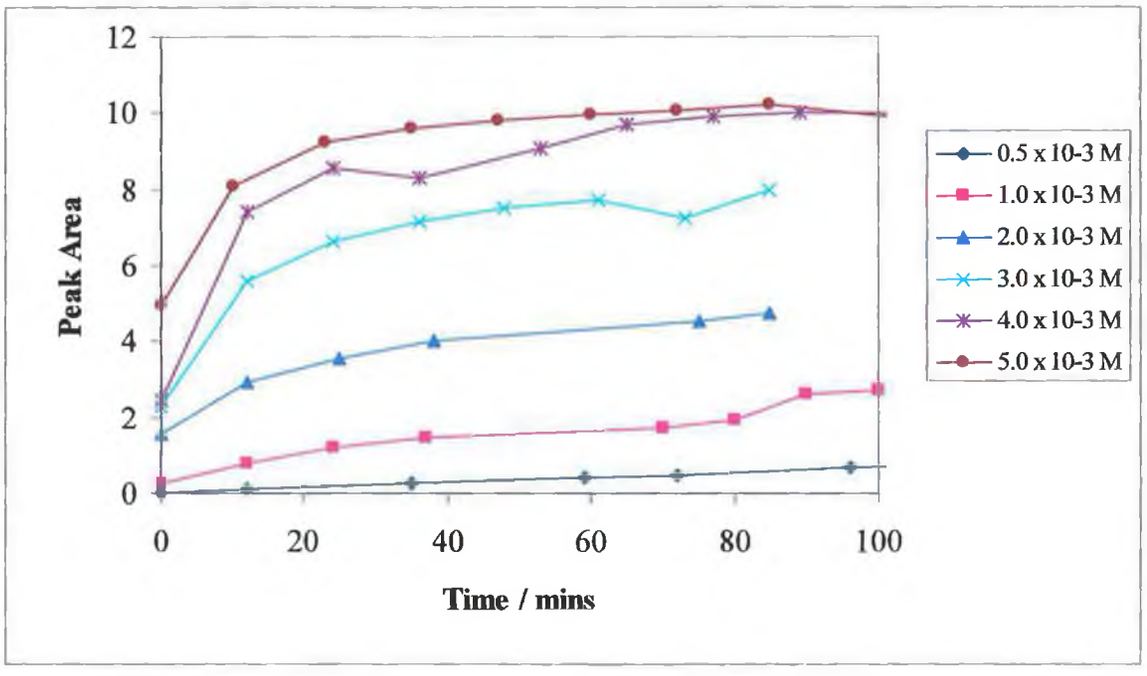


Fig. [4.10] (b): Comparison of the rate of formation of MeHgCl using trimethyltin chloride concentrations in the range 0.5 to 5.0 x 10^{-3} M, at a 1:3 ratio of Me_3SnCl:$HgCl_2$.

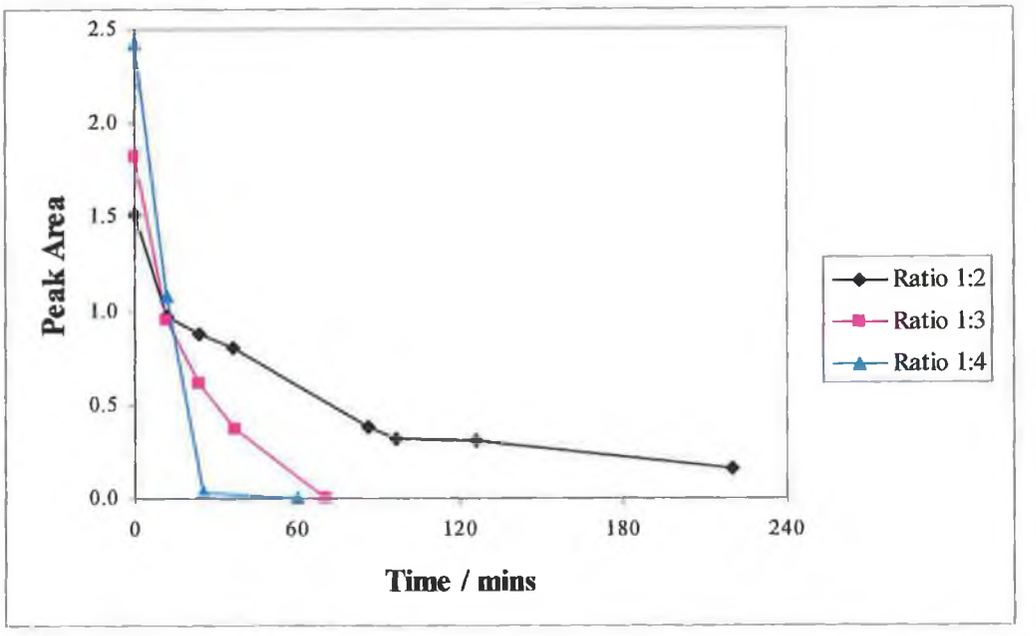


Fig. [4.11] (a): Comparison of the rate of decrease in Me_3SnCl using a trimethyltin chloride concentration of 1.0 x 10^{-3} M, at various ratios of Me_2SnCl_2:$HgCl_2$.

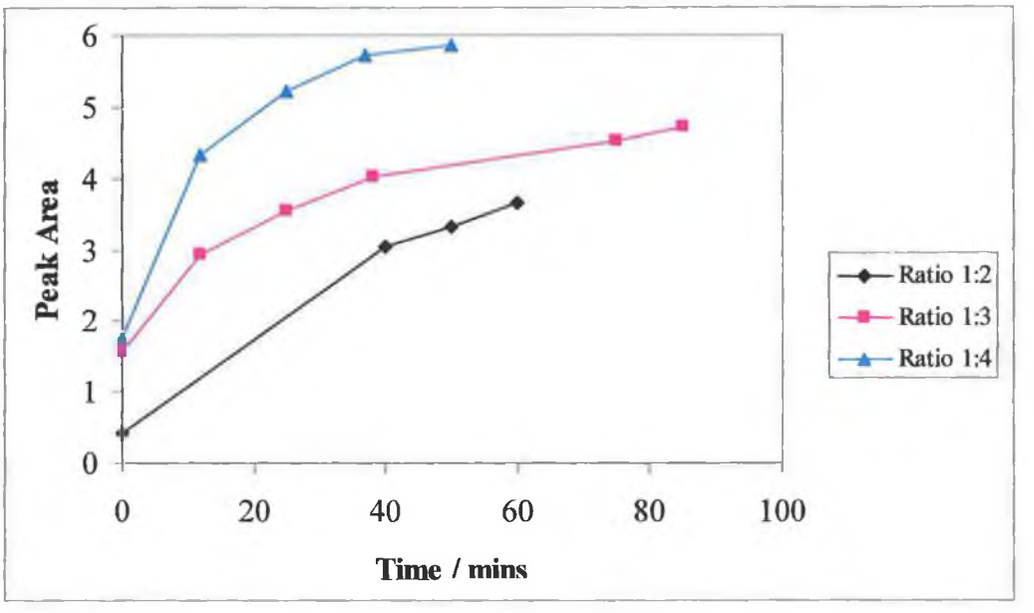


Fig. [4.11] (b): Comparison of the rate of increase in MeHgCl using a trimethyltin chloride concentration of 2.0 x 10^{-3} M, at various ratios of Me_2SnCl_2:$HgCl_2$.

However, increasing the $HgCl_2$ concentration from a 1:2 ratio to a 1:3 ratio has the effect of increasing the reaction rate – and thus all Me_3SnCl has reacted in less than 70 mins. Increasing the $HgCl_2$ concentration further increases the reaction rate even further and consequently the time it takes for all the Me_3SnCl to be totally consumed has decreased to ~ 20 mins.

Fig. [4.11] (b) shows the effect of an increase in $HgCl_2$ concentration on the levels of MeHgCl formed for a $2x10^{-3}$ M sample of Me_3SnCl. At the lowest $HgCl_2$ concentration examined ($3.33x10^{-3}$ M), a ratio of 1:2, MeHgCl continues to be formed as a product of the reaction over the time range examined which leads to the increases in peak area observed. Increasing the $HgCl_2$ concentration to $5x10^{-3}$ M (1:3 ratio) can be seen to increase the rate at which the MeHgCl is formed (which is the same as for Fig. [4.11] (a)) before a levelling out or equilibrium of the MeHgCl concentration occurs. This plateau may be due to either all the Me_3SnCl being consumed or all the $HgCl_2$ present in the reaction mixture being consumed. For the 1:4 ratio, the reaction proceeds at an even greater rate. Increasing the $HgCl_2$ concentration to $5x10^{-3}$ M (1:3 ratio), while maintaining the Me_3SnCl concentration at $2x10^{-3}$ M, leads to greater levels of MeHgCl being formed in the reaction than were seen for the reaction at a 1:2 ratio. A further increase in $HgCl_2$ concentration to $6.66x10^{-3}$ M leads to another significant increase in the levels of MeHgCl formed. It is apparent, from the comparison of these curves, that the rate of MeHgCl formation increases with increasing $HgCl_2$ concentration. This is seen in the form of a steeper initial portion of the graph with a subsequent plateau.

Overall, in the transmethylation reaction of Me_3SnCl with $HgCl_2$, under laboratory conditions, it appears that the greater the initial concentrations of the reactants, the faster the reaction proceeds, and the greater the levels of products formed. This series of experiments also revealed that it is most probable that only one methyl group is transferred in the reaction. This was determined due to the absence of dimethylmercury as a product of the reaction, and also any $MeSnCl_3$ present was most likely to be due to the secondary reaction of Me_2SnCl_2 with the excess $HgCl_2$.

4.3.2. Transformation of methyl groups from Me_2SnCl_2

From the above experiments it is clear that a transmethylation reaction did occur between Me_3SnCl and $HgCl_2$. However, as both dimethyltin dichloride and methyltin trichloride were formed as products of the reaction, it was not clear whether the process involved the transfer of one or two methyl groups from the trimethyltin chloride. Therefore, it was decided to study the transmethylation reaction for dimethyltin dichloride with mercury chloride and examine the products of the reaction along with the kinetics of the reaction.

The transmethylation reaction of dimethyltin dichloride with $HgCl_2$ was carried out over a range of concentrations of both dimethyltin dichloride and mercury chloride. Each reaction was monitored using CZE where the products and reactants were detected and identified, and their concentrations measured as a function of time. Time-decay plots were therefore established for each reaction. These plots were then examined and the relative rates of each reaction recorded. An insight into the kinetics of the reaction was thus provided.

The experimental procedures employed were similar to those used in the study of the transmethylation reaction of trimethyltin chloride with mercury chloride. The concentrations of solutions used are outlined in Table [4.2].

Fig. 4.12 shows the results of the transalkylation reaction of dimethyltin dichloride with mercury(II)chloride. The % transformed was calculated as per trimethyltin chloride. Fig. [4.12] (a) shows the reaction at a 1:2 mole ratio and the data collected suggests that at this ratio approximately 60% of the $HgCl_2$ present initially in the reaction mixture – regardless of the initial Me_2SnCl_2 concentration – is transformed in the reaction timeframe.

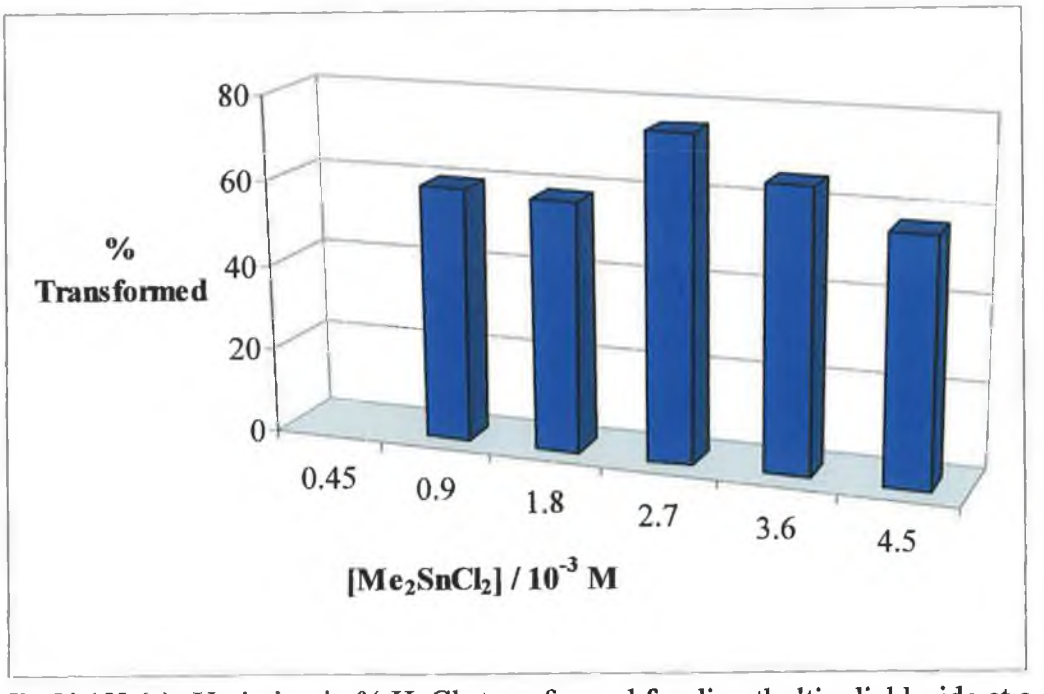


Fig [4.12] (a): Variation in % $HgCl_2$ transformed for dimethyltin dichloride at a Me_2SnCl_2:$HgCl_2$ ratio of 1:2.

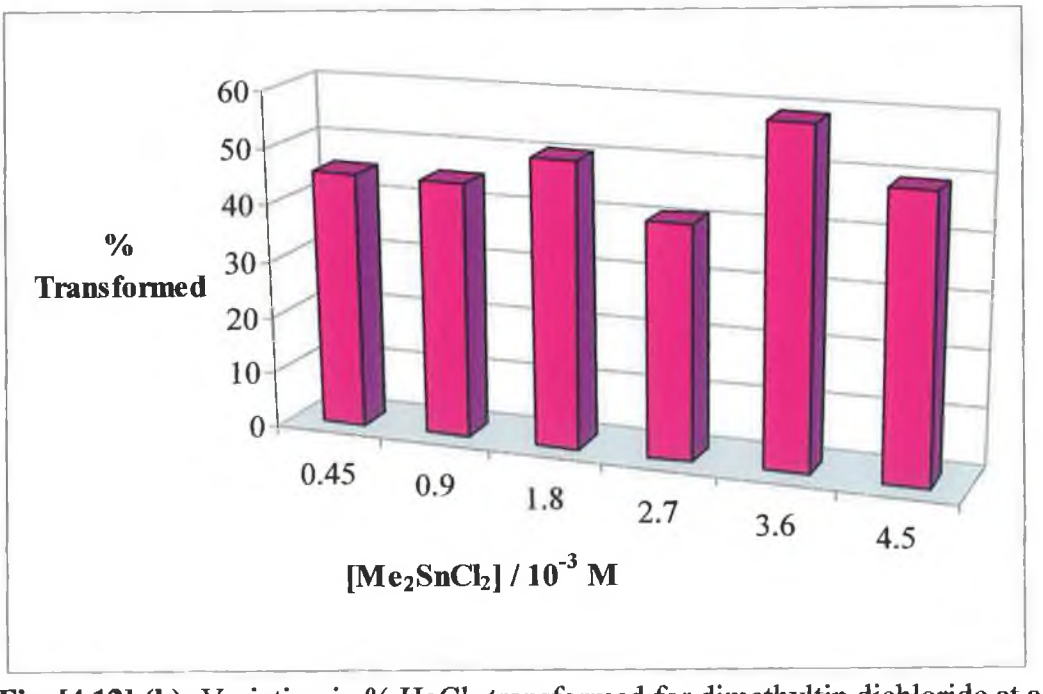


Fig. [4.12] (b): Variation in % $HgCl_2$ transformed for dimethyltin dichloride at a Me_2SnCl_2:$HgCl_2$ ratio of 1:3.

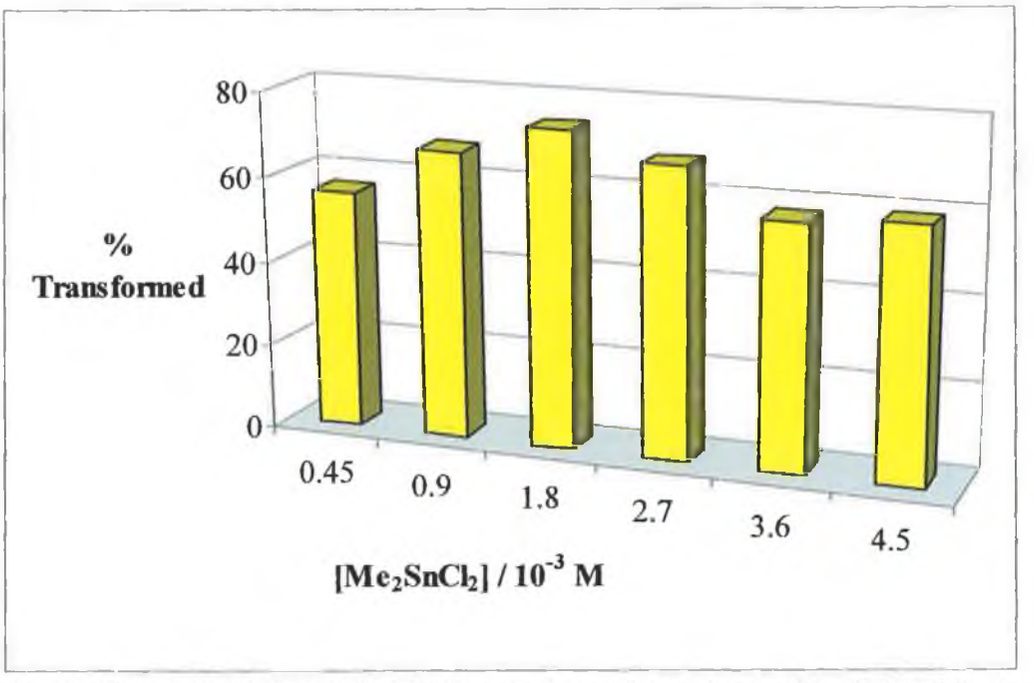


Fig. [4.12] (c): Variation in % $HgCl_2$ transformed for dimethyltin dichloride at a Me_2SnCl_2:$HgCl_2$ ratio of 1:4.

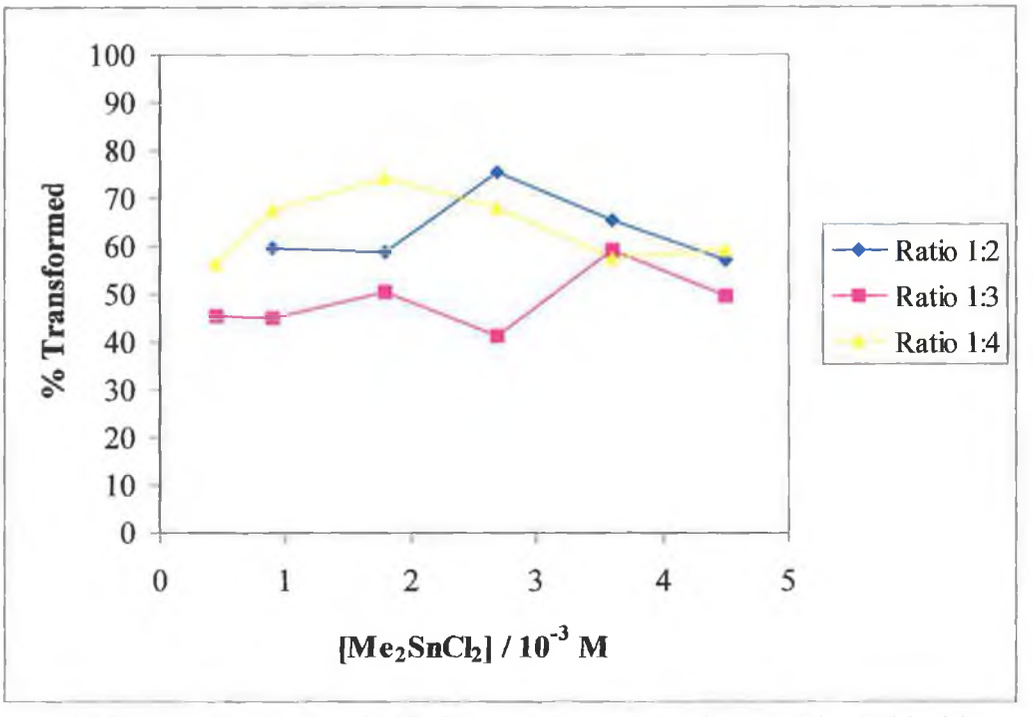


Fig. [4.12] (d): Variation in % $HgCl_2$ transformed for dimethyltin dichloride over a dimethyltin dichloride concentration range of 0.45 to 4.5 x 10^{-3} M at Me_2SnCl_2:$HgCl_2$ ratios of 1:2, 1:3 and 1:4.

The % $HgCl_2$ transformed for the transmethylation reaction of Me_2SnCl_2 and $HgCl_2$ with a 1:3 ratio reaction mixture is depicted in Fig. [4.12] (b). Here ~45% of the $HgCl_2$ present is transformed, and again the initial concentration of Me_2SnCl_2 seems to be irrelevant. In Fig. [4.12] (c) the reactions monitored consisted of a 1:4 mole ratio of Me_2SnCl_2:$HgCl_2$. The % $HgCl_2$ transformed seems to increase to a maximum of ~70% at a Me_2SnCl_2 concentration of $1.8x10^{-3}$ M and then decreases again for all other Me_2SnCl_2 concentrations. The average % transformed was ~60%.

Fig. [4.12] (d) graphically depicts all these transformations and it can be seen that for each ratio the % $HgCl_2$ transformed is in the region of 40-80%, with no other obvious trends apparent – i.e. increasing the relative $HgCl_2$ concentration doesn't correspond to a greater level of $HgCl_2$ being transformed.

It is therefore apparent that dimethyltin dichloride also has the ability to transfer a methyl group to mercury chloride resulting in the formation of methylmercury chloride and methyltin trichloride. It is also clear that dimethyltin dichloride only transfers one methyl group, and not two groups as has been suggested[4]. This is the case because, firstly, methyltin trichloride is formed as a product of the reaction, which would be absent if two methyl groups were transferred, and secondly, there is no evidence to suggest the formation of dimethylmercury. Standard dimethylmercury samples were run and were eluted last, after $HgCl_2$. However, no dimethylmercury was detected during the experiments. This lack of detection may be due to the volatile nature of Me_2Hg, so the presence of $MeSnCl_3$ is the more valid argument.

Figure [4.13] shows the individual electropherograms superimposed for a 1:3 mole ratio reaction of dimethyltin dichloride and mercury chloride. It is very clear that as time progresses there is a significant decrease in the levels of dimethyltin dichloride and also of mercury (II) chloride. However the rate of the reaction seems to be less than for Me_3SnCl – the kinetics of the reaction will be discussed later.

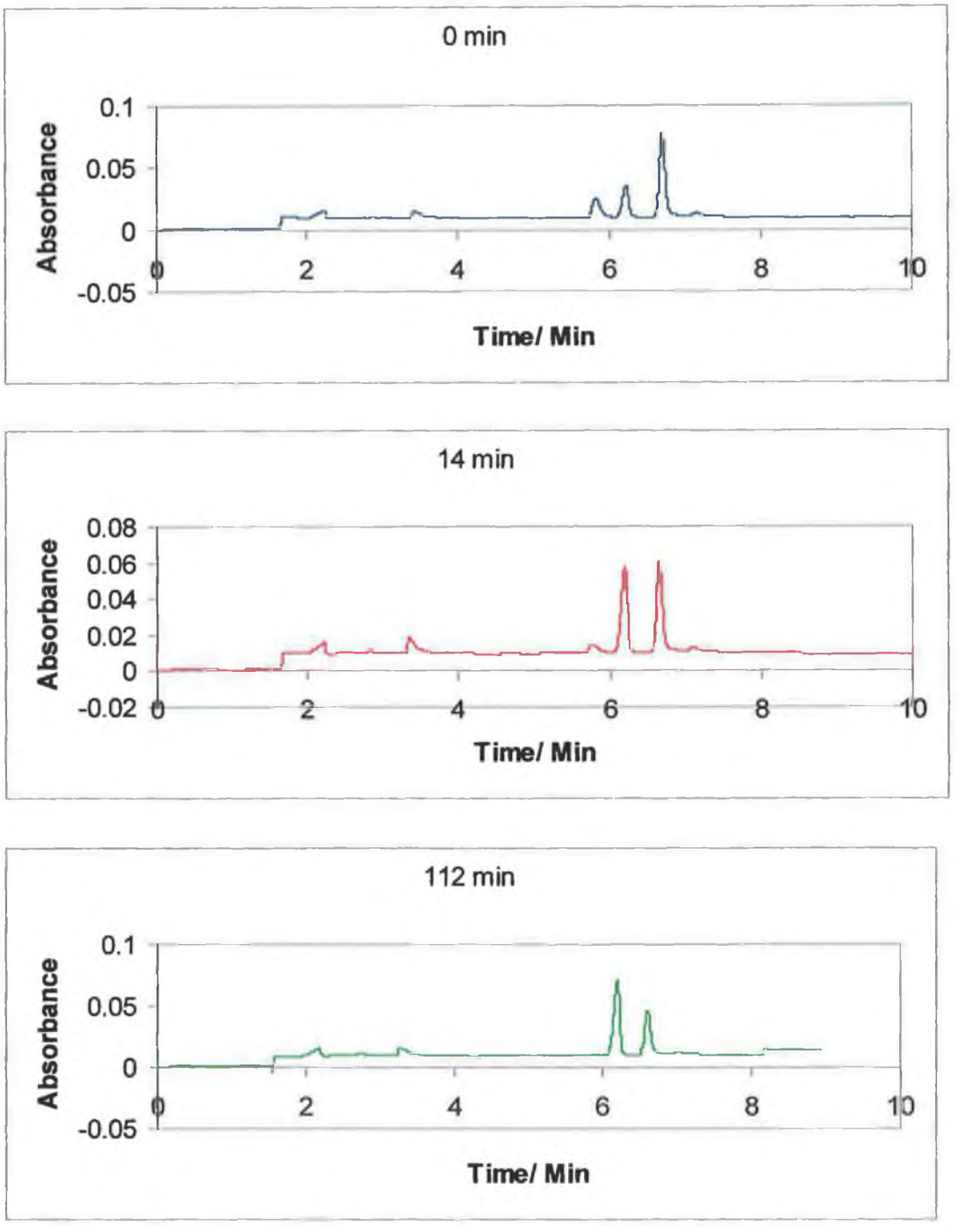


Fig. [4.13]: Electropherograms showing the transmethylation reaction of 3.6×10^{-3} M Me_2SnCl_2 with a Me_2SnCl_2:$HgCl_2$ ratio of 1:3. The figure shows the levels of both reactants and products present in the system at three stages of the reaction, i.e. at 0 min, 14 min and 112 min.

The dependence of the concentrations of reactants and products with time for each concentration of dimethyltin dichloride was examined for each reaction carried out with mercury chloride. Figs. [4.14] – [4.16] show the dependence curves for dimethyltin dichloride samples at 1:2, 1:3 and 1:4 reaction ratios. It is clear from these graphs that the rate of the reaction is dependent on the initial concentrations of the reactants.

Fig. [4.14] shows the reaction of Me_2SnCl_2 with $HgCl_2$ in a 1:2 mole ratio at two different Me_2SnCl_2 concentrations; (a): 9×10^{-4} M, and (b): 3.6×10^{-3} M. Similar dependencies were found for the reaction of Me_2SnCl_2 with $HgCl_2$ as were previously found for the reaction of Me_3SnCl with $HgCl_2$. For the lower Me_2SnCl_2 concentration (Fig. [4.14] (a)), a gradual reaction of the Me_2SnCl_2 with the $HgCl_2$ was seen with the formation of MeHgCl and $MeSnCl_3$ as products of the reaction. However, at the higher Me_2SnCl_2 concentration (Fig. [4.14] (b)), the reaction is seen to proceed at a much greater rate, i.e. the initial rate of consumption of Me_2SnCl_2 is far more rapid than for the 9×10^{-4} M Me_2SnCl_2 reaction. Here, the Me_2SnCl_2 concentration has been totally consumed in 80 mins. In this reaction the formation of $MeSnCl_3$ is again seen as a product of the transmethylation reaction. However, it can be seen that as the reaction progresses the levels of $MeSnCl_3$ formed are seen to decrease again, presumably due to its reaction with $HgCl_2$ still present in the reaction mixture. No further reaction product was detected, but it was thought that the most likely product would be $SnCl_4$. A small increase in the levels of MeHgCl are seen which corresponds with the proposed reaction.

Now it is apparent that a transmethylation reaction occurs between Me_2SnCl_2 and $HgCl_2$ and also occurs between Me_3SnCl and $HgCl_2$ and may also occur between $MeSnCl_3$ and $HgCl_2$. The kinetics of each of these reactions must be examined to determine the relative rates of product formation with each of these compounds.

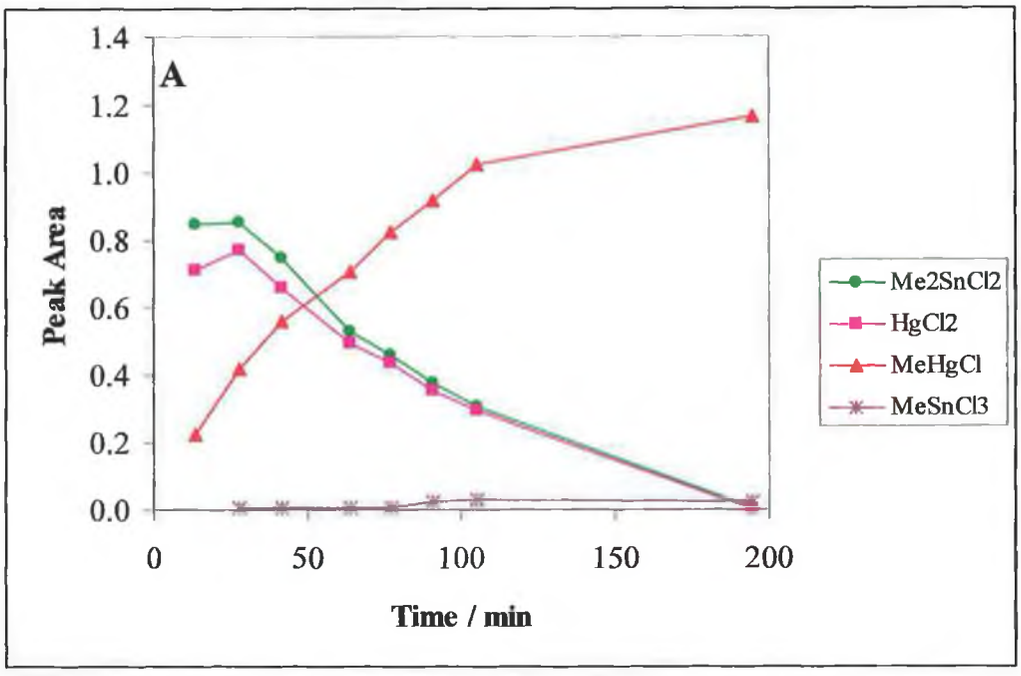


Fig. [4.14] (a): Time dependence curves for 0.9 x 10^{-3} M Me_2SnCl_2 at a Me_2SnCl_2:$HgCl_2$ ratio of 1:2.

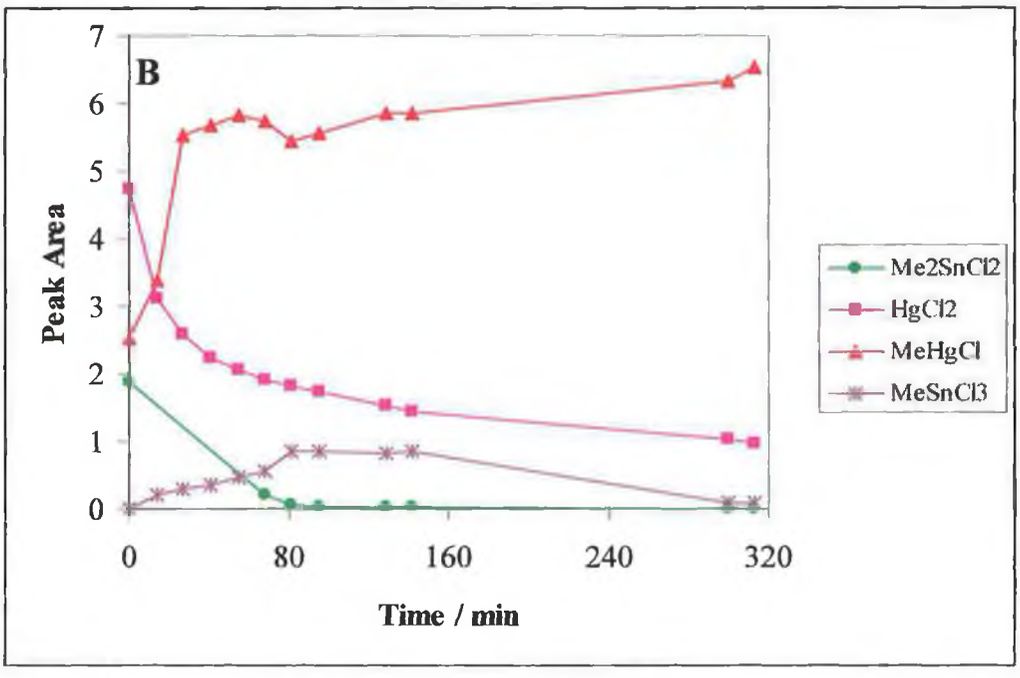


Fig. [4.14] (b): Time dependence curves for 3.6 x 10^{-3} M Me_2SnCl_2 at a Me_2SnCl_2:$HgCl_2$ ratio of 1:2.

Fig. [4.15] shows the transmethylation reaction of Me_2SnCl_2 with $HgCl_2$, for a 1:3 mole ratio of Me_2SnCl_2:$HgCl_2$ at two different Me_2SnCl_2 concentrations; (a): 9×10^{-4} M and (b): 3.6×10^{-3} M. It can be seen that at the lower Me_2SnCl_2 concentration the reaction proceeds at a slower rate than at the higher Me_2SnCl_2 concentrations. However, the rate of the reaction has increased from the 1:2 ratio, as can be seen by the fact that all the Me_2SnCl_2 has been consumed within 100 mins. Again MeHgCl and $MeSnCl_3$ are seen to be formed as products of the reaction. No further reaction of $MeSnCl_3$ was observed. For the reaction of 3.6×10^{-3} M Me_2SnCl_2 with a 1:3 mole ratio of $HgCl_2$, the reaction rate has again increased, and the Me_2SnCl_2 is consumed in ~30 mins. The initial formation and subsequent reaction of $MeSnCl_3$ is again observed. The levels of $HgCl_2$ decrease throughout the duration of the reaction.

Fig. [4.16] shows similar reactions, but in this instance at a reaction ratio of 1:4. Here, however, the initial concentration of Me_2SnCl_2 in (a) was 1.8×10^{-4} M and the concentration in (b) was 3.6×10^{-3} M. As expected, the reaction proceeds at an even greater rate with the higher Me_2SnCl_2 and $HgCl_2$ concentrations. Increasing the $HgCl_2$ concentration from 1.8×10^{-4} M to 3.6×10^{-3} M has the effect of apparently increasing the rate of the transmethylation reaction. The levels of Me_2SnCl_2 decrease very rapidly and has fully reacted within 50 mins, at both concentrations. There are significant levels of $HgCl_2$ present in the reaction at this stage – thus allowing further reactions of the products of the reaction to occur.

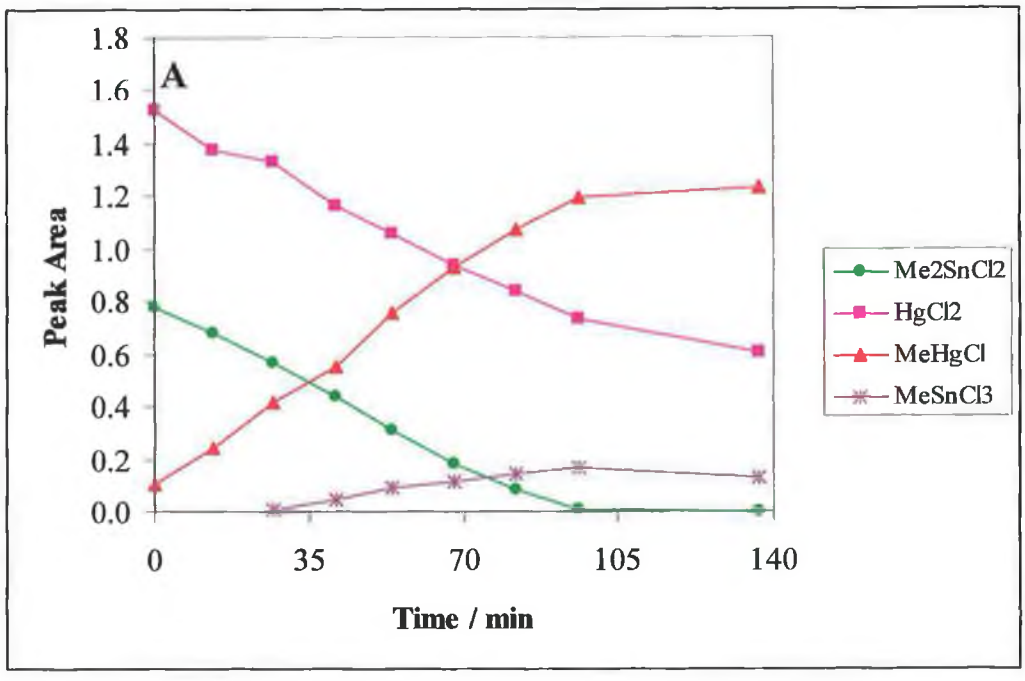


Fig. [4.15] (a): Time dependence curves for 0.9 x 10^{-3} M Me_2SnCl_2 at a Me_2SnCl_2:$HgCl_2$ ratio of 1:3.

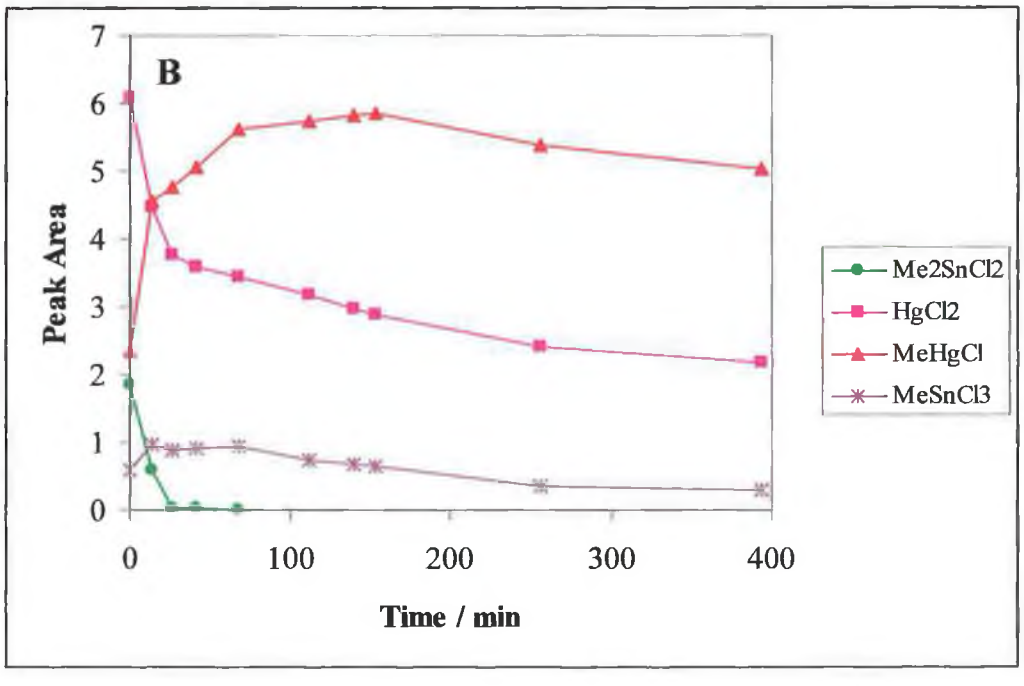


Fig. [4.15] (b): Time dependence curves for 3.6 x 10^{-3} M Me_2SnCl_2 at a Me_2SnCl_2:$HgCl_2$ ratio of 1:3.

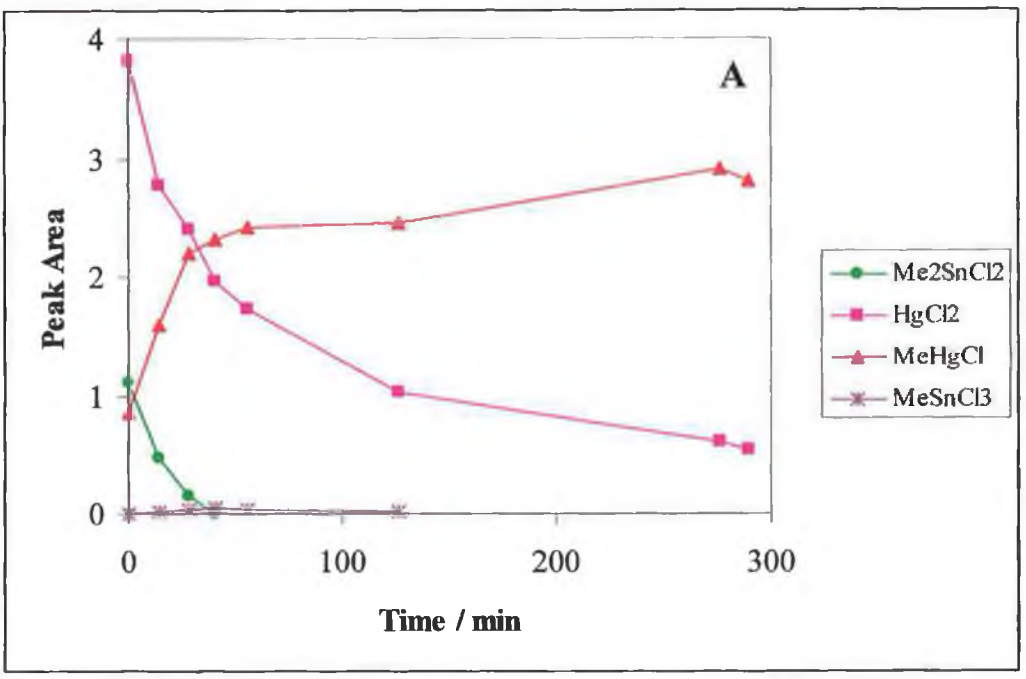


Fig. [4.16] (a): Time dependence curves for 1.8 x 10^{-3} M Me_2SnCl_2 at a Me_2SnCl_2:$HgCl_2$ ratio of 1:4.

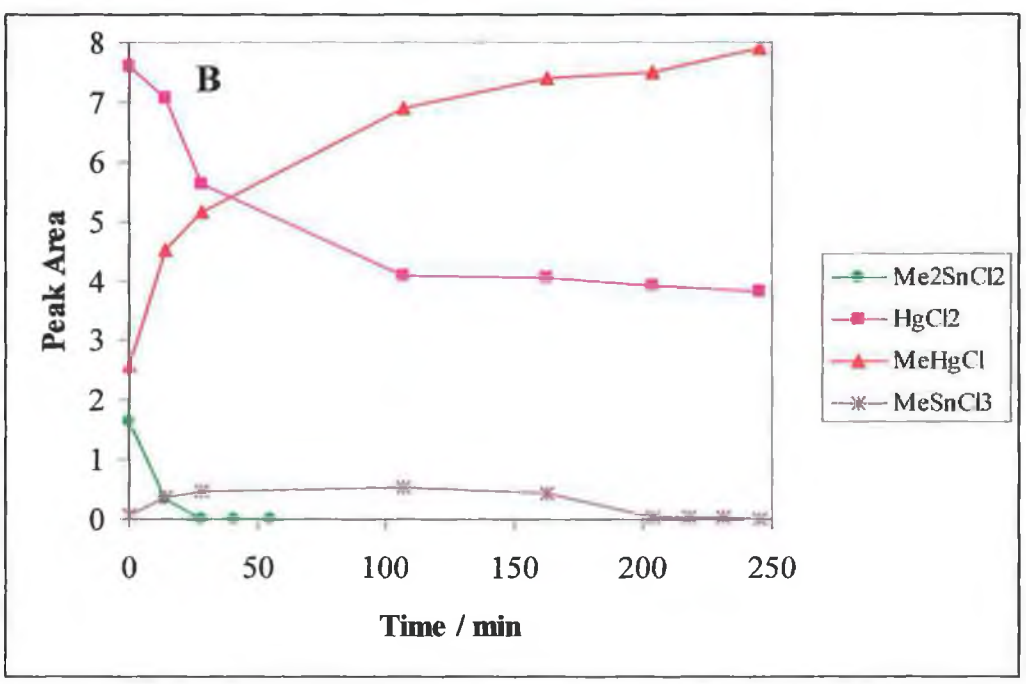


Fig. [4.16] (b): Time dependence curves for 3.6 x 10^{-3} M Me_2SnCl_2 at a Me_2SnCl_2:$HgCl_2$ ratio of 1:4.

Table [4.4] shows the transmethylation reactions which were attempted in buffer solution and also shows the concentrations of reactants which remained in solution after 35 mins reaction time had elapsed. As the concentration of $HgCl_2$ is increased for each Me_2SnCl_2 concentration the rate at which the dimethyltin dichloride is used up is increased. Similarly, as the concentration of Me_2SnCl_2 is increased the rate of the transmethylation reaction is also increased.

$[Me_2SnCl_2]_0$	Me_2SnCl_2: $HgCl_2$	$[HgCl_2]_0$	$[Me_2SnCl_2]_{35\ min}$	$[HgCl_2]_{35\ min}$
0.45×10^{-3} M	1:2	0.9×10^{-3} M	-	0.214
	1:3	1.3×10^{-3} M	-	-
	1:4	1.8×10^{-3} M	-	-
0.9×10^{-3} M	1:2	1.8×10^{-3} M	0.833	0.052
	1:3	2.7×10^{-3} M	0.556	0.425
	1:4	3.6×10^{-3} M	0.111	0.404
1.8×10^{-3} M	1:2	3.6×10^{-3} M	0.535	0.393
	1:3	5.4×10^{-3} M	0.111	1.390
	1:4	7.2×10^{-3} M	0.171	1.093
2.7×10^{-3} M	1:2	5.4×10^{-3} M	0.111	0.553
	1:3	8.1×10^{-3} M	0.173	1.788
	1:4	10.8×10^{-3} M	0.111	1.728
3.6×10^{-3} M	1:2	7.2×10^{-3} M	0.891	1.249
	1:3	10.8×10^{-3} M	0.136	2.159
	1:4	14.4×10^{-3} M	0.120	3.47
4.5×10^{-3} M	1:2	9.0×10^{-3} M		
	1:3	13.5×10^{-3} M		
	1:4	18.0×10^{-3} M		

TABLE [4.4]: Transmethylation reactions attempted in aqueous solution

Fig. [4.17] shows a comparison of the various rates of decrease of mercury (II) chloride in the transmethylation reaction of Me_2SnCl_2 and $HgCl_2$ at different initial dimethyltin

dichloride concentrations. The rate of decrease was monitored with increasing time, as the initial concentration of Me_2SnCl_2 is increased from 0.45 to 4.5 x 10^{-3} M. Each reaction was carried out at a mole ratio of Me_2SnCl_2:$HgCl_2$ of 1:3.

Fig. [4.17] (a) shows the rate of decrease of $HgCl_2$ at a 1:3 ratio of Me_2SnCl_2:$HgCl_2$ at various Me_2SnCl_2 concentrations. Increasing the Me_2SnCl_2 concentration led to greater levels of $HgCl_2$ remaining in the reaction mixture when the experimental time period had expired. The rate of decrease for the lower concentrations of Me_2SnCl_2 is much slower than for the higher concentrations. In fact, for 1.8 – 4.5 x 10^{-3} M reactions the level of $HgCl_2$ has decreased by ~50% in the first 25 mins but for 0.45 – 0.9 x 10^{-3} M reactions, the decrease was considered to be less than 10%.

Fig. [4.17] (b) compares the rate of formation of MeHgCl, also at a 1:3 mole ratio of Me_2SnCl_2:$HgCl_2$ at increasing dimethyltin dichloride concentrations. It can be seen that the higher the concentration of Me_2SnCl_2 the greater the levels of MeHgCl formed. The initial formation of MeHgCl also increases as the concentration of Me_2SnCl_2 increases and an equilibrium is established faster.

Fig. [4.18] shows a similar study. Here, however, the Me_2SnCl_2 concentration remained constant and the rate of increase/decrease of the products/reactants in the transmethylation reaction are examined at various mole ratios of Me_2SnCl_2:$HgCl_2$. Firstly, the rate of decrease of Me_2SnCl_2 with time was examined, as shown in Fig. [4.18] (a). It can be clearly seen that as the levels of $HgCl_2$ present in the reaction are increased then the rate at which Me_2SnCl_2 is removed from the reaction is also increased. The decrease in $HgCl_2$ with time was also examined (Fig. [4.18] (b)), at the three different ratios of Me_2SnCl_2:$HgCl_2$. Increasing the ratio of the reaction led to greater levels of $HgCl_2$ remaining in the vial after the reaction was complete. No greater initial rate of decrease was apparent with increasing concentration.

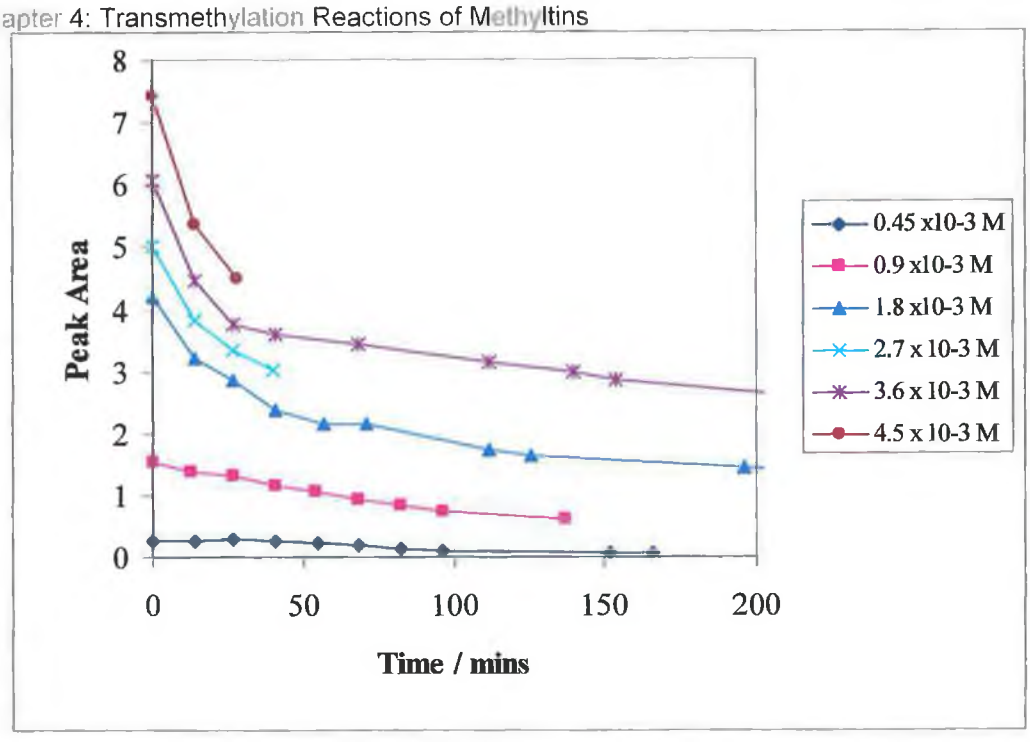
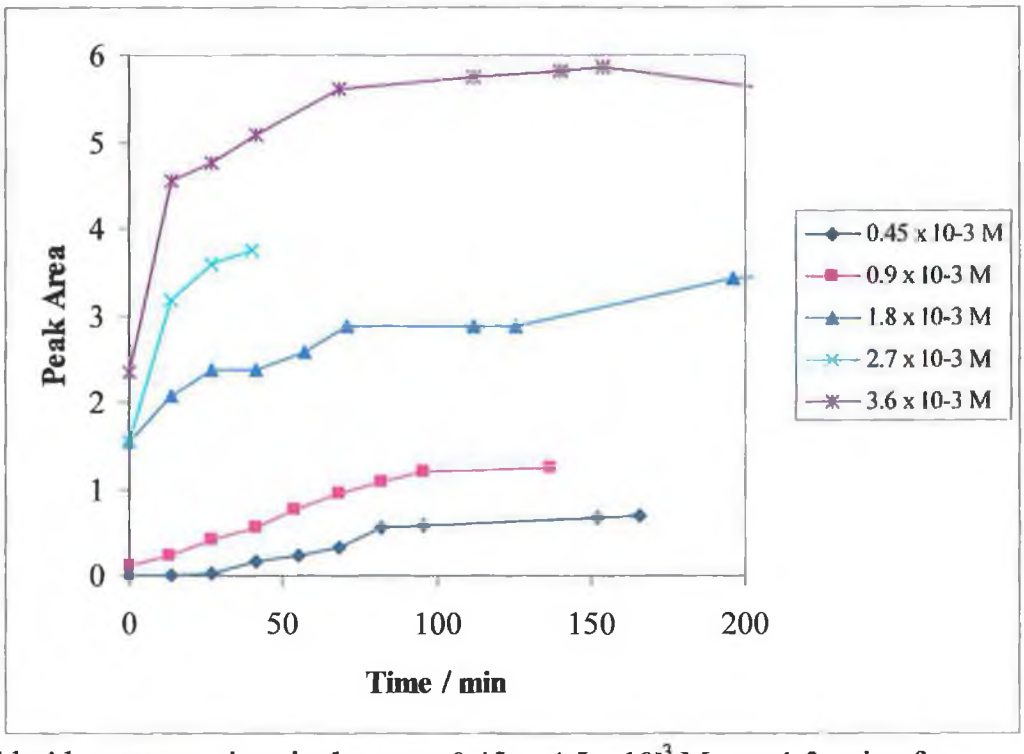


Fig. [4.17] (a): Comparison of the rate of decrease in $HgCl_2$ using dimethyltin dichloride concentrations in the range 0.45 to 4.5 x 10^{-3} M, at a 1:3 ratio of Me_2SnCl_2:$HgCl_2$.

Fig. [4.17] (b): Comparison of the rate of increase in MeHgCl using dimethyltin



dichloride concentrations in the range 0.45 to 4.5 x 10^{-3} M, at a 1:3 ratio of Me_2SnCl_2:$HgCl_2$.

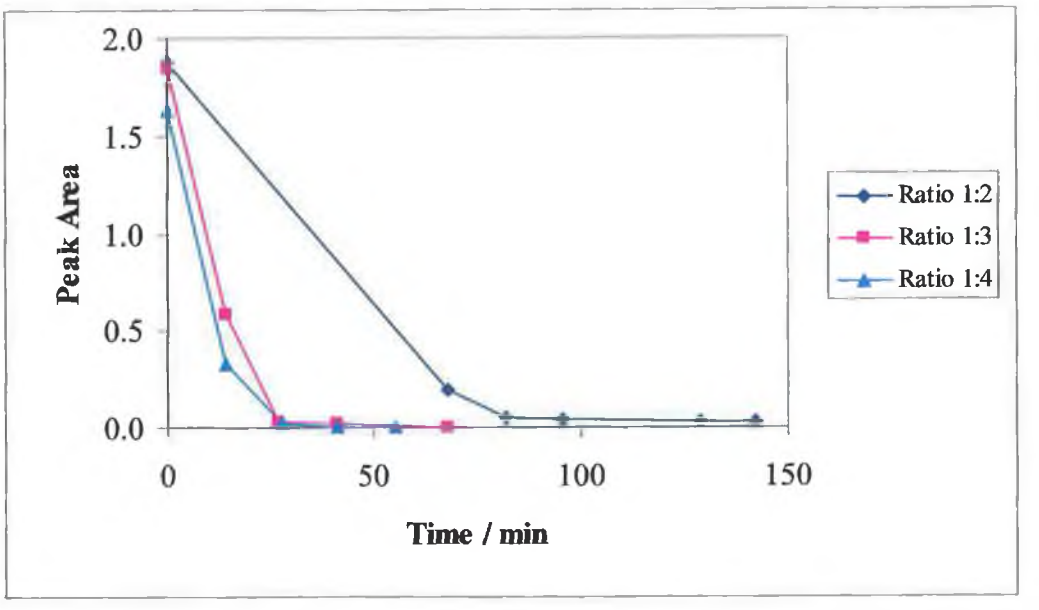


Fig. [4.18] (a): Comparison of the rate of decrease in Me_2SnCl_2 using a dimethyltin dichloride concentration of 3.6 x 10^{-3} M, at various ratios of Me_2SnCl_2:$HgCl_2$.

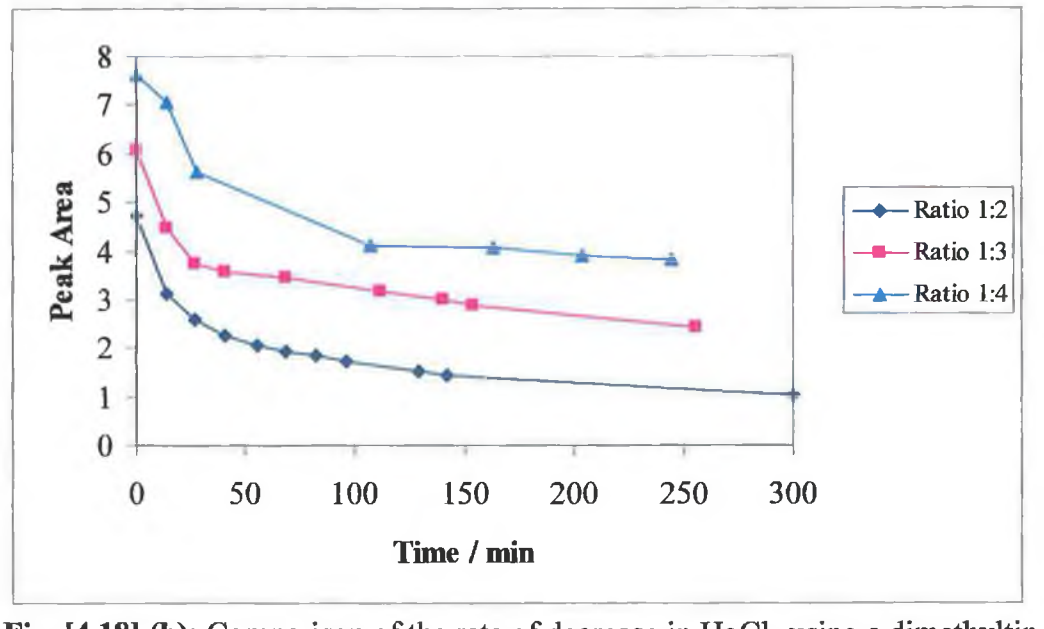


Fig. [4.18] (b): Comparison of the rate of decrease in $HgCl_2$ using a dimethyltin dichloride concentration of 3.6 x 10^{-3} M, at various ratios of Me_2SnCl_2:$HgCl_2$.

4.3.3. Transformation of methyl groups from $MeSnCl_3$

Studies thus far have shown that a transmethylation reaction occurs between Me_3SnCl and $HgCl_2$ which led to the formation of both Me_2SnCl_2 and $MeSnCl_3$ as products of the reaction, along with MeHgCl. This led to the question over the number of methyl groups that were transferred in the reaction; one, two or all three? By looking at the dependence of the concentrations of the reactants and products upon time for the transmethylation reaction of Me_3SnCl with $HgCl_2$ it became clear that although both Me_2SnCl_2 and $MeSnCl_3$ were formed as products of the reaction, it was, in reality, the secondary reaction of Me_2SnCl_2 with $HgCl_2$ which led to the formation of $MeSnCl_3$ as a product in the reaction. As a result it was decided to determine the extent of this transmethylation reaction of Me_2SnCl_2 with $HgCl_2$, and a number of reactions were performed, in which both the initial concentration of $HgCl_2$ and Me_2SnCl_2 were varied. Looking at the dependence of the concentrations of the reactants and products upon time for the transmethylation reaction of Me_2SnCl_2 with $HgCl_2$, it became apparent that, although $MeSnCl_3$ was formed as a product of the reaction, it too underwent further reaction, presumably with $HgCl_2$, and thus its concentration was seen to decrease, over the duration of the experimental period.

Therefore, it was decided to examine the potential of a transmethylation reaction occurring between $MeSnCl_3$ and $HgCl_2$, to form MeHgCl and an inorganic tin species, most likely $SnCl_4$. As before, the initial concentrations of both $MeSnCl_3$ and $HgCl_2$ were varied to determine the concentration at which the reaction is maximised. The % transformation was also examined.

As no tin-containing product of the reaction was detected during the "dimethyl" series of reactions, it was quite possible that none would be detected here, and so emphasis was placed on monitoring the products of the reaction, along with examining the rate of decrease of the reactants, $MeSnCl_3$ and $HgCl_2$.

Now that we know that the transmethylation reaction occurs with both Me_3SnCl and $HgCl_2$ and also Me_2SnCl_2 and $HgCl_2$, the final stage in the analysis of the transmethylation reaction would be to find if the transmethylation reaction occurs for $MeSnCl_3$ and $HgCl_2$. This task was a little more difficult than the previous reactions. Firstly, using the capillary electrophoresis method developed, the detection of $MeSnCl_3$ was quite insensitive. This was due to the poor UV absorbance of $MeSnCl_3$ at 190 nm, the selected UV detection wavelength. Secondly, as it was assumed that the products of the reaction would be methylmercury chloride and inorganic tin, it would not be possible to detect inorganic tin using CZE, and therefore not possible to determine the concentrations formed.

Fig [4.19] shows the % $HgCl_2$ transformed in the transmethylation reaction of methyltin trichloride with mercury(II)chloride. The results obtained exemplify the difficulties encountered in the analysis of this reaction. No clear pattern emerged regarding the relationship between concentrations of reactants present and percentage transformation. However, in all cases examined, the % transformed was consistently below 20%, and this suggests a slower reaction than was evident for either dimethyltin dichloride or trimethyltin chloride.

Fig [4.20] shows the individual electropherograms superimposed for a 1:4 mole ratio reaction of methyltin trichloride and mercury (II)chloride. It is very clear that as time progresses there is a significant decrease in the levels of methyltin trichloride and also of mercury (II) chloride. However the rate of the reaction seems to be less than for Me_3SnCl – the kinetics of which will be discussed later.

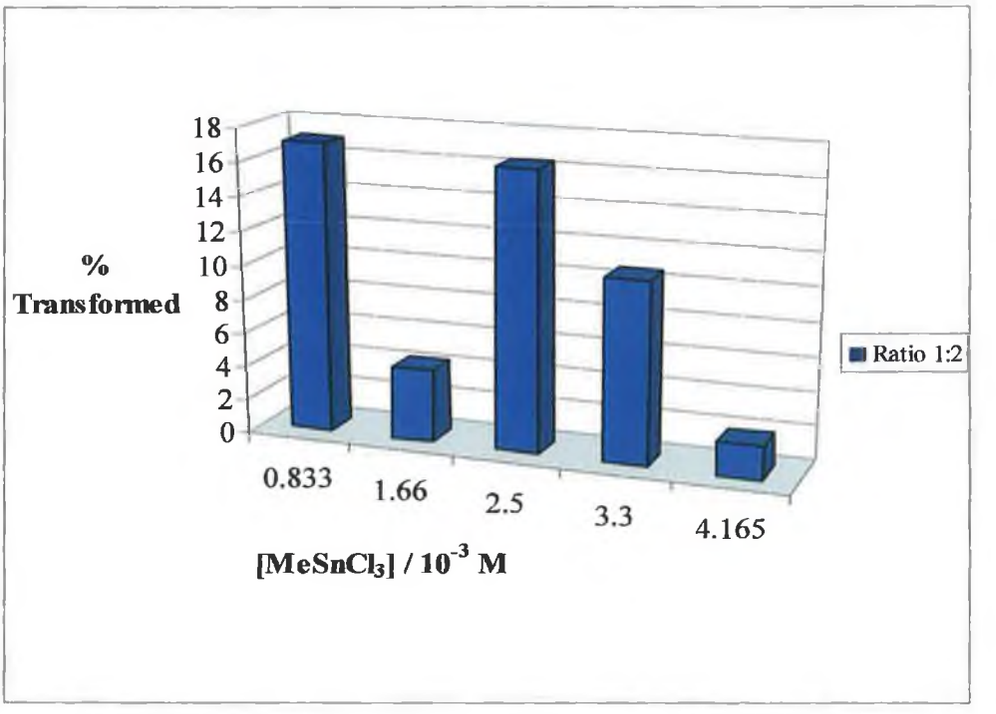


Fig [4.19] (a): Variation in % $HgCl_2$ transformed for methyltin trichloride at a $MeSnCl_3$:$HgCl_2$ ratio of 1:2.

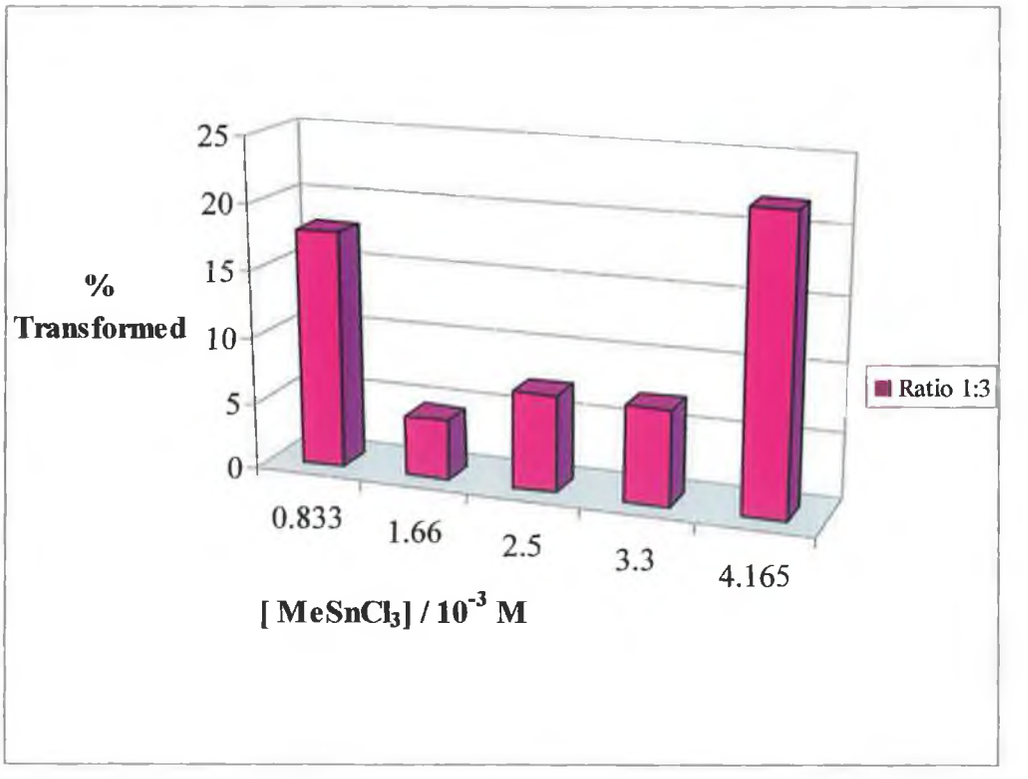


Fig [4.19] (b): Variation in % $HgCl_2$ transformed for methyltin trichloride at a $MeSnCl_3$:$HgCl_2$ ratio of 1:3.

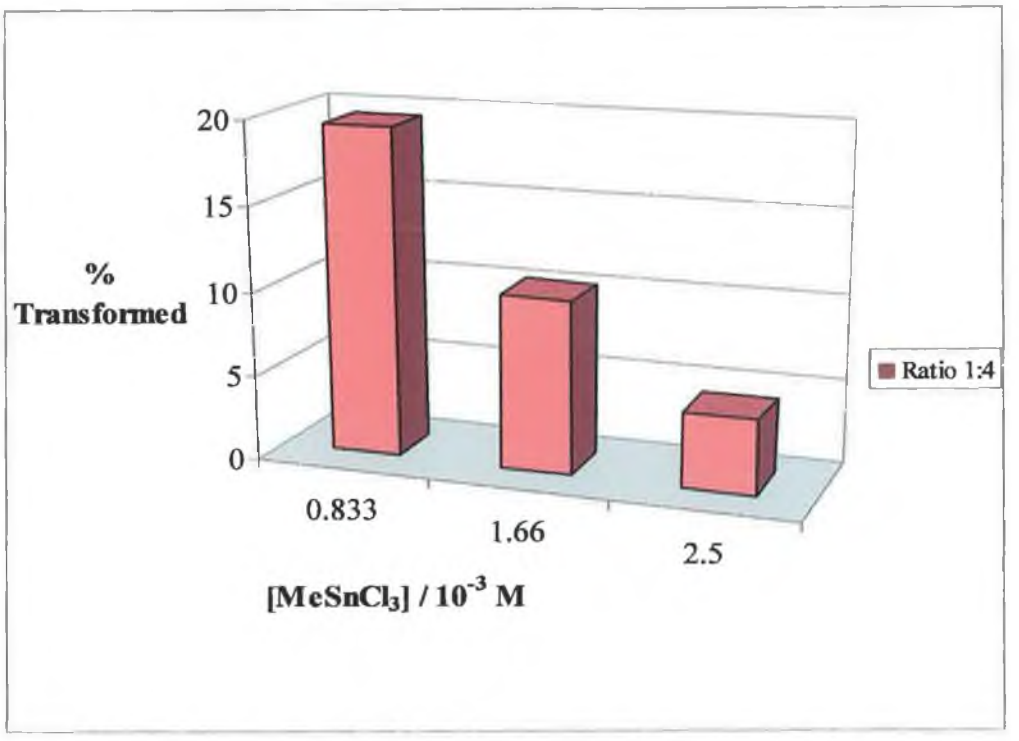


Fig [4.19] (c): Variation in % $HgCl_2$ transformed for methyltin trichloride at a $MeSnCl_3$:$HgCl_2$ ratio of 1:4.

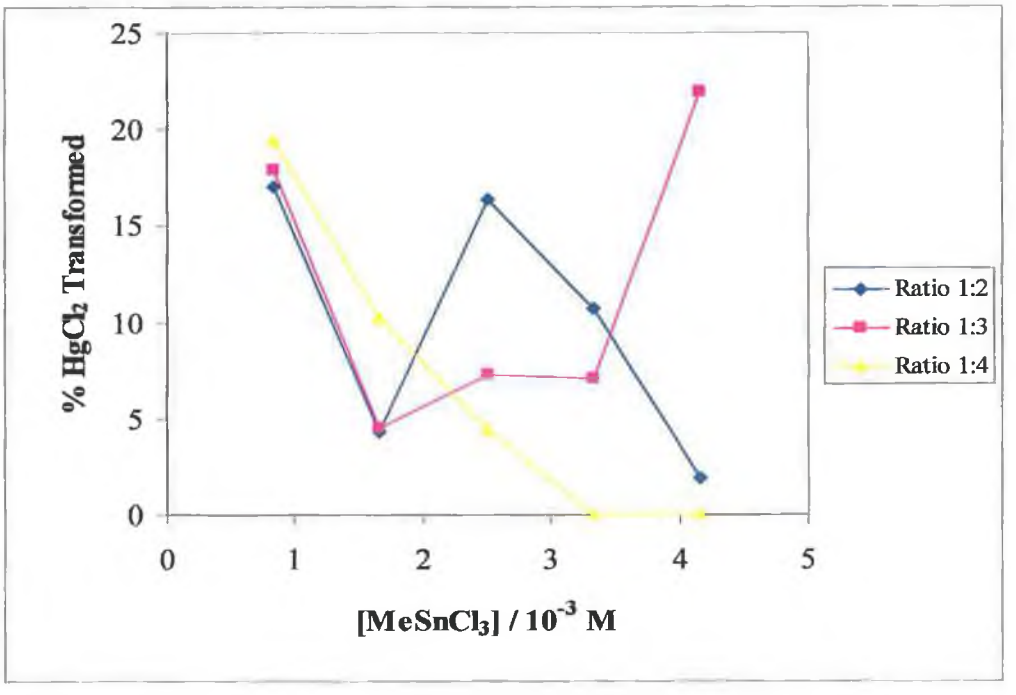


Fig [4.19] (d): Variation in % $HgCl_2$ transformed for methyltin trichloride over a $MeSnCl_3$ concentration range of 0.833 to 4.165 x 10^{-3} M, at $MeSnCl_3$:$HgCl_2$ ratios of 1:2, 1:3 and 1:4.

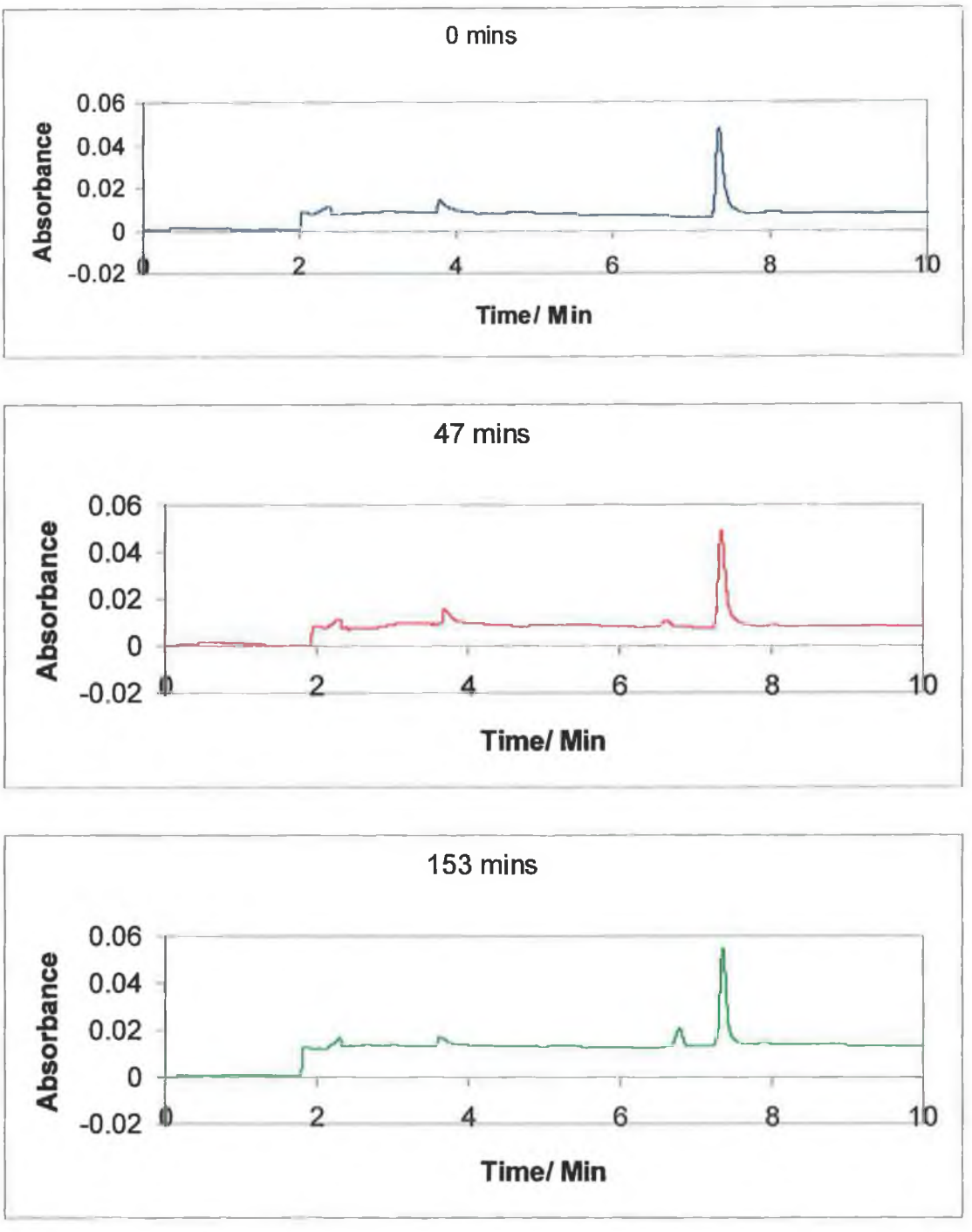


Fig. [4.20]: Electropherogram showing the transmethylation reaction of 1.66 x 10^{-3} M $MeSnCl_3$ with a $MeSnCl_3$:$HgCl_2$ ratio of 1:4. The figure shows the levels of both reactants and products present in the system at (i) 0 min; (ii) 47 min and (iii) 153 min.

Fig. [4.21] shows the concentrations of both the reactants and the products of the transmethylation reaction of $MeSnCl_3$ with $HgCl_2$ in a time decay plot. In Fig. [4.21] (a) a lower concentration (1.66×10^{-3} M) of $MeSnCl_3$ was reacted with a 1:2 mole ratio of $MeSnCl_3$:$HgCl_2$. The figure shows a decrease in both reactants is apparent, as is an increase in one product of the reaction, namely MeHgCl. It can be seen that the reaction does not proceed immediately on mixing, but takes approx. 60 mins for the first signs of product formation to appear. No decrease in reactants was noticed in this time. Increasing the initial $MeSnCl_3$ concentration from 1.66×10^{-3} M to 4.16×10^{-3} M, -over a two fold increase- led to a similar pattern emerging, as shown in Fig. [4.21] (b). Again, no product formation was seen in the first 60 minutes of the reaction, only a slight decrease in peak area for $HgCl_2$ was seen, over the entire reaction period.

Increasing the reaction ratio of $MeSnCl_3$:$HgCl_2$ from 1:2 to 1:3 had the effect of increasing the reaction rate. This is depicted clearly in Fig. [4.22] (a). Here, 0.83×10^{-3} M $MeSnCl_3$ was reacted with a 1:3 mole ratio of $HgCl_2$. Again, however, the reaction is delayed, but it can be seen that the reaction proceeds and products begin to form at around 40 minutes. Increasing the initial $MeSnCl_3$ concentration to 1.66×10^{-3} M, a four fold increase, led to an increase in the rate of the reaction. In this instance, the transmethylation reaction begins in under 30 minutes. Again only slight decreases in the peak areas of $HgCl_2$ are observed.

At a 1:4 reaction ratio, shown in Fig [4.23], a further increase in reaction rate with increased $HgCl_2$ concentration is observed. At the lower $MeSnCl_3$ concentration (Fig. [4.23] (a)), it can be seen that the MeHgCl doesn't begin to form until after 50 minutes reaction time has elapsed. At the higher $MeSnCl_3$ concentration, however, no delay is seen and the reaction is seen to proceed almost immediately. No decrease in the concentration of $HgCl_2$ is seen with increasing time.

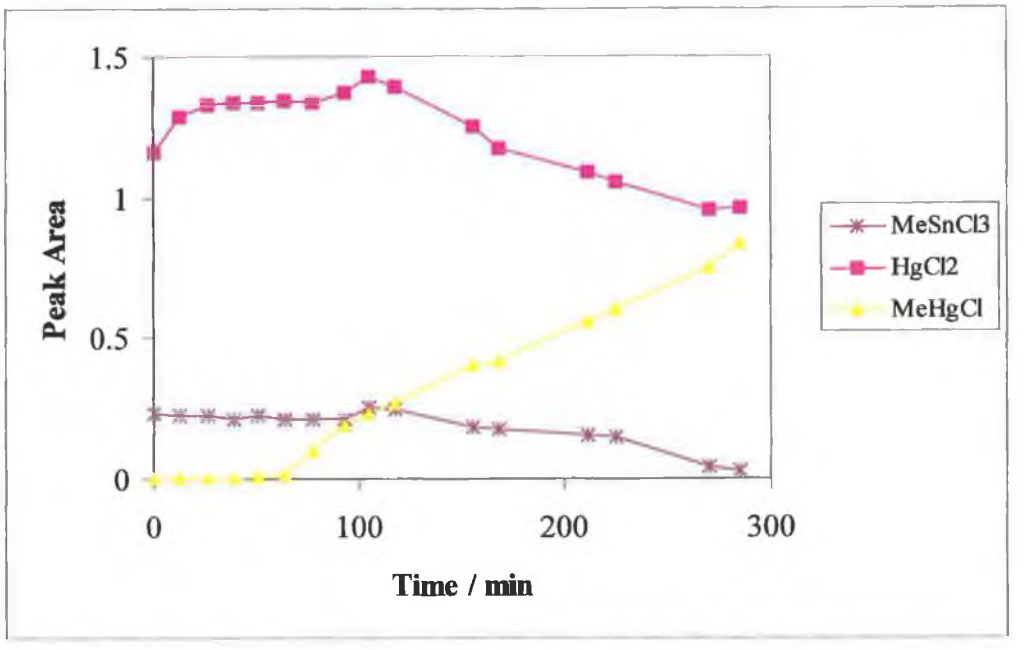


Fig. [4.21] (a): Time dependence curves for 1.66 x 10^{-3} M $MeSnCl_3$ at a $MeSnCl_3$:$HgCl_2$ ratio of 1:2.

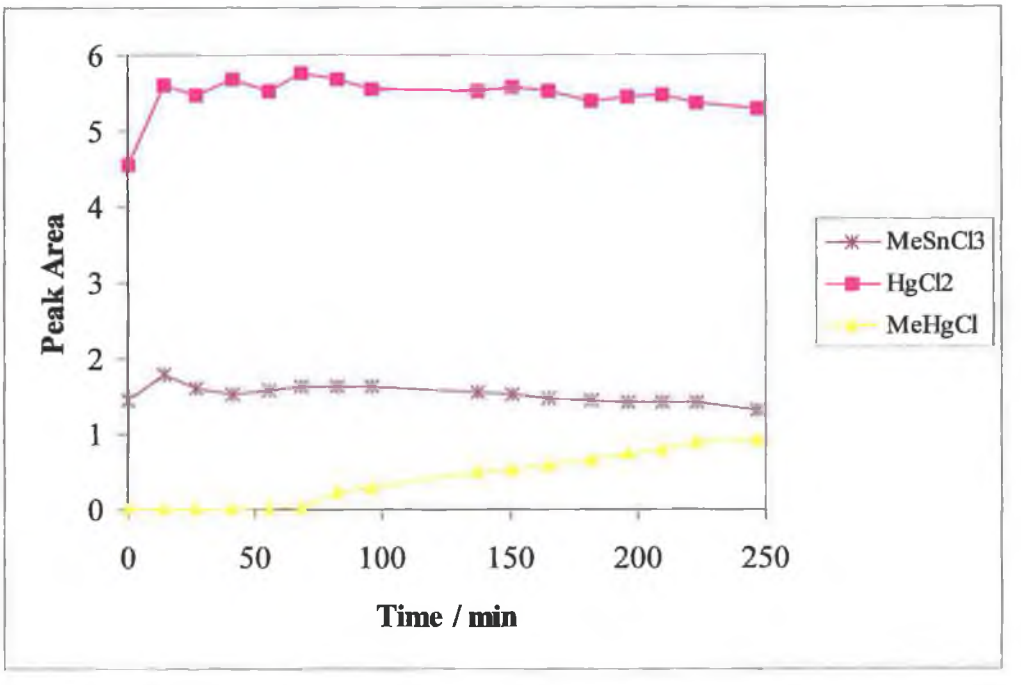


Fig. [4.21] (b): Time dependence curves for 4.165 x 10^{-3} M $MeSnCl_3$ at a $MeSnCl_3$:$HgCl_2$ ratio of 1:2.

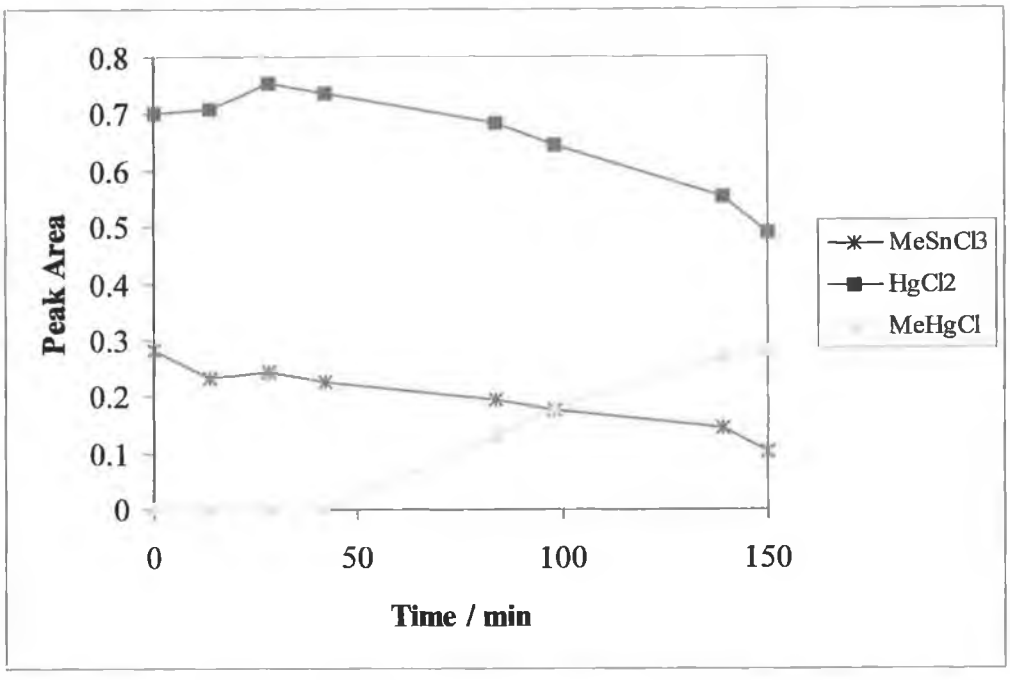


Fig. [4.22] (a): Time dependence curves for 0.833 x 10^{-3} M $MeSnCl_3$ at a $MeSnCl_3$:$HgCl_2$ ratio of 1:3.

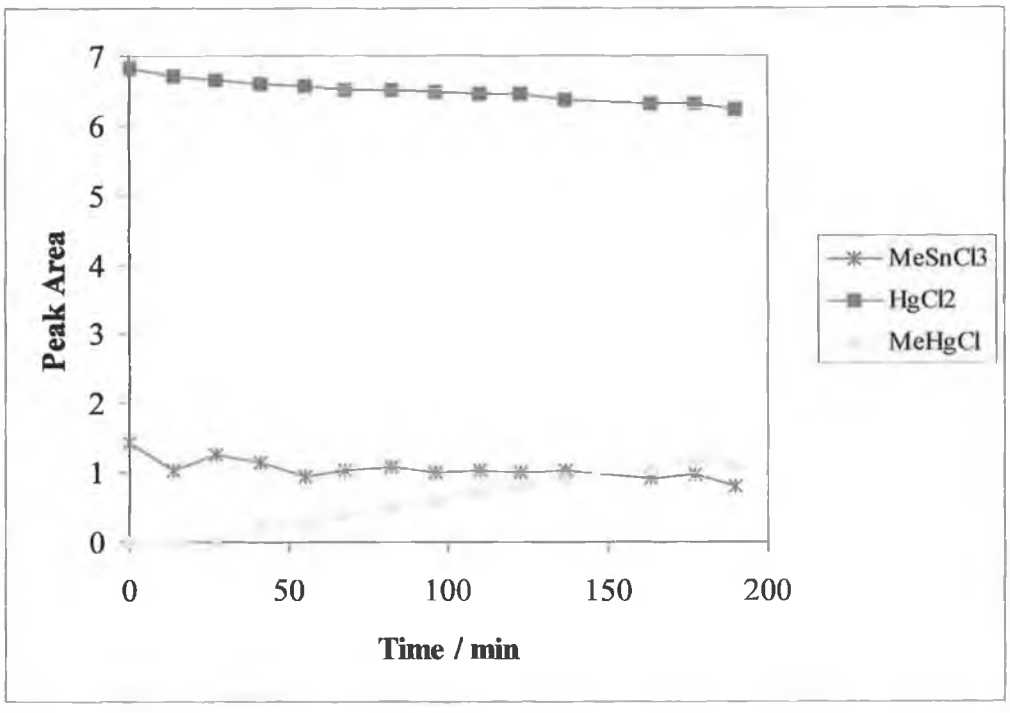


Fig. [4.22] (b): Time dependence curves for 3.3 x 10^{-3} M $MeSnCl_3$ at a $MeSnCl_3$:$HgCl_2$ ratio of 1:3.

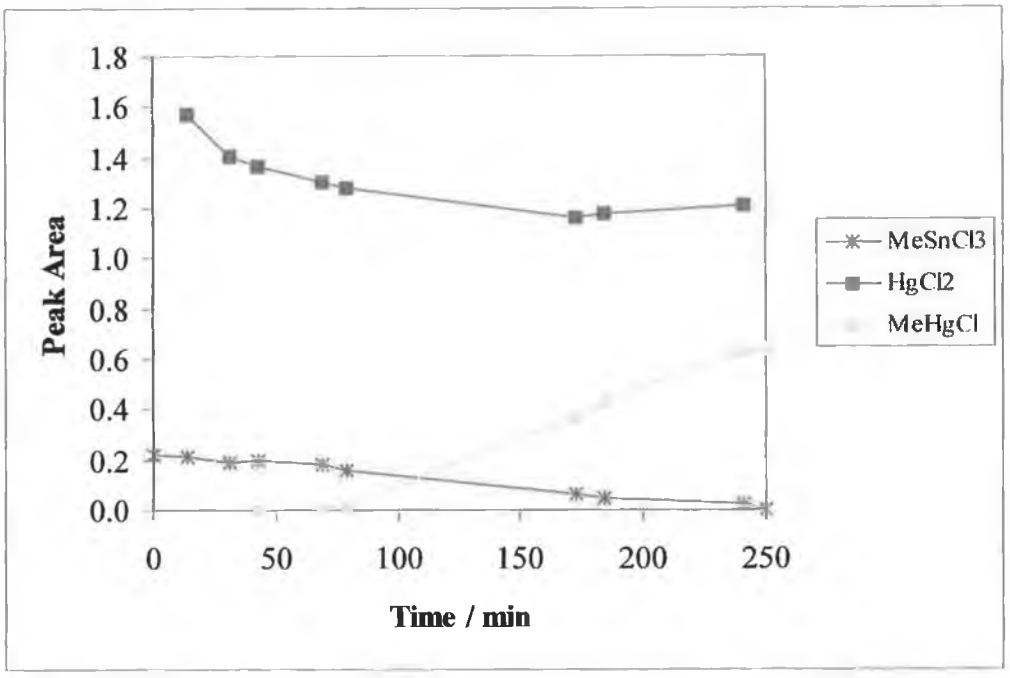


Fig. [4.23] (a): Time dependence curves for 0.833 x 10^{-3} M $MeSnCl_3$ at a $MeSnCl_3$:$HgCl_2$ ratio of 1:4.

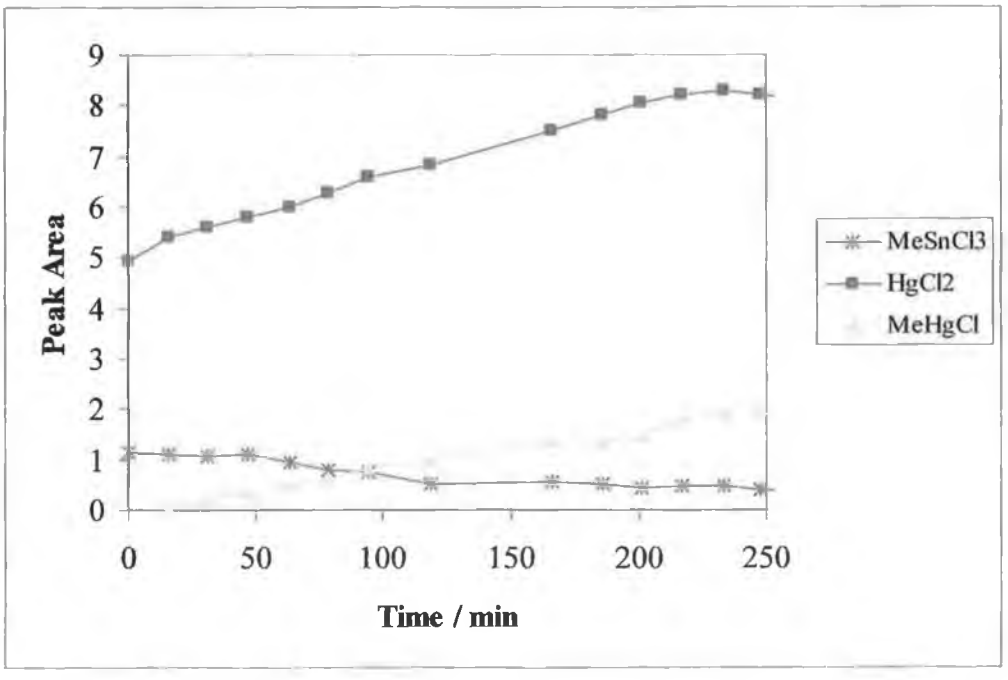


Fig. [4.23] (b): Time dependence curves for 3.33 x 10^{-3} M $MeSnCl_3$ at a $MeSnCl_3$:$HgCl_2$ ratio of 1:4.

$MeSnCl_3$	$MeSnCl_3$: $HgCl_2$	$HgCl_2$	$[MeSnCl_3]_{150\ min}$	$[HgCl_2]_{150\ min}$
8.33×10^{-4} M	1:2	1.66×10^{-3} M	-	-
	1:3	2.5×10^{-3} M	-	-
	1:4	3.33×10^{-3} M	-	-
1.66×10^{-3} M	1:2	3.33×10^{-3} M	-	0.435
	1:3	5.0×10^{-3} M	-	0.896
	1:4	6.66×10^{-3} M	-	2.48
2.5×10^{-3} M	1:2	5.0×10^{-3} M	-	0.686
	1:3	7.5×10^{-3} M	0.129	2.625
	1:4	1.0×10^{-2} M	0.118	5.129
3.33×10^{-3} M	1:2	6.66×10^{-3} M	0.140	1.827
	1:3	1.0×10^{-2} M	0.196	4.090
	1:4	1.33×10^{-2} M	0.047	4.914
4.165×10^{-3} M	1:2	8.33×10^{-3} M	0.425	3.542
	1:3	1.25×10^{-2} M	0.288	4.769
	1:4	1.66×10^{-2} M	0.387	9.355

TABLE [4.5]: Transmethylation reactions attempted in aqueous solution

Table [4.5] shows the various transmethylation reactions attempted for methyltin trichloride and mercury (II) chloride. As the concentration of mercury(II)chloride added is increased then the residual concentration remaining after the specified time period also increases. This is also the case as the initial $MeSnCl_3$ concentration is increased.
A comparison of the time dependence curves for particular species at the various $MeSnCl_3$ concentrations was examined in Fig. [4.24]. Here a comparison of the rate of decrease of methyltin trichloride in the transmethylation reaction of $MeSnCl_3$ and $HgCl_2$ is shown. The rate of decrease is monitored with increasing time, as the initial concentration of $MeSnCl_3$ is increased from 8.33×10^{-4} M to 4.165×10^{-3} M. Each reaction was carried out at a $MeSnCl_3$:$HgCl_2$ ratio of 1:3. Similar rates of reaction are apparent at each $MeSnCl_3$ concentration with none of the reactions showing a total consumption of the reactant.

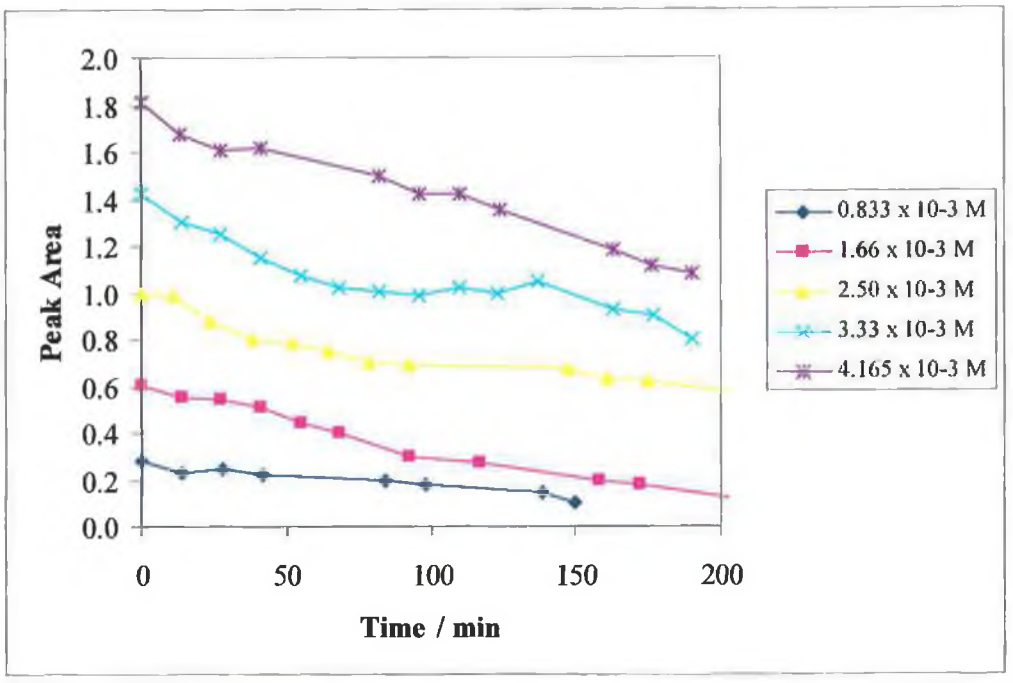


Fig. [4.24] (a): Comparison of the rate of decrease in $MeSnCl_3$ using methyltin trichloride concentrations in the range 0.833 to 4.165 x 10^{-3} M, at a 1:3 ratio of $MeSnCl_3$:$HgCl_2$.

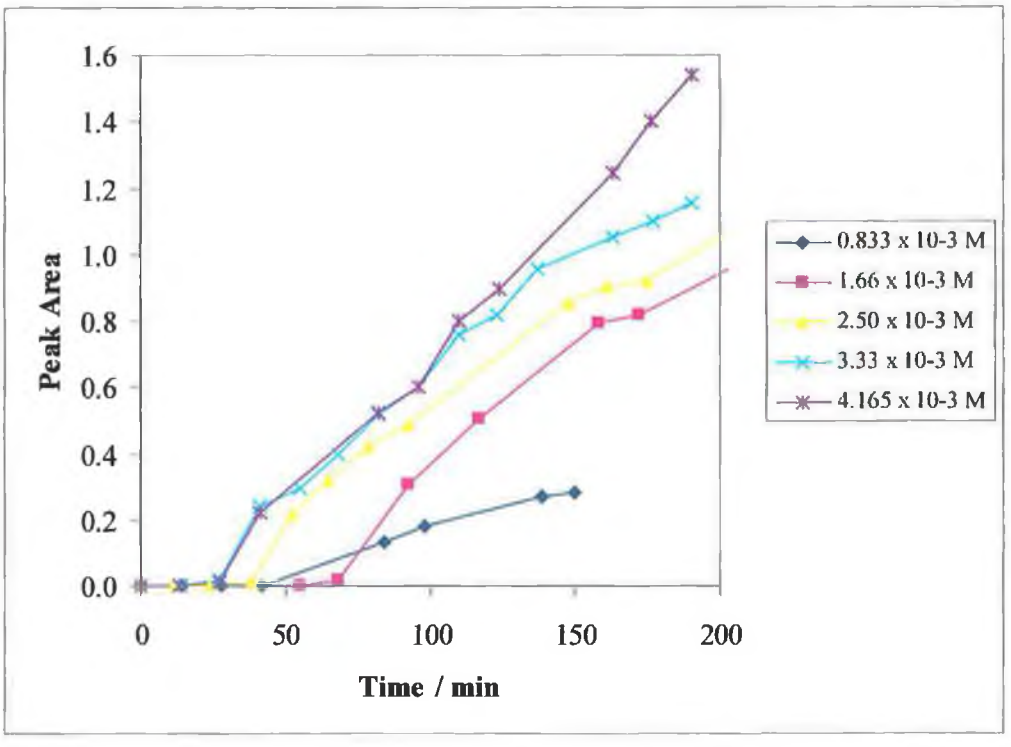


Fig. [4.24] (a): Comparison of the rate of increase in MeHgCl using methyltin trichloride concentrations in the range 0.833 to 4.165 x 10^{-3} M, at a 1:3 ratio of $MeSnCl_3$:$HgCl_2$.

Fig. [4.24] (b) shows the increase in MeHgCl formed as a product of the reaction for the entire range of $MeSnCl_3$ concentrations examined. Increasing the $MeSnCl_3$ concentration has the effect of decreasing the length of time taken for the reaction to begin. An equilibrium was not established for any of the reactions studied, indicating a longer timescale was necessary for the reaction to reach completion.

Fig. [4.25] shows a study of the effect of increasing the mercury(II)concentration at one methyltin trichloride concentration. Here, the $MeSnCl_3$ concentration remained constant and the rate of increase/decrease of the products/reactants in the transmethylation reaction are examined at various mole ratios of Me_2SnCl_2:$HgCl_2$. Firstly, the rate of increase of MeHgCl with time was examined, as shown in Fig. [4.25] (a). Clearly, as the relative ratio of $MeSnCl_3$:$HgCl_2$ is increased there is an accompanying decrease in the time taken for a reaction to begin. The greater the $HgCl_2$ concentration, the sooner the transmethylation reaction begins. Fig. [4.25] (b) shows the decrease in methyltin trichloride over the course of the reaction. It is notable that as the concentration of $HgCl_2$ added to the reaction is increased, the rate of decrease in $MeSnCl_3$ also appears to increase.

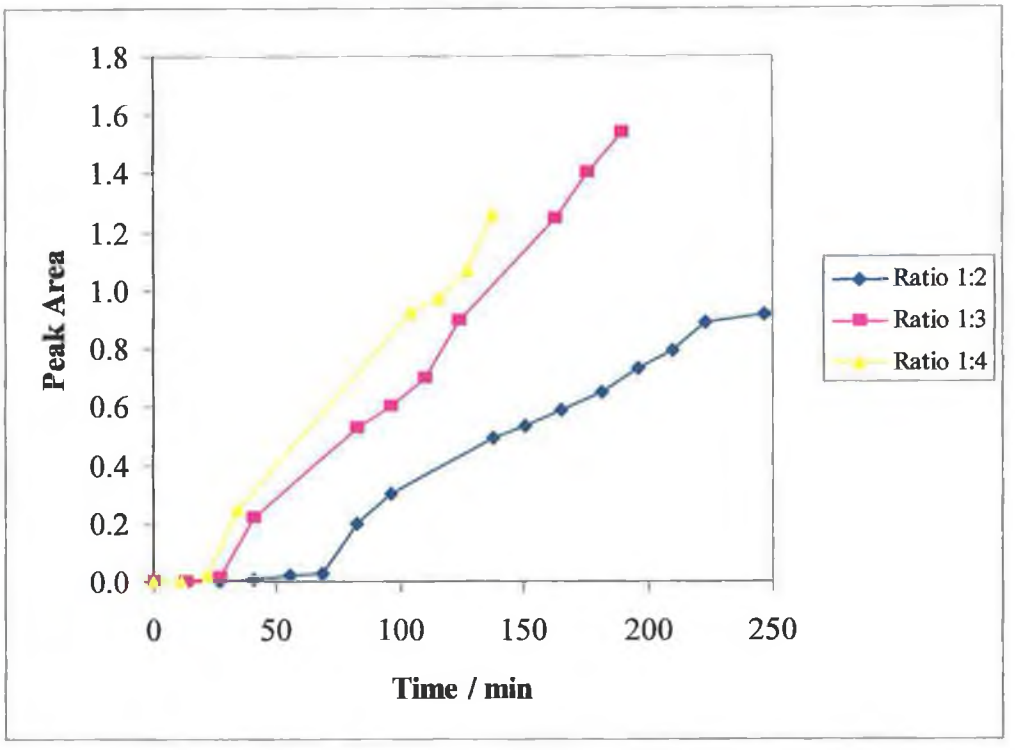


Fig. [4.25] (a): Comparison of the rate of decrease in $MeSnCl_3$ using a methyltin trichloride concentration of 4.165 x 10^{-3} M, at various ratios of $MeSnCl_3$:$HgCl_2$.

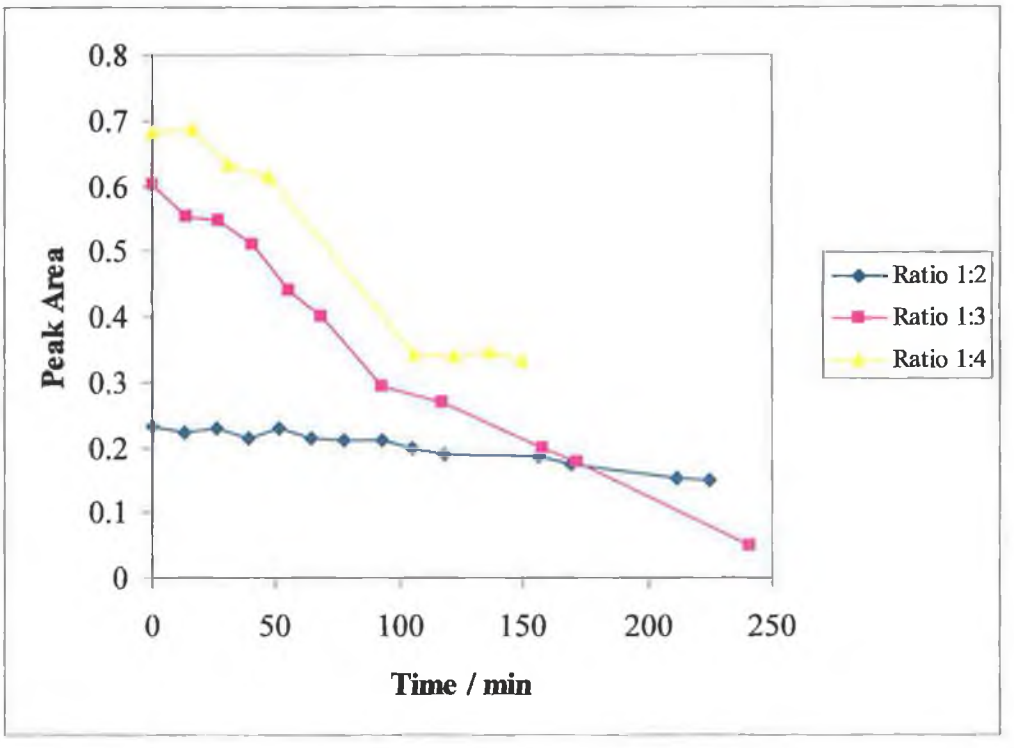


Fig. [4.25] (b): Comparison of the rate of decrease in $MeSnCl_3$ using a methyltin trichloride concentration of 1.66 x 10^{-3} M, at various ratios of $MeSnCl_3$:$HgCl_2$.

4.3.4. Kinetic studies

The rate at which one species may be turned into another in a chemical reaction is of central importance both practically and theoretically. Chemical kinetics reveal information about the mechanism of the interconversion of molecules: to discover whether the reaction takes place in one step or a sequence of steps, and discover whether or not the solvent plays a significant role. Experiments give this information and theories attempt to explain it. Methods are needed that can monitor the reaction in the timescale in which it is complete.

Rate data, (i.e. decay plots for reactant concentration or growth plots for products) are the basic raw material of reaction kinetics. The shape of these plots depends on the rate constant for the reaction, and on the reactant concentrations. The functional form of the dependence on concentration enables us to determine the reaction order and hence categorise the reaction, and possibly say something about its mechanism.

In these kinetic studies, the experimental data consists of reactant concentrations as a function of time. The first task was to obtain from these a reaction order and a rate constant. Experimental conditions were designed to enforce pseudo first order kinetics on the reactions being studied. Pseudo-first-order conditions mean that one reactant is in sufficient excess to have approximately the same concentration after 100% reaction.

Thus, the rate law given by Eq. [4.1] applies:

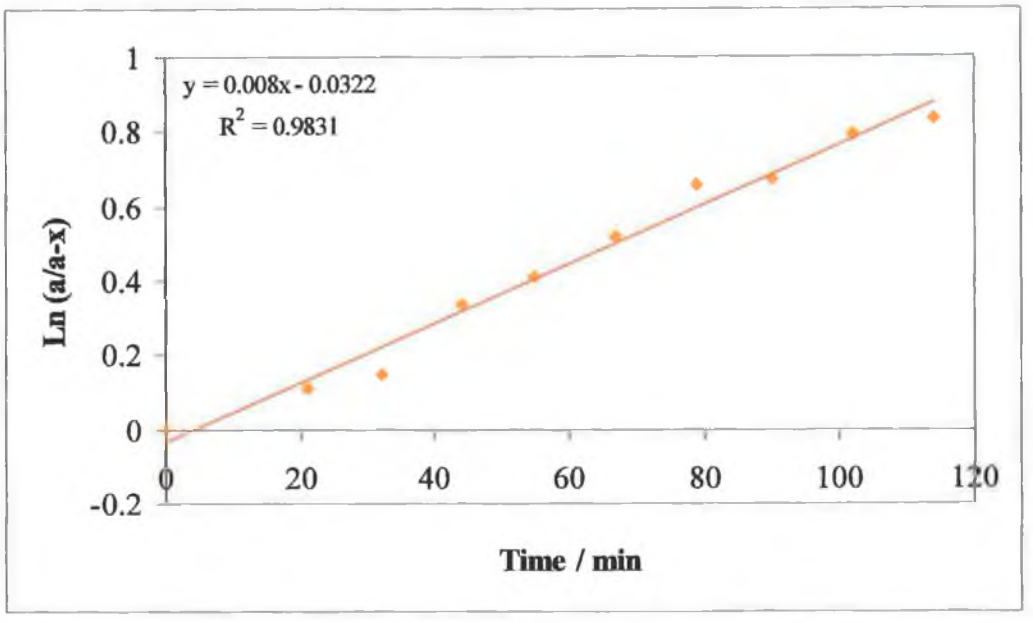
$$-kt = \ln(a/a-x) \qquad [4.1]$$

where a is the initial concentration of the methyltin species and a-x is the fraction remaining, k is the pseudo-first-order rate constant and t is the time (min). The constant k yields the second-order-rate constant (k_2), at the given pH value, given by Eq [4.2].

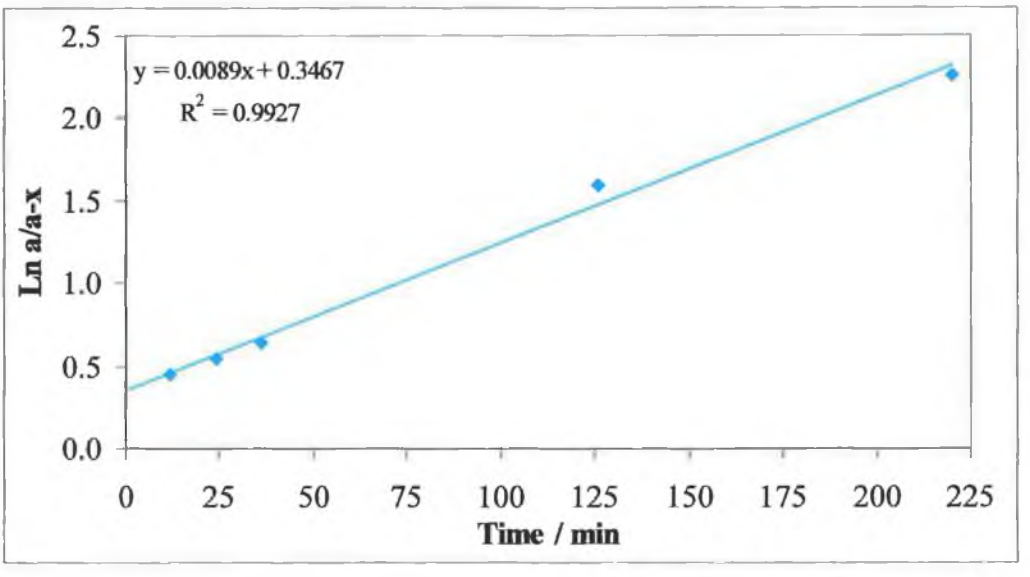
$$k = k_2[HgCl_2]_0 \qquad [2]$$

where $[HgCl_2]_0$ is the initial concentration (mol dm^{-3}) of $HgCl_2$.

A series of experiments were run at pH 8.25, at concentration ratios of 1:2, 1:3 and 1:4 of (a) trimethyltin chloride, (b) dimethyltin dichloride and (c) methyltin trichloride with mercury(II)chloride. Even the lowest $HgCl_2$ excess is sufficient for pseudo-first order kinetics, and so we can calculate the second order rate constant. The pseudo-first-order rate constants for the transmethylation reactions of were determined by linear regression analysis. A typical example of the calculation of the pseudo-first-order rate constant is shown in Figs. [4.26] and [4.27]. Here the transmethylation reactions of two different trimethyltin chloride concentrations with a 1:2 and 1:3 mole ratio of Me_3SnCl:$HgCl_2$ are shown, respectively. From the slope of the line obtained it was then possible to determine k_2, the second order rate constant.

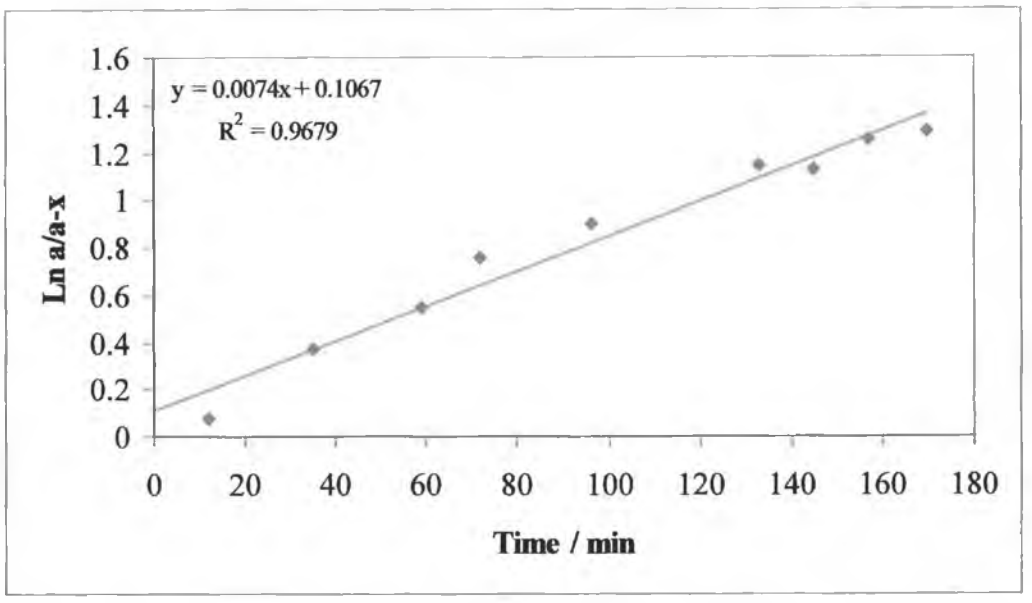


(A)

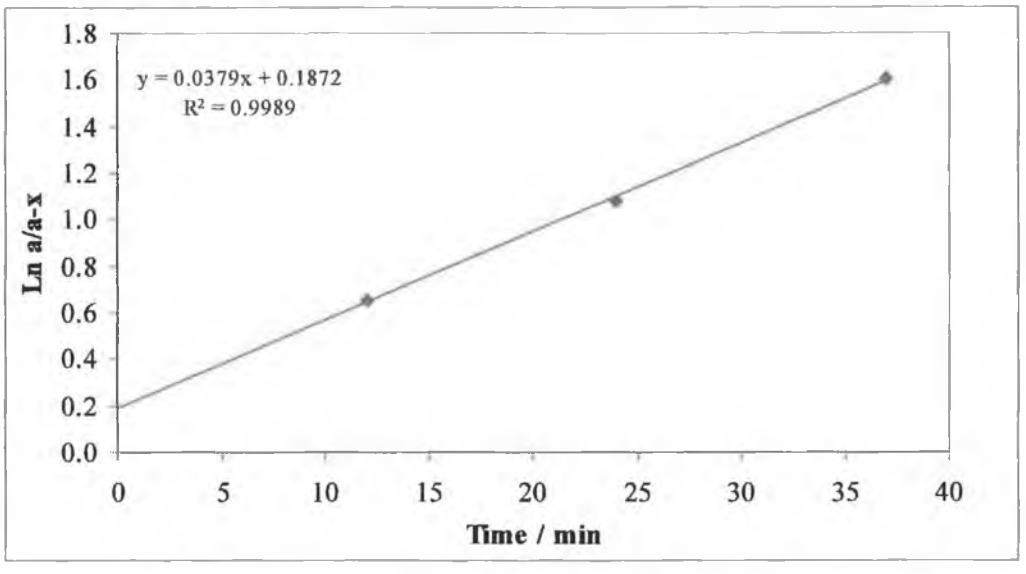


(B)

Fig. [4.26]: First order plots of (A) 0.5 x 10^{-3} M Me_3SnCl and (B) 1.0 x 10^{-3} M Me_3SnCl at a 1:2 mole ratio of $Me_3SnCl:HgCl_2$.



(A)



(B)

Fig. [4.27]: First order plots of the transmethylation reaction at a 1:3 reaction ratio of $Me_3SnCl:HgCl_2$ for (A) 0.5 x 10^{-3} M Me_3SnCl and (B) 1.0 x 10^{-3} M Me_3SnCl.

Expt. No.	Methyl Donor	[Methyl Donor]/M	Methyl Donor:$HgCl_2$	[$HgCl_2$]/ M	k (min^{-1})	k_2 ($dm^3\ mol^{-1}\ min^{-1}$)
1	Me_3SnCl	$0.5x10^{-3}$	1:2	$1.0x10^{-3}$	0.0035	3.5
2		$0.5x10^{-3}$	1:3	$1.5x10^{-3}$	0.0074	4.9
3		$0.5x10^{-3}$	1:4	$2.0x10^{-3}$	0.0227	11.35
4	Me_3SnCl	$1.0x10^{-3}$	1:2	$2.0x10^{-3}$	0.0089	4.45
5		$1.0x10^{-3}$	1:3	$3.0x10^{-3}$	0.0379	12.63
6		$1.0x10^{-3}$	1:4	$4.0x10^{-3}$	0.1745	43.63
7	Me_3SnCl	$2.0x10^{-3}$	1:2	$4.0x10^{-3}$	0.0082	2.05
8		$2.0x10^{-3}$	1:3	$6.0x10^{-3}$	0.1081	18.02
9		$2.0x10^{-3}$	1:4	$8.0x10^{-3}$	0.2946	36.83
10	Me_3SnCl	$3.0x10^{-3}$	1:2	$6.0x10^{-3}$	0.136	22.67
11		$3.0x10^{-3}$	1:3	$9.0x10^{-3}$	0.2731	30.34

(a) Trimethyltin chloride

Expt. No.	Methyl Donor	[Methyl Donor]/ M	Methyl Donor:$HgCl_2$	[$HgCl_2$]/ M	k (min^{-1})	k_2 ($dm^3\ mol^{-1}\ min^{-1}$)
12	Me_2SnCl_2	$0.9x10^{-3}$	1:2	$1.8x10^{-3}$	0.0135	7.5
13		$0.9x10^{-3}$	1:3	$2.7x10^{-3}$	0.0192	7.11
14	Me_2SnCl_2	$1.8x10^{-3}$	1:2	$3.6x10^{-3}$	0.0277	7.69
15		$1.8x10^{-3}$	1:4	$7.2x10^{-3}$	0.0724	10.06
16	Me_2SnCl_2	$3.6x10^{-3}$	1:3	$10.8x10^{-3}$	0.1162	10.76
17		$3.6x10^{-3}$	1:4	$14.4x10^{-3}$	0.1652	11.47

(b) Dimethyltin dichloride

Expt. No.	Methyl Donor	[Methyl Donor]/M	Methyl Donor:$HgCl_2$	[$HgCl_2$]/ M	k (min^{-1})	k_2 ($dm^3\ mol^{-1}\ min^{-1}$)
18	$MeSnCl_3$	$0.833x10^{-3}$	1:2	$1.66x10^{-3}$	0.0029	1.75
19		$0.833x10^{-3}$	1:3	$2.5x10^{-3}$	0.0054	2.16
20		$0.833x10^{-3}$	1:4	$3.33x10^{-3}$	0.0095	2.85
21	$MeSnCl_3$	$1.66x10^{-3}$	1:2	$3.33x10^{-3}$	0.0019	0.57
22		$1.66x10^{-3}$	1:3	$5.0x10^{-3}$	0.0068	1.36
23		$1.66x10^{-3}$	1:4	$6.66x10^{-3}$	0.0053	0.80
24	$MeSnCl_3$	$2.5x10^{-3}$	1:2	$5.0x10^{-3}$	0.0036	0.72
25		$2.5x10^{-3}$	1:3	$7.5x10^{-3}$	0.0038	0.41
26		$2.5x10^{-3}$	1:4	$10.0x10^{-3}$	0.0011	0.11
27	$MeSnCl_3$	$3.33x10^{-3}$	1:2	$6.66x10^{-3}$	0.0006	0.09
28		$3.33x10^{-3}$	1:3	$10.0x10^{-3}$	0.0017	0.17
29		$3.33x10^{-3}$	1:4	$13.33x10^{-3}$	0.0067	0.50

(c) Methyltin trichloride

Table [4.6]: Rate constants for methyl transfer from methyltin compounds to mercury (II) chloride. Ref. Appendix 1 for further details

Looking at each group of reactions separately, it can be seen that the transmethylation reaction between Me_3SnCl and $HgCl_2$ occurs rapidly and that the reaction does indeed occur more aggressively if the initial concentration of $HgCl_2$ is increased. This is seen as the rate constant increases with increasing $HgCl_2$ concentration. An increase in the rate constant of at least one order of magnitude was found for expt.'s 1→3, 4→6, and 7→9. An increase in k_2 was also observed on comparison of similar concentration series of reactions. For example, a 1:3 ratio of Me_3SnCl:$HgCl_2$ gives k_2 values of 4.9, 18.02 and 30.34 $dm^3\ mol^{-1}\ min^{-1}$, on increasing the Me_3SnCl concentration from $0.5x10^{-3}$M to $2.0x10^{-3}$M to $3.0x10^{-3}$M, respectively.

Examination of the rate constants for the transmethylation reaction of Me_2SnCl_2 and $HgCl_2$ reveals small increases in k_2 values for each series of reactions on increasing both Me_2SnCl_2 and $HgCl_2$ concentrations. The k_2 values obtained range from 7.5 to 11.47 dm^3 mol^{-1} min^{-1}, which corresponds to the lowest Me_2SnCl_2 and $HgCl_2$ concentration to the highest Me_2SnCl_2 and $HgCl_2$ concentrations. It can be seen that the actual rate of the transmethylation reaction of Me_2SnCl_2 with $HgCl_2$ is greater than for the reaction of Me_3SnCl with $HgCl_2$ at both 1:2 and 1:3 mole ratios but is less than for the reaction of Me_3SnCl with $HgCl_2$ at the 1:4 ratio suggesting that Me_2SnCl_2 is a faster methyl donor at lower $HgCl_2$ concentrations and that Me_3SnCl is a faster methyl donor than Me_2SnCl_2 at the higher $HgCl_2$ concentrations.

Similar observations were made for the reaction of $MeSnCl_3$ with $HgCl_2$. However, here it can be seen that increasing the $MeSnCl_3$ concentration leads to a decrease in the rate constants recorded. Keeping the ratio of $MeSnCl_3$:$HgCl_2$ at a ratio of 1:4, it can be seen that increasing the $MeSnCl_3$ concentration from $0.83x10^{-3}$ M to $1.66x10^{-3}$ M to 2.5×10^{-3} M to $3.33x10^{-3}$ M gives rate constants from 2.85 to 0.80 to 0.11 to 0.50

Keeping the $MeSnCl_3$ concentration constant but increasing the $HgCl_2$ concentration leads to an increased reaction rate, as exemplified by reactions 31→33. Again the k_2 values recorded, ranging from 0.11 to 2.85 dm^3 mol^{-1} min^{-1}, are less than those obtained for Me_2SnCl_2 and also Me_3SnCl.

Comparing similar reactions for each methyltin compound (Ratio 1:2; expt.'s 7, 14, 21) reveals the following order of k_2 (dm^3 mol^{-1} min^{-1}): Me_2SnCl_2 (7.69) > Me_3SnCl (2.05) > $MeSnCl_3$ (0.57). Increasing the reaction ratio to 1:4 (expt.'s 9, 15, 23) reveals the following order of k_2 (dm^3 mol^{-1} min^{-1}): Me_3SnCl (36.83)> Me_2SnCl_2 (10.06)> $MeSnCl_3$ (0.80). Therefore a difference in order of k_2 values occurs depending on the concentration of $HgCl_2$ present. At the low concentration series of reactions, at pH 8.25, the relative k_2 values were in the order Me_2SnCl_2>Me_3SnCl>$MeSnCl_3$, whereas at the high concentration series of reactions, at pH 8.25, the relative k_2 values were in the order Me_3SnCl>Me_2SnCl_2>$MeSnCl_3$.

The transmethylation reaction of the various methyltin species with mercury(II)chloride was found to be a second order reaction. If a reaction is second order this means that the rate of the reaction is dependent on the concentrations of both reactants. The order of a reaction is based directly on experimental observations of the dependence of the reaction rate on concentration, as shown above.

The order of a reaction, however, says nothing about the mechanism of the reaction. The mechanism may be found by careful examination of the reaction, i.e. by having a detailed knowledge of the nature of the reactants and also of the nature of the products. The detection (direct or indirect) of a suspected intermediate also aids in the determination of a reaction mechanism.

4.4. Discussion

This series of experiments have shown that the transformation of alkyl groups from one species to the other is possible for those species used in our experiments. Under laboratory conditions, the main factors influencing this transformation is the methyltin species present i.e. $Me_3SnCl/Me_2SnCl_2/MeSnCl_3$ and also the concentration of reactants present and their relative ratios. Reaction rates have shown that Me_3SnCl is the fastest methyl donor at high methyltin and /or high mercury (II) chloride concentrations. It should also be noted that the transformation of one methyl group was favoured over the transfer of two methyl groups which would have led to the formation of dialkylated mercury species. The amount of the transformation seems to depend on the concentrations of the relevant species present.

Methyl transfer to mercury has long been known. However, individual complexes show large differences in reactivity as methyl donors. The mechanism and rates of reactions depend not only on the nature of the methyl acceptor, but also on the nature of groups present other than the methyl being transferred. The methyl transfer reactions from methylcobalamin ($MeCoB_{12}$) to mercury (II) acetate have been studied by many groups. The transfer of a methyl group from a methyl tin species to mercury (II) chloride was therefore not in question. The aim of this work was to determine the conditions which promote this transfer, and if possible, to determine the mechanism in which the methyl transfer occurs. Although performed at higher than environmentally significant concentrations these methyl transfer reactions are still of environmental importance because organometallic compounds are much more toxic than their inorganic counterparts.

It was necessary in the early stages of this investigation to determine the number of methyl groups which were transferred in the transmethylation process. Initial suggestions were that under certain concentration conditions two methyl groups could be transferred, leading to the formation of dimethylmercury as a product of the reaction. This, however, was not found experimentally. Nevertheless, both dimethyltin dichloride and methyltin trichloride were formed as products of the reaction of trimethyltin chloride with mercury (II) chloride, which would suggest the possible transfer of one or two methyl groups. Subsequent experiments in which both dimethyltin dichloride and methyltin trichloride were each reacted with mercury (II) chloride reveal that only one methyl group is transferred, but that secondary reactions do occur. This contradicts evidence put forth by Brinckman[7], who noted that the reaction of trimethyltin chloride with mercury (II) chloride was a second-order process, and that the dimethyltin species formed as product did not react with mercury (II) chloride.

The percentage transformation of the methyl groups was also considered. It was found that, for both trimethyltin chloride and dimethyltin dichloride, in each case examined, a minimum of 40% of the $HgCl_2$ species present were transformed, with the highest % transformation values being recorded for trimethyltin chloride at mole ratios greater than

1:3. However, for methyltin trichloride, the maximum percentage transformation was approximately 20%, notably lower than for either of the other two species examined. This low % transformation was thought to be due to the difficulty encountered by the molecule in losing its final methyl group, which would be tightly bound to the tin atom.

Time dependence curves for each transmethylation reaction studied were drawn and compared. These decay plots for reactant concentrations show that the rate at which the reaction proceeds depends on the reactant concentrations. This dependence allows the rate of the reaction to be determined. The mechanism of the reaction may also be established from these curves.

It was found that the transmethylation reaction of trimethyltin chloride with mercury (II) chloride was thought to be a second order process. Similarly, the transmethylation reactions of dimethyltin dichloride and methyltin trichloride with mercury (II) chloride were also thought to be second order processes. The rate of methyl transfer was found, at high $HgCl_2$ concentrations, to be fastest for the trimethyltin reaction, followed by dimethyltin dichloride and finally the methyltin trichloride reaction. At lower $HgCl_2$ concentrations it was found that methyl transfer was fastest for the dimethyltin dichloride reaction followed by the trimethyltin chloride reaction and again the reaction of methyltin trichloride was found to be slowest. The rate of the transmethylation reaction was found to be affected by both the initial concentration of the particular methyltin species and also the concentration of mercury (II) chloride present in the reaction mixture. This suggests a second order process.

4.5. References

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5. The Behaviour of the Methyltin Chlorides when bound to a Solid Support in the Presence of Inorganic Mercury (II): Transalkylation of Mercury Species and their analysis by CZE

5.1. Introduction

Much attention has focussed on the chemistry of organotins in the aquatic environment. Indeed, the previous chapters have discussed the chemistry of the transmethylation reaction of organotin compounds with mercury (II) chloride in liquid media. However, it is important to note that organotins exist in other compartments in the environment. These would include soils due to their application as biocides in agriculture and sediments where organotins have also been found. Therefore, in examining their chemistry it is important not to neglect these areas in determining their persistence and fate in the environment.

Previous investigations have shown that a transmethylation reaction occurs between tetramethyltin and mercury (II) chloride in solution at pH 8.25. The greatest rate of reaction was found to occur for trimethyltin chloride at the highest concentrations of both trimethyltin chloride and of mercury (II) chloride examined. However, this result would be of greater significance if the transmethylation reaction could be shown to occur under simulated environmental conditions.

Here, the effect of binding the organotin species to a solid support on the transmethylation reaction of the organotin species with mercury (II) chloride was examined. It was the intention that binding the organotin species to a resin would mimic its binding in the environment to soils or sediments. Having ensured that the methyltin species had been adsorbed onto the solid material, it was then reacted with mercury (II) chloride. The quantities of both species reacted were similar to those used in Chapter 4 for the reaction of these species in a liquid medium. Using these similar quantities allows the comparison of the relative rates of the transmethylation reaction in both the liquid and solid phase.

Rosenkranz et al.[1] analyzed a contaminated soil sample to ensure that results obtained for the transalkylation of mercury species in aqueous solutions were of fundamental

importance. One part of the sample was spiked with a stock solution of inorganic mercury. The samples were then stored for four days at room temperature. On analysis the predominant species were found to be dimethylmercury and inorganic mercury but methyl- and ethyl- mercury were also detected. The transformation rate of the alkyl groups was found to increase with the increase in the concentration of inorganic mercury and took place in the same way as was found in aqueous solution.

Considerable evidence[2] based mainly on experiments with sediment samples in the laboratory, suggests that environmental methylation of Hg(II) occurs by a biological process. Typically researchers have added Hg(II) to active and sterilized sediments and observe that MeHg occurs only in active sediments. In addition, Compeau and Bartha[3] found little or no methylation of Hg(II) in the presence of MnO_4^{2-}, which inhibits silphate-reducing bacteria, and concluded that those bacteria are the main methylating agents of Hg(II) in sediments. However, it is clear that evidence for the exclusive biological methylation of Hg(II) in the environment is quite weak and abiotic methylation may play an important role in MeHg production.

Chau et al.[4] studied the feasibility of methyl group transfer from methyl derivatives of arsenic, mercury and lead to Sn(II), Sn(IV) and Pb(II) species. These studies were conducted in abiotic chemical systems using distilled water and in biological systems containing sediment. It was found that alkyllead compounds could transfer their alkyl groups to Sn(II) and Sn(IV) ions to form various methyltin compounds in biotic and abiotic systems. The presence of sediment was found to enhance the transmethylation reactions. Methylarsenic acids transfer their methyl groups to Sn(II) and Sn(IV) in an abiotic system, but not in a biotic system containing sediment. The strong adsorption of tin onto sediment was the reason for the non-availability of tin ions for methylation.

Preliminary studies on the effect of using a solid support were carried out using TLC plates as a solid support in the UV degradation of tetramethyltin. Tetramethyltin samples developed in ethyl acetate prior to UV exposure had R_f values of 0.8. However, following UV exposure for ~6 hrs, the sample had turned yellow, and, on developing the

plate a spot remained on the baseline, while there was also a spot with an R_f of 0.8 indicating that Me_4Sn was still present.

In order to identify the spot on the baseline, a sample of tetramethyltin, which previously had been subjected to UV exposure for 114 hrs, was analyzed by TLC. This sample had already been analyzed by ^1H NMR to reveal the presence of Me_3SnCl, and Me_2SnCl_2, along with Me_4Sn. On developing the TLC plate in ethyl acetate, three spots were apparent. One spot remained on the baseline, a second spot could be seen two thirds of the way up the plate (R_f =0.62), and the third spot had the same R_f as tetramethyltin. Samples of both trimethyltin chloride and dimethyltin dichloride were then developed in the same TLC system to reveal R_f values of 0.62 and 0 respectively. These results indicate that UV degradation of tetramethyltin to both trimethyltin chloride and dimethyltin dichloride did occur while tetramethyltin was bound to the silica.

Having shown that UV degradation of tetramethyltin could occur while bound to silica, the next step was to determine if a transmethylation reaction could occur while the tin species was bound to a solid material. In order to do this an appropriate material had to be found and a method developed for loading the tin species onto the material, its subsequent reaction with mercury (II) chloride and finally the elution of all products of the reaction from the material.

In developing the method for binding the tin species to a solid material a number of factors were examined, namely:

- Separation strategy i.e. reverse phase, normal phase or ion exchange
- Packing material
- Sample loading method
- Elution protocol

5.1.1. Separation strategy/packing material

The selection of a separation strategy involves careful consideration of the task to be undertaken. Here, chromatographic conditions were chosen that caused the component of interest, the tin species, to be retained while other species pass through the material. The polar organotin species behave as cations in solution. A number of authors have therefore used cation exchange columns in the HPLC separation of organotin compounds. The mobile phase used, in most instances, consisted of a mixture of ammonium acetate or ammonium citrate and methanol and water.

Ion exchange chromatography is carried out on ionizable analytes by using column-packing materials that possess charge bearing functional groups. The majority of organotin speciation studies employing ion-exchange chromatography are carried out with silica based columns, although both pellicular and porous microparticles have been adapted for use with ion-exchange chromatography (IEC) by coating the rigid silica particles with a thin layer of relatively non-porous ion-exchange resin. The capacity is a hundred-fold less than for the resins used in batch separations, but separation is considerably improved making the technique able to resolve even very similar species of the same element.

The simplest IEC of metal species is based on affinity differences of the native analytes for the column. Separation is controlled by pH and ionic strength of the eluent, which competes with sample species for ion-exchange sites and elutes the sample from the column. Eluents in IEC are aqueous solutions containing 0.05-0.3 mol/l of an ionic solute in the mobile phase. Proton (provided by acids) is a favourite eluting agent for the separation of cations because its binding capability can easily be adjusted by the use of pH buffers. Small fractions of water-miscible polar solvents (lower alcohols) are sometimes added to increase the solubility of the analytes.

The ion exchange resin will bind all ions of the opposite charge type. The range of applications of IEC can be expanded by converting metal cations to anions through

complexation with a negatively charged ligand, which also makes the technique applicable to simultaneous separation of cations and anions.

To date, in the speciation of methyltin compounds by HPLC a number of authors have used cation exchange columns. In 1988, Whang and Yang[5] used an SCX column in the speciation of organotins. A methanol (70%), sodium acetate (10 mM) and benzyltrimethylammonium chloride (2 mM) mobile phase was used with indirect photometric (UV) detection. This method was effectively used to speciate trimethyltin, triethyltin, tripropyltin and triphenyltin in estuarine waters.

Suyami et al.[6] also used SCX columns to speciate trimethytin, tributyltin and triphenyltin standard samples. However they used a methanol (85%) and ammonium acetate (0.1 M) mobile phase with ICP-AES detection.

An SCX column was again used in 1991 by Walton et al.[7] in the speciation of trimethyl-, triethyl- and tributyltin species. Their mobile phase contained methanol (80%) and ammonium acetate (0.2 M) at pH 4. Detection was by laser-excited atomic fluorescence spectrometry (LEAFS) with a flame as an atom reservoir.

As cation exchange columns have been most commonly used in the HPLC separation of methyltins it was decided to use a cation exchange resin in our work, for the solid phase extraction of the tin species. It was necessary also to find a suitable solvent to extract all bound materials, i.e. the products of the reaction along with any remaining unreacted products and therefore ammonium acetate was examined as a potential eluting agent. A number of experiments were performed to obtain the optimum absorption and elution conditions. The percentage recovery of the organotin from the resin was also evaluated. It was essential that the method be reproducible with the minimum amount of error. Careful attention had to be paid to the separation conditions as an improperly packed cartridge could result in poor separation and reproducibility. Analysis of the extracted solutions was performed by CZE, so it was important that the eluting agent used to

extract the products of the reaction did not interfere with any of the product peaks obtained.

5.1.2. Sample loading/elution protocol

In loading the sample onto the resin, care must be taken to ensure that the entire sample is retained by the resin and not allowed to pass through. As such, after loading the tin species onto the resin, a wash procedure was performed and the eluate was analyzed for methyltins. No methyltin was found and so it was concluded that all the methyltin had been adsorbed onto the resin.

Once the methyltin species had been adsorbed onto the resin, it was important to ensure that they could be removed again, when required. Therefore, a number of percentage recovery experiments were performed for each of the methyltin species to ascertain the optimum conditions for complete recovery. The amount of resin used and the concentration of ammonium acetate solution were varied to obtain these conditions.

The amount of sample that may be loaded is a function of the number of active sites available on the packing material. The amount of sample that can be loaded depends on

1. The concentration of all components in the sample
2. The choice of packing material and
3. The quantity of packing material

A finite volume can be loaded onto the column before breakthrough occurs. Breakthrough is the term used to describe the situation when sample overload occurs and sample components pass through the resin because all the available active sites on the resin are already occupied.

5.1.3. Solid Phase Extraction of Methyltin chloride

Five steps were involved in the transmethylation reaction under investigation. These were:

1. Absorption of the methyltin to the cation exchange resin
2. Reaction of the methyltin with mercury(II) chloride
3. Elution of the products of the reaction
4. Desorption of all remaining methyltin species
5. Analysis, by CZE, of all collected fractions

Loading the methyltin sample onto the cation exchange resin involved a number of steps. Firstly the resin was conditioned with deionized water and poured into a syringe, which had been stoppered with a piece of filter paper. Next the methyltin sample was dissolved in deionized water and loaded onto the resin. The sample was then washed with deionized water to ensure the methyltin species remained adsorbed to the resin. The required concentration of mercury (II) chloride was then dissolved in the minimum volume of water and added to the resin. This mixture was left to sit for particular periods of time at which stage the resin was washed with more deionized water to remove all the mercury compounds from the resin. One-millilitre fractions were collected and analyzed by CZE. An ammonium acetate solution was then used to elute all the tin compounds from the resin. Again, one-millilitre fractions were collected and analyzed by CZE.

5.2. Experimental

5.2.1. Materials

The following tin compounds were investigated: trimethyltin chloride (TMT), dimethyltin dichloride (DMT) and methyltin trichloride (MMT), all from Aldrich. The mercury (II) chloride was also obtained from Sigma Aldrich. The ammonium acetate used was also obtained from Aldrich. The C_{18} 30-70 micron 608, cyanopropyl, anion (SAX) and cation (SCX) exchange packings were all supplied by Alltech Associates/Applied Science. Solid phase extraction columns were prepared using 2 cm^3 syringe barrels obtained from Bond Elut, Analchem International. Sodium dihydrogen phosphate (Merck) and disodium hydrogen phosphate (Riedel de Haen) were employed as buffers.

Unless otherwise stated, all solutions were prepared in distilled deionised water obtained from a Milipore water purification system.

5.2.2. Apparatus

Methyltin determinations were carried out using the capillary electrophoresis system described in Chapter 2.

5.2.3. Initial Preparation of Packing Material

Prior to carrying out each extraction the chromatographic packing in question was washed with an appropriate solvent. In the case of the reverse phase type material (C_{18}, CN, C_2), packing was first washed with methanol followed by water. The ion-exchange packings required only a water wash. This washing step was carried out by loading approximately 1 g of the appropriate packing material into the barrel of a 2 cm^3 plastic syringe packed with a filter and passing 5 cm^3 of solvent through it.

5.2.4. Formation of solutions

All methyltin solutions were prepared in deionized water. Stock standard solutions of 5 x 10^{-2} M trimethyltin chloride and dimethyltin dichloride were prepared and diluted to give the final concentrations of Me_3SnCl and Me_2SnCl_2 required for each reaction. A number of reactions were performed at each concentration of trimethyltin chloride and dimethyltin dichloride to correspond to a 1:2 and a 1:4 mole ratio of Me_3SnCl:$HgCl_2$ and Me_2SnCl_2:$HgCl_2$ respectively. The experiments performed were as outlined in Table [5.1] below.

Experiment Number	**Methyltin Compound**	**Methyltin Concentration**	**Mole Ratio of MeSn:$HgCl_2$**	**$HgCl_2$ Concentration**
1	Me_3SnCl	2.0 x 10^{-3} M	1:2	4.0 x 10^{-3} M
2	Me_3SnCl	2.0 x 10^{-3} M	1:4	8.0 x 10^{-3} M
3	Me_3SnCl	5.0 x 10^{-3} M	1:2	1.0 x 10^{-2} M
4	Me_3SnCl	5.0 x 10^{-3} M	1:4	2.0 x 10^{-2} M
5	Me_2SnCl_2	4.5 x 10^{-3} M	1:2	9.0 x 10^{-3} M
6	Me_2SnCl_2	4.5 x 10^{-3} M	1:4	1.8 x 10^{-2} M

Table [5.1]: Solutions prepared for the transmethylation reactions of trimethyltin chloride or dimethyltin dichloride with mercury (II) chloride.

Each experiment was performed in triplicate and the results presented are an average of the three experiments. So, for Experiment 1 above, 0.2 g resin was conditioned with water in the 2-ml disposable syringe. A 2.0 x 10^{-3} M sample of Me_3SnCl was then loaded onto the resin. All waste was collected and analyzed at a later stage. Following this, 4.0 x 10^{-3} M $HgCl_2$ was dissolved in water and added to the syringe. This sample was then left to react for either 10, 20, 40 or 60 minutes. When the particular time had elapsed the

mixture was washed with water to remove all the mercury components. A subsequent wash with ammonium acetate removed all methyltin compounds from the resin.

5.2.5. Extraction Procedure

Each extraction was carried out using 0.2 g of packing. This packing was placed in a small beaker and spiked directly with an appropriate concentration of specific tin species. The resulting matrix was then quantitatively transferred into the barrel of a 2 cm^3 syringe which had been plugged with a filter. After the material was allowed to settle it was washed with 5 cm^3 of water, which resulted in the removal of any unbound components or interferants. In this case flow through the column was gravity controlled.

The tin compounds were subsequently eluted with a specific volume of ammonium acetate buffer. Again the flow rate of this eluting buffer was controlled by gravity filtration. A 0.5 cm^3 sample of the resulting extracts were removed and dissolved in 4.5 cm^3 of Na_2HPO_4/NaH_2PO_4 buffer at pH 8.25, and analysed by CZE. Blank controls were treated in the same manner except these were spiked with distilled water. The entire extraction procedure is summarised below.

Tin sample

↓

Add to pre-weighed packing material

↓

blend

↓

Pack into the barrel of a 2 cm^3 syringe

↓

Remove contaminants or unbound components with water wash

↓

Elute with ammonium acetate buffer

↓

Collect 1 ml fractions

↓

Remove 0.5 ml sample

↓

Dilute to 4.5 ml in phosphate buffer, pH 8.25

↓

Analyse by CZE

5.2.6. Preliminary Optimisation of Extraction Conditions

Initial experiments focussed on establishing the most suitable parameters for the extraction of the tin species from the packing material. The effectiveness of C_{18}, CN, anion exchange and cation exchange packing materials were primarily examined. The most favourable elution buffer and concentration of buffer required for eluting each tin species was established. Following this, the influence of the amount of packing used on the recovery was studied. Extractions were carried out using 0.05, 0.1, 0.2, and 0.5g of packing.

Methanol and water were used as washing solvents and ammonium acetate and sodium acetate were examined for their suitability as eluting solvents. The effect of each solvent on recovery, peak shape and reproducibility was monitored.

5.2.7. Comparison of Solid Phase Extraction Materials

Initial experiments indicated that C_{18} was ineffective in extracting tin species from the loading solution. Therefore the efficiency of alternative solid-phase extraction materials such as anion (SAX) and cation (SCX) exchange resins and a cyanopropyl stationary phase were studied along with C_2.

Experiments were carried out using the extraction conditions listed below.

Concentration Tin Species	5×10^{-3} M
Weight of Packing	1 g
Washing Solvent	Methanol
Elution Buffer	Ammonium Acetate
Concentration of Elution Buffer	0.2 M

The high buffer concentration was used to ensure complete elution.

5.2.8. Elution Volume Tests

For the purposes of optimisation, this procedure was treated as an off-line extraction technique. Tin compounds were eluted with a large volume of buffer solution to ensure maximum possible recovery. However, for routine determinations, it would be preferable if the extractions were carried out on line with the analytical technique of choice, in this case CZE. This would require the volume of eluting buffer to be kept to a minimum, ideally below 1 cm^3. Tin compounds could then be completely eluted from the extraction column and subsequently be introduced to the analytical column in a small plug of solvent.

It was examined whether increasing the concentration of ammonium acetate buffer would allow for a reduction in the minimum elution volume required to achieve maximum recovery of tin species from the solid phase extraction columns. The combined effects of elution volume and ammonium acetate concentration on recovery were initially studied in respect to trimethyltin chloride. The tin level used was 5×10^{-3} M. Ammonium acetate buffer at pH 2 and pH 4 was used as the eluting buffer and buffer concentrations of 0.05, 0.1, and 0.5 M were investigated. In each case the percentage recovery of trimethyltin chloride after various elution stages was recorded (i.e. after 1 ml, 3 ml, 5 ml etc. elution buffer). All eluted solutions were collected into 1 ml test tubes, 0.5 ml of which was removed for analysis by CZE.

Subsequent elution experiments were carried out using varying methanol to ammonium acetate concentration percentage ratios and the effects of increasing elution volume on the recoveries of trimethyltin chloride were investigated. The same trimethyltin chloride concentration was used in each instance and all elution volume tests were carried out in triplicate.

5.2.9. Validation of Extraction Procedure

This extraction of tin species from various packing materials requires statistical validation. This was accomplished by methods analogous to those used for validating chromatographic techniques, using intra (within day) and inter (between day) variability assays.

Extractions were performed using a range of trimethyltin chloride concentrations (1.0 – 5.0 x 10^{-3} M). An ammonium acetate concentration of 0.1 M was used along with 0.2 g of SCX resin. Comparison of extracted samples for each concentration of tin to that of its respective pure standard run under identical analytical conditions allowed the calculation of percentage recoveries.

Intra-assay variabilities were determined as follows; the percentage recovery for three replicate extractions at each concentration all measured within one day were averaged resulting in mean ± standard deviation which gave the relative standard deviation (R.S.D.). When this value is expressed as a percentage it is termed the coefficient of variation (C.V.). The mean percentage coefficient of variation ± the standard deviation was then defined as the intra-assay variability. Mean percentage C.V. relates to the precision of the method and its corresponding standard deviation to the error associated with it.

For intra-assay variability a separate calibration curve was generated and the extraction procedure carried out using 5 x 10^{-3} M for each tin compound, every day over a five day period. The percentage coefficient of variation for the mean of these replicates was then calculated as their inter-assay variability. Once the inter- and intra-assay variabilities and their standard derivatives were below 10% the technique was deemed valid.

5.3. Results

5.3.1. Preliminary Optimisation of Extraction Conditions

The initial conditions chosen for the extraction of the tin species are listed in Table [5.2] below.

Type of Packing Material	Strong Cation Exchange
Weight of Packing Material	0.2 g
Washing solvent	Water with methanol
Elution Buffer	Ammonium acetate
Concentration of Elution Buffer	0.1 M

Table [5.1]: Most suitable conditions for the extraction of methyltin species.

The initial step in the design of this procedure involved the determination of an appropriate packing material on which to retain the required tin species. A number of different materials were examined and the results are reported in section 5.3.2.

A washing stage was included to determine if all the tin species was bound to the packing material. The unbound portion would be removed and therefore detected in this wash. Although experiments proved that all the tin species did bind to the SCX resin it was decided to retain this step as a check.

In the preliminary stages of this project, ammonium acetate buffer (0.2 M) combined with methanol at various ratios was chosen to elute the tin species. This was chosen as this system is commonly used in the chromatographic separation of methyltin species. It was subsequently examined whether the use of ammonium acetate alone could effect the recovery of the tin species. Increasing the ammonium acetate concentration was also examined. In the case of the strong cation exchange resin, the use of ammonium acetate

as the eluting buffer resulted in higher recoveries. On increasing the ammonium acetate concentration it was found that 0.1 M gave optimum results. Buffer concentrations below this were not sufficiently strong to remove all bound species from the packing material.

Conc. Ammonium Acetate Buffer	Average % Recovery
0.05 M	92.82
0.1 M	97.60
0.2 M	103.52
0.5 M	104.74

Table [5.2]: Effect of increasing concentration of Ammonium Acetate Buffer

The weight of packing material used also affected the results obtained. It was necessary to determine the quantity of cation exchange resin needed to allow all the trimethyltin chloride to be successfully loaded onto the resin without breakthrough occurring. Initially 0.05 g of the cation exchange resin was examined. An aqueous solution containing the trimethyltin chloride (5.0×10^{-3} M) was then loaded onto the resin. A 0.1 M ammonium acetate solution was used to elute the tin from the resin. The percentage trimethyltin chloride found in the waste sample was determined in each case. The weight of material used and the resultant recoveries are listed in Table [5.3]. It was found that a minimum of 0.2 g of SCX resin was required to obtain a reasonable recovery. Increasing the amount of material further would lead to increased time required for each washing and elution stage as the flow rate would also decrease, and thus a resin weight of 0.2 g was used in all subsequent experiments. As no breakthrough was found to occur, 0.2 g resin was found to be effective in retaining all the methyltin species on the resin

Weight of Packing Material	Average % Found in Waste Sample	Average % Recovery
0.5 g	0	113.04
0.2 g	0	93.45
0.1 g	2.71	84.12
0.05 g	6.45	83.07

Table [5.3]: Effect of increasing the weight of packing material used for extraction of TMT from SCX resin.

Therefore 0.2 g resin and 0.1 M ammonium acetate were chosen as optimum conditions for the adsorption and subsequent removal of trimethyltin chloride from the cation exchange resin.

5.3.2. Comparison of solid-phase extraction materials

The variation in percentage recoveries for trimethyltin chloride, dimethyltin dichloride and methyltin trichloride obtained from cation exchange (SCX) packing, anion exchange (SAX) packing, cyanopropyl (CN) packing, C_2 and C_{18} packing materials was investigated. However, it was soon discovered that the methyltin compounds examined were not retained by any of these packing materials, with the one exception being the SCX resin which was found to retain each of the species examined.

5.3.3. Effects of elution volume on recoveries of methyltin species

The effects of increasing the concentration of the eluting buffer on recovery were primarily investigated for the extraction of trimethyltin chloride from the column. Elution was carried out using 1 cm^3, 3 cm^3, 5 cm^3 and 10 cm^3 respectively of 0.05 M, 0.1

M and 0.5 M ammonium acetate buffer. The percentage recoveries obtained with each elution volume at each buffer concentration investigated are compared in Fig. [5.1].

The percentage recovery in all instances was above 99%. For each buffer concentration examined it is clear that an elution volume of 1 cm^3 is insufficient to remove all bound components from the resin. A minimum elution volume of 3 cm^3 is necessary in each instance to remove all the tin species from the resin. Only in the case of the 0.5 M ammonium acetate buffer was as increase in recovery found on moving from 3 cm^3 to 5 cm^3. This result was deemed an anomaly and therefore ignored. An elution volume of 5 cm^3 was used in all experiments.

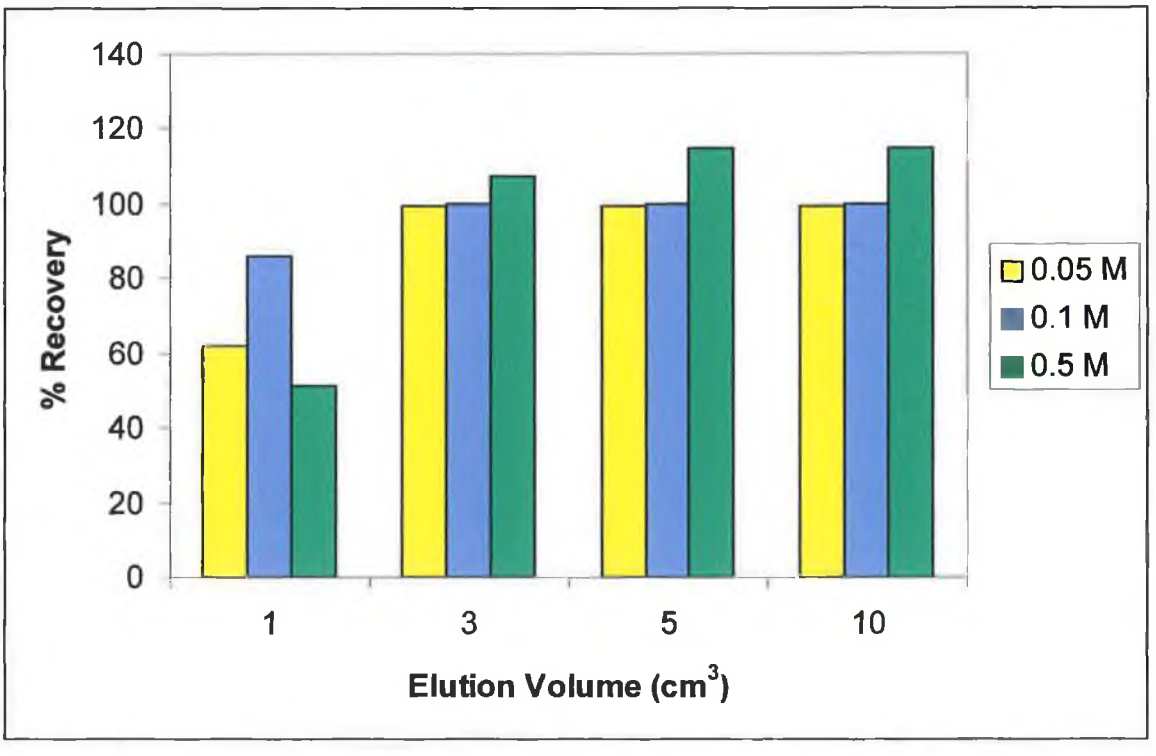


Fig. [5.1]: Comparison of the percentage recoveries of trimethyltin chloride achieved using 1 cm^3, 3 cm^3, 5 cm^3 and 10 cm^3 respectively of 0.05 M, 0.1 M and 0.5 M ammonium acetate buffer. In all cases the extractions were carried out using 0.2 g SCX resin, and 5.0 x 10^{-3} M TMT.

Additional experiments on the effects of elution volume on percentage recovery were carried out using the ammonium acetate buffer at a concentration of 0.1 M. In this case dimethyltin dichloride and methyltin trichloride were the species under investigation. The percentage recoveries for each species achieved following elution with 1 cm^3, 3cm^3, 5 cm^3 and 10 cm^3 are illustrated in Fig. [5.2].

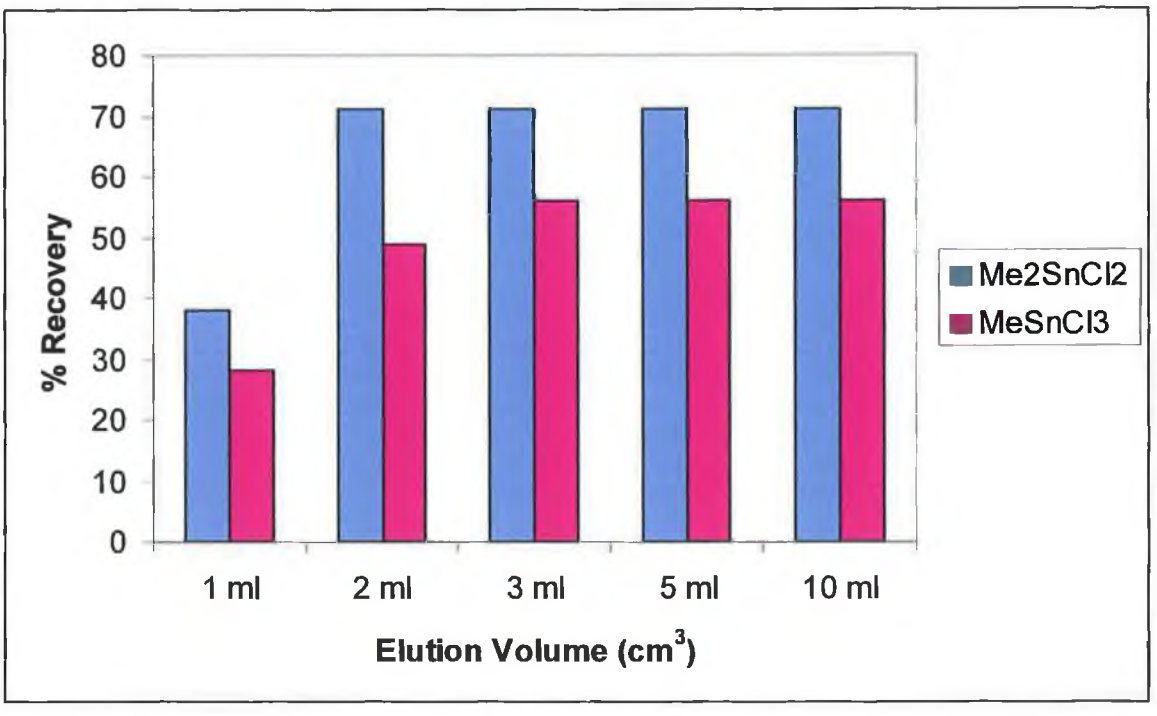


Fig. [5.2]: Variation in percentage recovery of dimethyltin dichloride and methyltin trichloride using increasing elution volumes. In all cases extractions were carried out using 0.2 g SCX resin and 0.1 M ammonium acetate buffer.

It can be seen from Fig [5.2] that the minimum volume of eluting buffer required for maximum recovery of dimethyltin dichloride was 2 cm^3. This volume is sufficiently low to allow all extractions to be performed on-line. However, the minimum elution volume required to achieve maximum recovery of MMT is 3 cm^3. As such, it was decided, due to the nature of the experiments being performed to perform all extractions off-line to

allow for greater flexibility in washing steps etc. All species were therefore eluted separately prior to analysis.

5.3.4. Validation of extraction procedure

Validation of the extraction of trimethyltin chloride, dimethyltin dichloride and methyltin trichloride was carried out using the optimised conditions listed in Table [5.4].

Type of packing	Strong cation exchange
Weight of packing	0.2 g
Washing solvent	Water
Elution buffer	0.1 M ammonium acetate buffer
Elution volume	5 cm^3

Table [5.4]: Optimum conditions for the extraction of methyltin compounds from SCX resin.

In each case the intra assay variability of the optimised extraction was carried out using a concentration range of each species from 1.0 to 5.0 x 10^{-3} M. The between day variation in recovery (inter assay variability) which is related to the precision of the method was examined for each tin species using a concentration of 5 x 10^{-3} M.

The intra and inter-assay variabilities obtained for trimethyltin chloride, dimethyltin dichloride and methyltin trichloride are listed in Tables [5.5] –[5.10].

5.3.4.1. Intra-assay variability for trimethyltin chloride

TMT concentration (ppm)	% Recovery	Mean % Recovery ± Std. Dev.	Relative Standard Deviation
1×10^{-3} M (a)	91.17		
(b)	89.06	90.84 ± 1.64	0.0180
(c)	92.28		
2×10^{-3} M (a)	93.40		
(b)	92.59	93.66 ± 1.22	0.0129
(c)	94.98		
3×10^{-3} M (a)	93.54		
(b)	91.90	92.93 ± 0.90	0.0097
(c)	93.36		
4×10^{-3} M (a)	95.29		
(b)	98.45	96.68 ± 1.61	0.0167
(c)	96.31		
5×10^{-3} M (a)	90.10		
(b)	92.70	91.39 ± 1.30	0.0142
(c)	91.37		

Table [5.5]: Recoveries of Trimethyltin chloride from SCX resin.

Mean percentage recovery of TMT over the concentration range examined:

93.1% ± 2.42%

Mean coefficient of Variation (intra assay variability) for TMT: 1.43 ± 0.33

5.3.4.2. Inter-assay variability for trimethyltin chloride

Day	**Mean percentage recovery**
1	90.10
2	99.68
3	92.61
4	91.04
5	92.09

Table [5.6]: Mean percentage recoveries of TMT over five days

Mean percentage recovery of TMT over a five day period: 93.10% ± 3.80%

Relative Standard Deviation: 0.0408

Coefficient of Variation (Inter-assay variability): 4.08%

5.3.4.3. Intra-assay variability for dimethyltin dichloride

DMT concentration (ppm)	% Recovery	Mean % Recovery ± Std. Dev.	Relative Standard Deviation
1×10^{-3} M (a)	68.12		
(b)	82.33	72.63 ± 8.41	0.1158
(c)	67.43		
2×10^{-3} M (a)	74.14		
(b)	72.66	73.17 ± 0.84	0.0115
(c)	72.70		
3×10^{-3} M (a)	66.13		
(b)	62.92	66.09 ± 3.16	0.0477
(c)	69.23		
4×10^{-3} M (a)	74.19		
(b)	77.90	73.48 ± 4.81	0.0655
(c)	68.35		
5×10^{-3} M (a)	67.34		
(b)	70.14	68.17 ± 1.71	0.0251
(c)	67.03		

Table [5.7]: Recoveries of dimethyltin dichloride from SCX resin.

Mean percentage recovery of DMT over the concentration range examined:

70.71% ± 5.00%

Mean coefficient of Variation (intra assay variability) for DMT: 5.314% ± 4.07%

5.3.4.4. Inter-assay variability for dimethyltin dichloride

Day	**Mean percentage recovery**
1	71.07
2	65.56
3	77.60
4	67.31
5	70.88

Table [5.8]: Mean percentage recoveries of DMT over five days

Mean percentage recovery of DMT over a five day period: 70.48% ± 4.62%

Relative Standard Deviation: 0.06558

Coefficient of Variation (Inter-assay variability): 6.56%

5.3.4.5. Intra-assay variability for methyltin trichloride

MMT concentration (ppm)	% Recovery	Mean % Recovery ± Std. Dev.	Relative Standard Deviation
1×10^{-3} M (a)	60.86		
(b)	54.36	56.37 ± 3.89	0.06911
(c)	53.88		
2×10^{-3} M (a)	64.75		
(b)	60.21	58.2 ± 7.75	0.1332
(c)	49.64		
3×10^{-3} M (a)	54.48		
(b)	47.88	49.09 ± 4.89	0.09969
(c)	44.92		
4×10^{-3} M (a)	46.31		
(b)	54.12	52.38 ± 5.41	0.10329
(c)	56.70		
5×10^{-3} M (a)	63.62		
(b)	57.29	62.26 ± 4.45	0.07152
(c)	65.88		

Table [5.9]: Recoveries of methyltin trichloride from SCX resin.

Mean percentage recovery of MMT over the concentration range examined:

55.66% ± 6.60%

Mean coefficient of Variation (intra assay variability) for MMT: 9.54% ± 2.63%

5.3.4.6. Intra-assay variability for methyltin trichloride

Day	**Mean percentage recovery**
1	57.32
2	64.28
3	52.18
4	59.65
5	60.14

Table [5.10]: Mean percentage recoveries of MMT over five days

Mean percentage recovery of MMT over a five-day period: 58.71% ± 4.43%

Relative Standard Deviation: 0.07546

Coefficient of Variation (Inter-assay variability): 7.55%

It can be seen that for each species examined the criteria for method validation discussed in Section 5.2.9, which states that both the within day and between day variation should not exceed 10%, have been met. The percentage recoveries achieved were quite high ranging from 55.66% for methyltin trichloride to 93.1% for trimethyltin chloride.

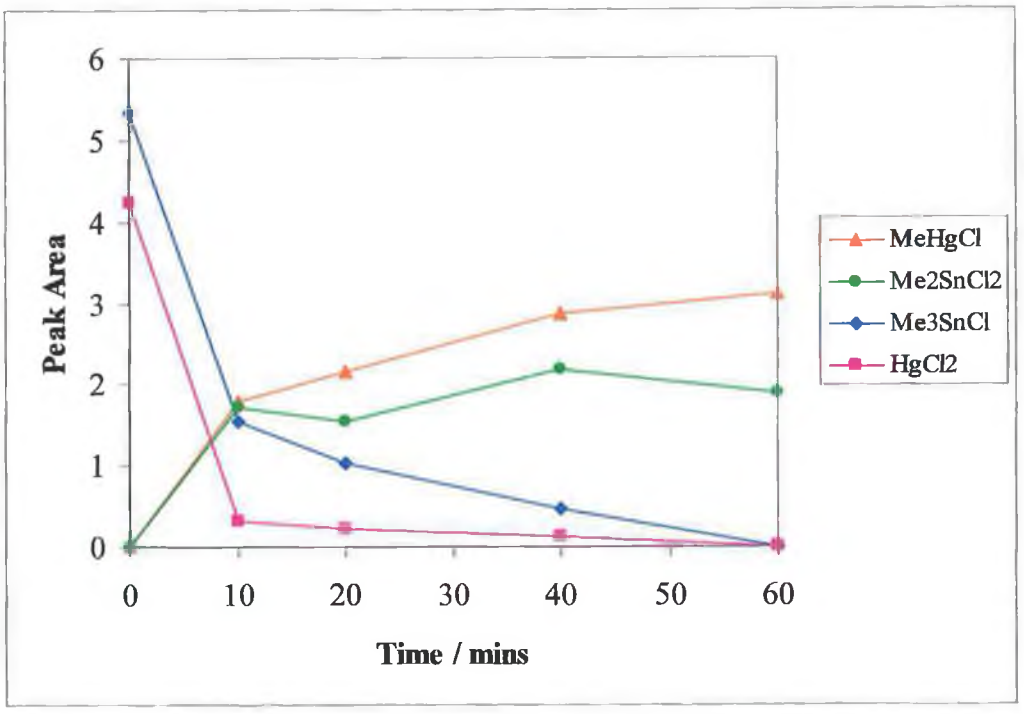
The technique met with a high degree of success when applied to the extraction of trimethyltin chloride from the resin. Recoveries in the range 89% - 98% were achieved in the concentration range examined. The results obtained were reproducible with an overall standard deviation of 2.42%. The mean coefficient of variation was 1.43%, which was well below 10%, the level acceptable for method validation. A greater degree of variation was found for inter-assay experiments. A result of 4.08% reveals a slight variability in precision for between day analysis, but these results are still indicative of a valid method.

The technique was less successful when applied to the extraction of both dimethyltin dichloride and methyltin trichloride. Percentage recoveries in the range 63% - 78% were achieved for DMT while recoveries in the range 45% - 66% were found for MMT. The coefficients of variation for within day analysis were found to be 5.3% for DMT and 9.54% for MMT. The coefficients of variation for between day analysis for DMT and MMT were found to be 6.56% and 7.55% respectively. Although these results are higher than those achieved for trimethyltin chloride, the results obtained indicate a valid method.

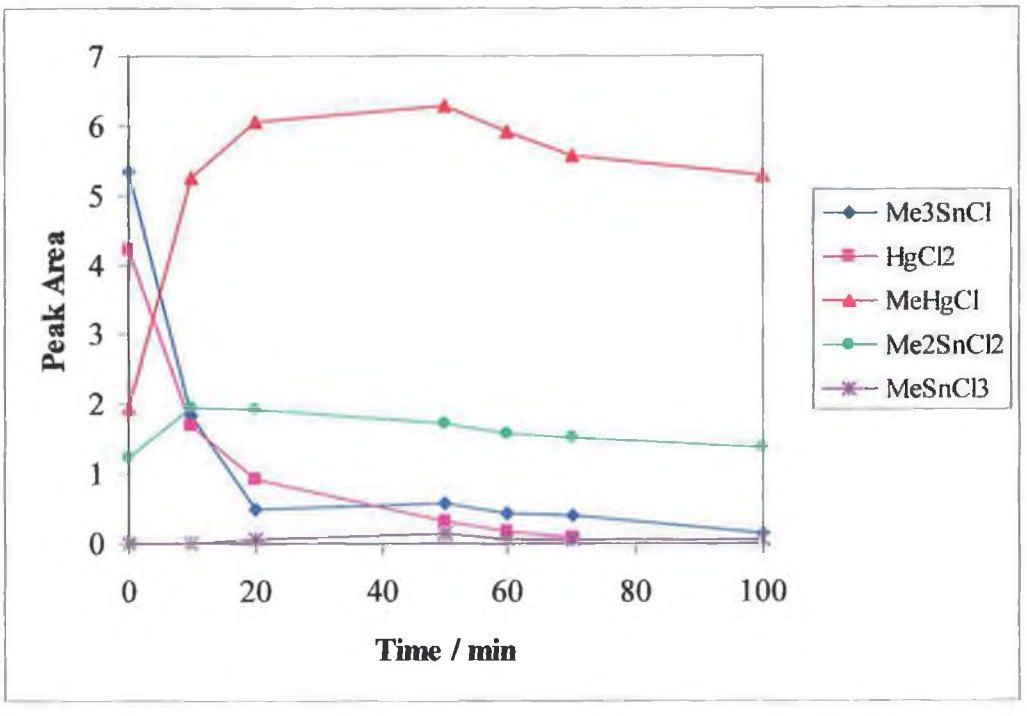
5.3.5. Transformation of methyl groups from Me_3SnCl

Experimental conditions were designed to monitor the transmethylation reaction of trimethyltin chloride with mercury (II) chloride, having previously adsorbed the trimethyltin chloride to a cation exchange resin. In order to monitor the reaction over sixty minutes it was necessary to perform four individual reactions which were stopped at 10, 20, 40 and 60 minutes respectively. The concentration of both reactants and products were recorded at each stage, which, when combined, gave a time dependence plot for the reaction. The concentrations of the reactants were initially recorded, and their peak areas were determined from the calibration curves shown in Chapter 4 (Figs. [4.3] to [4.6]). These peak area values are depicted at zero minutes in each of the time dependence plots presented. The initial reactions which were performed consisted of either a high or a low trimethyltin chloride concentration reacted in either a 1:2 or a 1:4 mole ratio of Me_3SnCl:$HgCl_2$. Previously when these reactions were performed in a liquid medium the highest rate of reaction was found for the highest concentration of both trimethyltin chloride and mercury (II) chloride examined. Therefore, it was hoped that, by performing these particular experiments a difference in reaction rate would be detected, due to the trimethyltin chloride being adsorbed to a solid support material.

In the first experiment 5.0×10^{-3} M Me_3SnCl was reacted in a 1:2 mole ratio with $HgCl_2$. A time dependence plot for this reaction is shown in Fig. [5.3] (a). Clearly a transmethylation reaction occurs, which is significant as the trimethyltin chloride is bound to a solid material. The reaction is indicated by the presence of both dimethyltin dichloride and methylmercury chloride, known products of this reaction. No methyltin trichloride was detected. The levels of both dimethyltin dichloride and methylmercury chloride are seen to increase throughout the reaction period, as both trimethyltin chloride and mercury (II) chloride decrease.



(a)



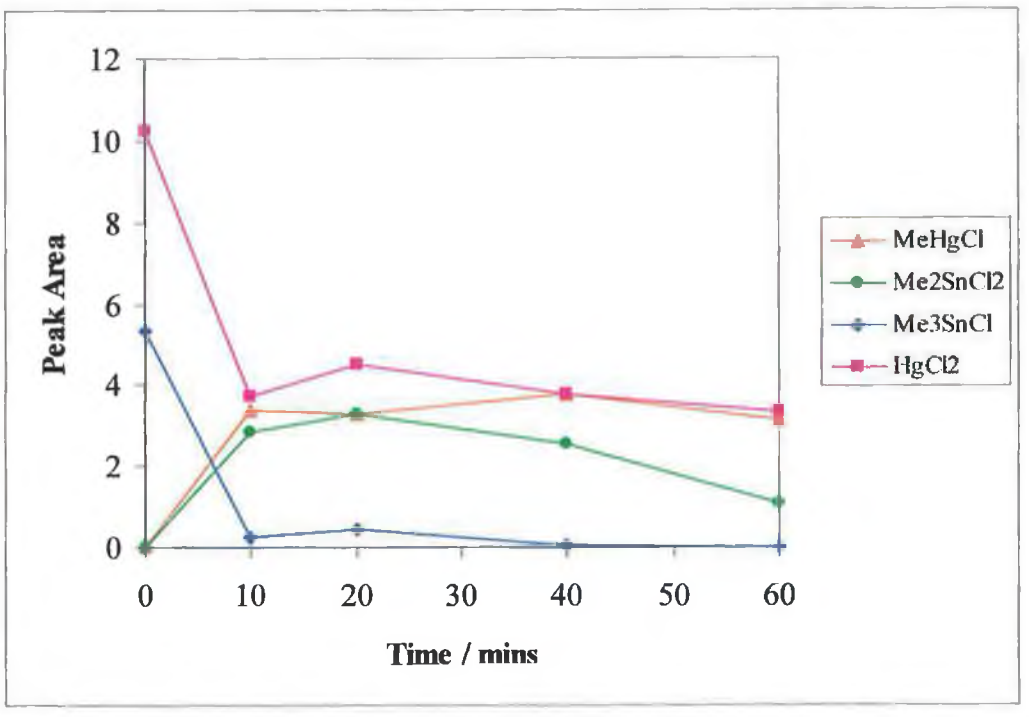
(b)

Fig. [5.3]: Time dependence plots of 5.0 x 10^{-3} M Me_3SnCl reacted in a 1:2 mole ratio with $HgCl_2$ in (a) a solid material and (b) a liquid medium.

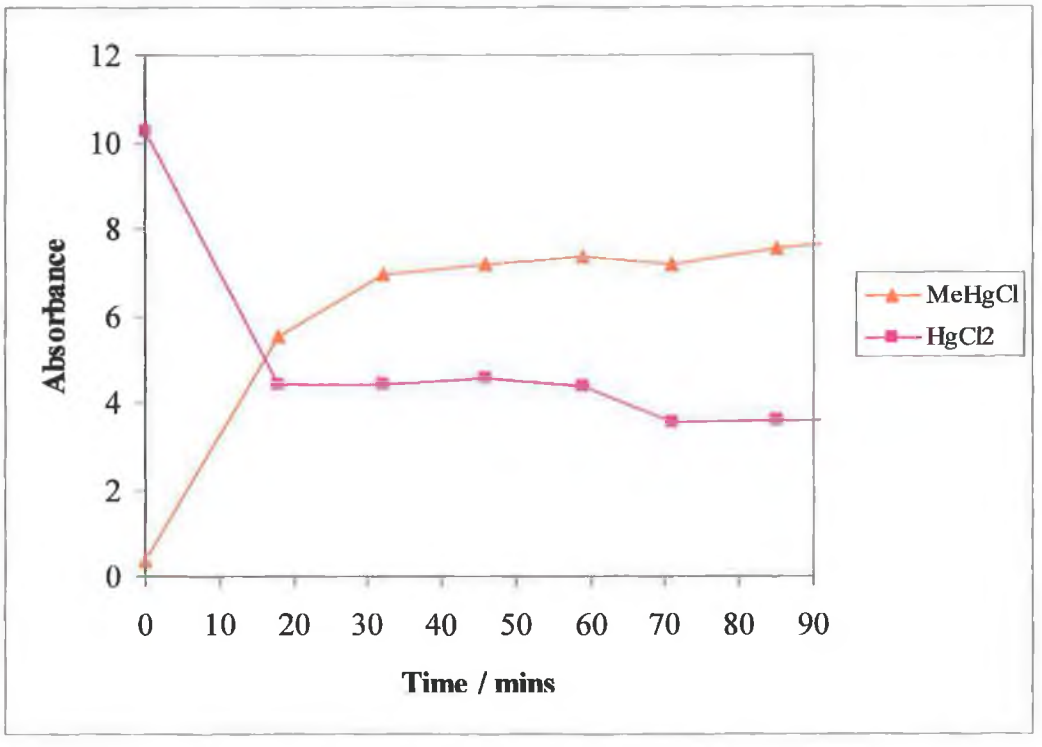
On comparison of the results of this transmethylation reaction with its equivalent reaction in the liquid media one main difference is apparent. The levels of MeHgCl formed in the "solid phase" reaction are much less than those formed in the "liquid phase". However, the levels of all other components remained similar and thus a possible reason for the reduced levels of MeHgCl may be a low percentage recovery of MeHgCl from the resin. Very similar patterns are seen for the formation of Me_2SnCl_2 in both the solid and liquid phases. This was also the case for the depletion of both Me_3SnCl and $HgCl_2$. The reaction of 5.0×10^{-3} M Me_3SnCl in a 1:2 mole ratio of Me_3SnCl: $HgCl_2$ in a liquid medium is shown in Fig. [5.3] (b).

The second reaction involved the same Me_3SnCl concentration as before but here it was reacted in a 1:4 ratio of Me_3SnCl:$HgCl_2$. It was thought from the previous reaction that the rate of this reaction would not differ substantially from that found in the liquid phase. If this were the case it could be concluded that the transmethylation reaction was not affected by the adsorption of Me_3SnCl onto the solid resin. Fig. [5.4] (a) shows the time dependence plot of the reaction in the solid phase while Fig. [5.4] (b) shows the same reaction performed in the liquid phase.

On comparison of both reactions the most notable difference is the presence of both Me_3SnCl and Me_2SnCl_2 in the solid reaction, which were not detected when the reaction was performed in the liquid phase. The pattern of $HgCl_2$ depletion was very similar in both reactions as indeed was the formation of MeHgCl. No real decrease/increase was seen for either compound after 10 minutes had elapsed in the solid reaction. The levels of Me_2SnCl_2 increased up to 20 minutes and decreased thereafter, indicating the ongoing transmethylation reaction. No $MeSnCl_3$ was detected. These results would tend to suggest that no increase in the rate of reaction occurs when one species is bound to a solid material.



(a)



(b)

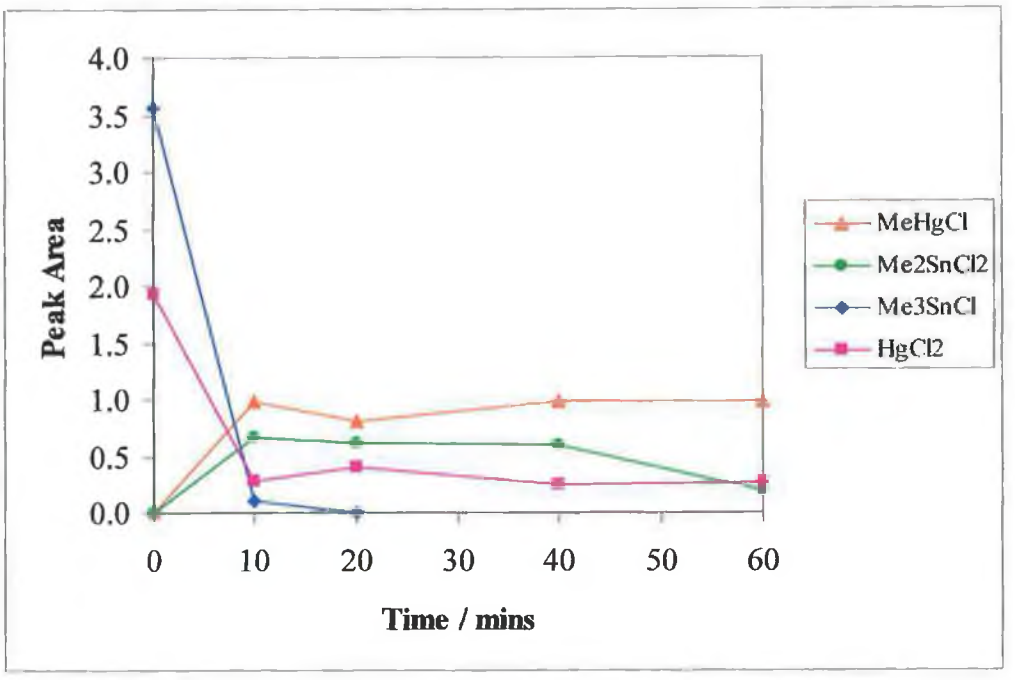
Fig. [5.4]: Time dependence plots of 5.0 x 10^{-3} M Me_3SnCl reacted in a 1:4 mole ratio with $HgCl_2$ in (a) a solid phase and (b) a liquid phase.

Literature has shown that the transmethylation reaction in soils and sediments proceeds at a greater rate than in a liquid medium. So far in this investigation this hasn't appeared to be the case. It was thought that maybe the high concentration of both trimethyltin chloride and mercury (II) chloride might be responsible for this in some way. Therefore, it was decided to examine the transmethylation reaction again using a smaller concentration of trimethyltin chloride but using the same mole ratios of Me_3SnCl: $HgCl_2$.

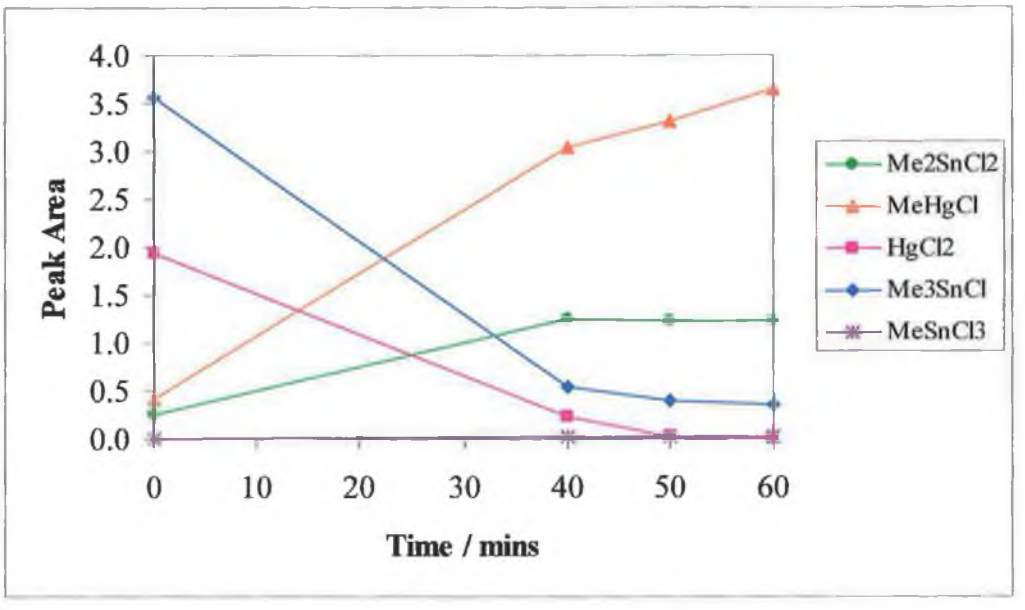
As a result the transmethylation reaction of a lower concentration (2×10^{-3} M) of Me_3SnCl with a 1:2 ratio of Me_3SnCl: $HgCl_2$ was examined. The results of the reaction are shown in Fig. [5.5] (a). There is a significant drop in both Me_3SnCl and $HgCl_2$ initially, which, after 10 minutes levels off, indicating that no further reaction occurs. At 10 minutes the level of MeHgCl formed also stabilises while Me_2SnCl_2 levels off and then decreases again at 60 minutes. Comparing these results with those in Fig. [5.5] (b) of the reaction in a liquid medium shows that the reaction in the solid material is complete in less time, therefore confirming a greater reaction rate in a solid phase reaction.

The final reaction performed in this section consisted of reacting this lower Me_3SnCl concentration (2×10^{-3} M) with a 1:4 mole ratio of Me_3SnCl: $HgCl_2$. It would be interesting to find out if the higher rate of reaction found at the 1:2 ratio is reproduced here. The time dependence plot of the reaction in the solid and liquid media are shown in Figs. [5.6] (a) and (b) respectively. On analysis of the results of the experiment the reaction performed in the solid resin appears to proceed at a greater rate than in the liquid. No Me_3SnCl was detected during the course of the reaction which would indicate that it was all reacted before analysis was performed (i.e. within 10 minutes). However, it appears that Me_2SnCl_2 continued to be formed throughout the reaction period, which contradicts this.

Overall it appears that using a high Me_3SnCl concentration there is no increase in the rate of the reaction using a solid material. However, at the lower Me_3SnCl concentration the transmethylation reaction proceeded at a greater rate in the solid phase.

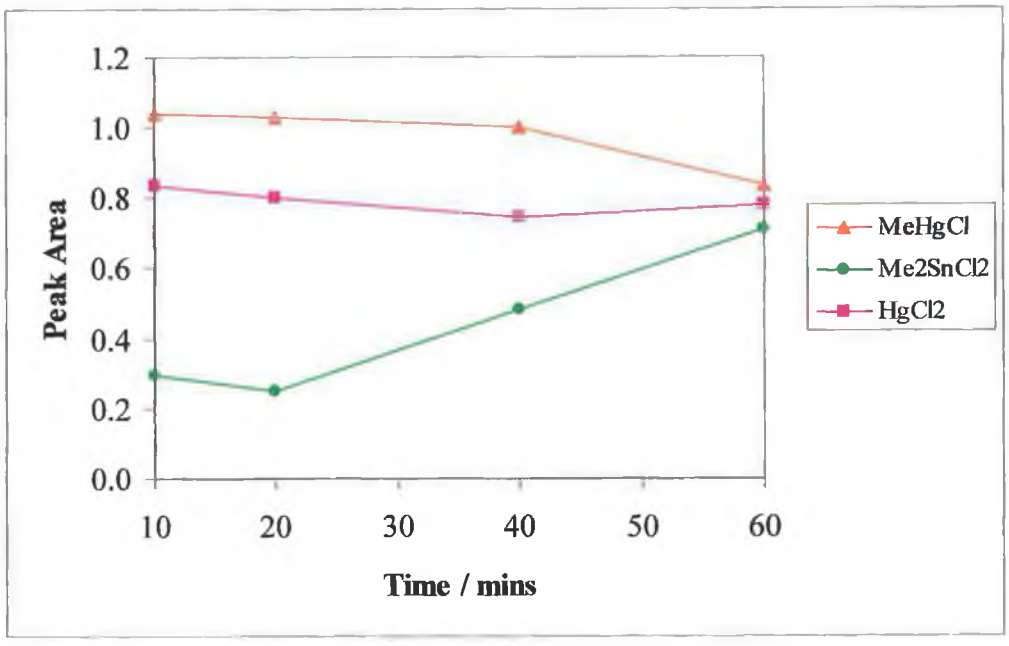


(a)

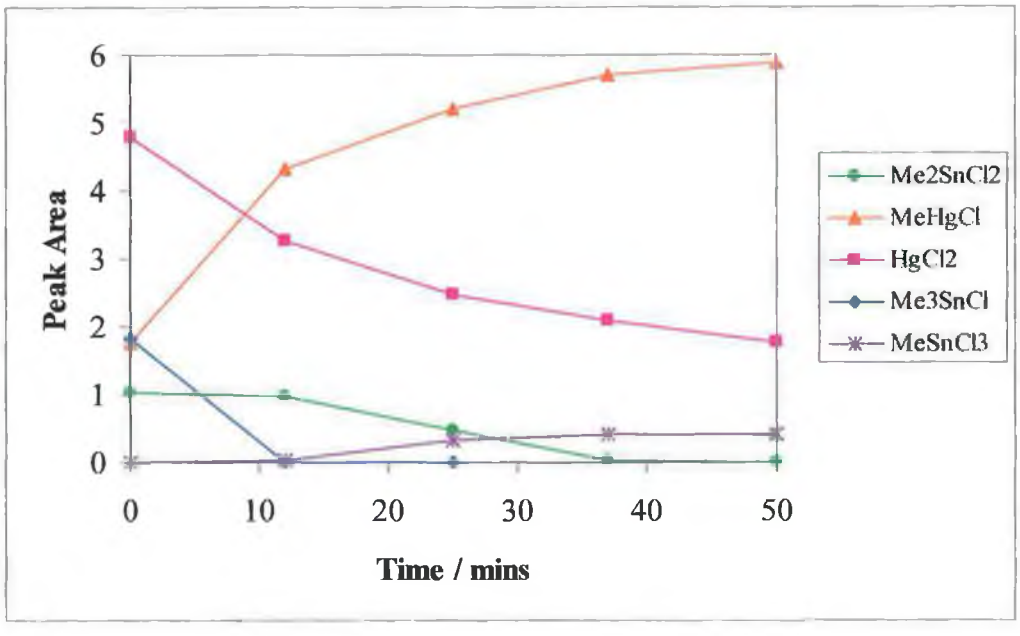


(b)

Fig. [5.5]: Time dependence plots of 2.0 x 10^{-3} M Me_3SnCl reacted in a 1:2 mole ratio with $HgCl_2$ in (a) a solid material and (b) a liquid phase.



(a)



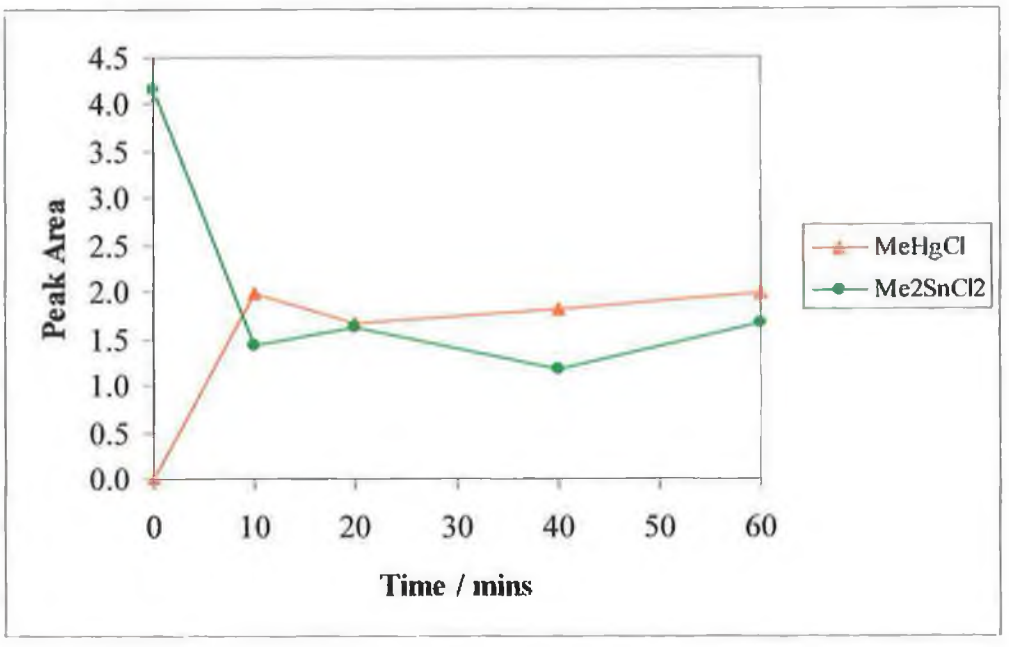
(b)

Fig. [5.6]: Time dependence plots of 2.0 x 10^{-3} M Me_3SnCl reacted in a 1:4 mole ratio with $HgCl_2$ in (a) a solid phase and (b) a liquid phase.

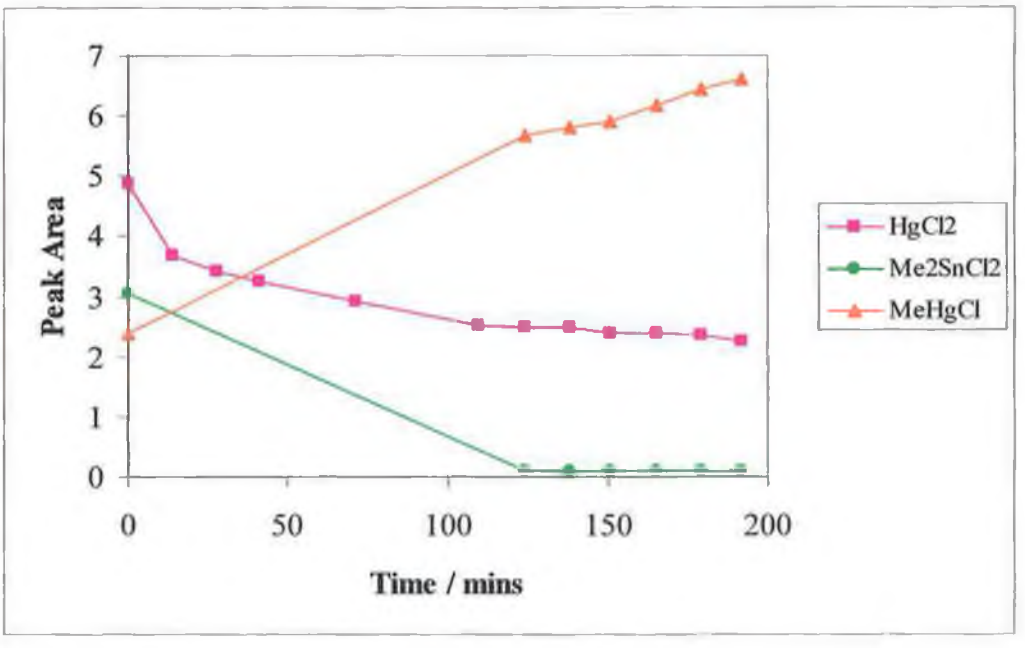
5.3.6. Transformation of methyl groups from Me_2SnCl_2

Having determined that the rate of reaction at high concentrations of Me_3SnCl is not affected by the presence of the resin it was decided to check if the rate of reaction is higher at high Me_2SnCl_2 concentrations. Therefore, in this set of experiments the dimethyltin dichloride was initially adsorbed onto the cation exchange resin and subsequently reacted with a 1:2 and a 1:4 ratio of Me_2SnCl_2: $HgCl_2$. The reaction was again monitored over 60 minutes, as was the case for trimethyltin chloride. After the reaction period ended all the products of the reaction were removed using ammonium acetate to desorb all the methyltin compounds. The concentration of each product of the transmethylation reaction was recorded at each stage and eventually compiled to give the time dependence plots for the reactions.

Fig. [5.7] (a) shows a time dependence plot for 4.5×10^{-3} M Me_2SnCl_2, which was reacted with 9.0×10^{-3} M $HgCl_2$. This corresponded to a 1:2 mole ratio of Me_2SnCl_2: $HgCl_2$. It is clear that a transmethylation reaction occurs here, even though the dimethyltin dichloride was adsorbed onto the cation exchange resin. On comparison of this transmethylation reaction with that performed in the liquid phase (shown in Fig. [5.7] (b)) there are several features of note. Firstly, no mercury (II) chloride was detected in the solid phase experiment. Secondly, after the initial 10 minute reaction no further change in the levels of either Me_2SnCl_2 or MeHgCl were detected, thus indicating the completion of the reaction. In the liquid phase the transmethylation reaction is seen to continue until over 100 minutes. This increase in reaction time for the reaction in the solid phase is in agreement with results found in the literature – i.e. an increase in reaction rate was found for reactions performed in soils and sediments.



(a)

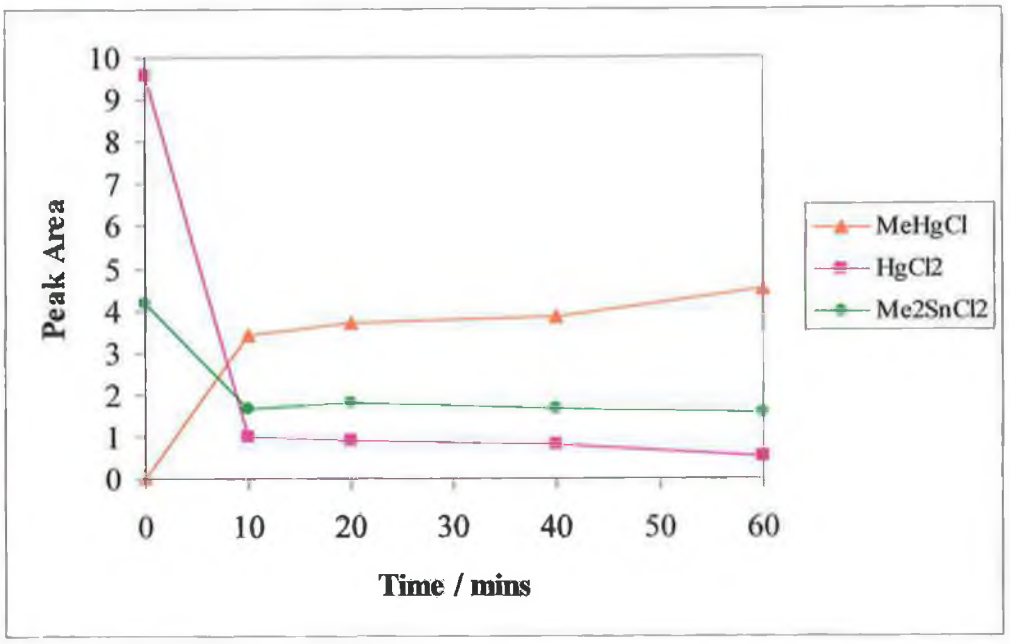


(b)

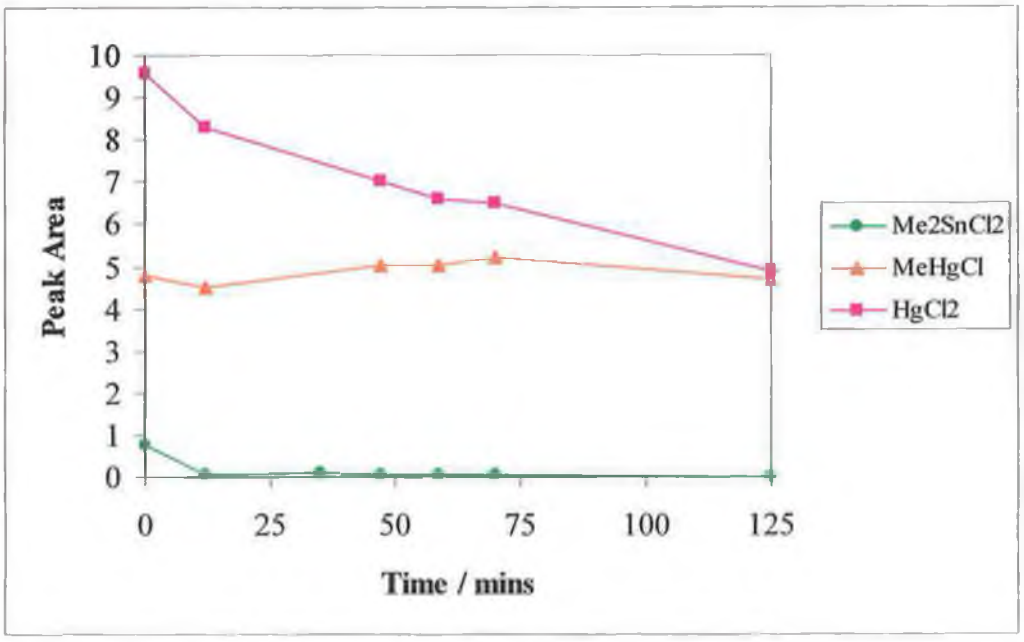
Fig. [5.7]: Time dependence plots of 4.5 x 10^{-3} M Me_2SnCl_2 reacted in a 1:2 mole ratio with $HgCl_2$ in (a) a solid phase and (b) a liquid phase.

The transmethylation reaction of a 1:4 ratio of Me_2SnCl_2:$HgCl_2$ was the final transmethylation reaction performed; the results of which are shown in Fig. [5.8] (a). A similar increase in the reaction rate was found for this reaction as was found for the 1:2 reaction ratio. Again, in the solid medium the reaction appears to be complete in less than 10 minutes – with little change in levels of reactants or products of the reaction after this time. The transmethylation reaction in liquid is shown in Fig. [5.7] (b). In the liquid medium the continuous decrease in the levels of mercury (II) chloride indicate the ongoing transmethylation reaction.

In summary, the transmethylation reaction, when performed in a solid medium leads to an increase in reaction rate when either trimethyltin chloride or dimethyltin dichloride are used as the reactants with inorganic mercury (II).



(a)



(b)

Fig. [5.6]: Time dependence plots of 4.5 x 10^{-3} M Me_2SnCl_2 reacted with $HgCl_2$ in a 1:4 mole ratio in (a) a solid medium and (b) a liquid medium.

5.4. Conclusions

The purpose of this study was to explore the feasibility of using solid phase extraction techniques to promote the binding of methyltin species to determine if this binding process inhibits or otherwise affects the transmethylation reaction known to occur when these compounds are in the presence of inorganic mercury compounds. First of all, however, this process demanded that the tin species not only be completely bound but also that it could be removed again in a form capable of analysis by capillary zone electrophoresis.

To be deemed a valid extraction technique it is required that values for intra- and inter-assay variabilities, which are associated with precision and accuracy respectively, be below 10%. For all the tin species examined the values obtained met this criteria.

For the purposes of this optimisation and validation the levels of the tin species used were quite high. This was necessary for the sake of comparison with the results obtained in Chapter 4 for the transmethylation reaction of these tin compounds with mercury chloride. However, to use this extraction technique for environmental applications a need would exist to then reduce the levels of the tin species used.

With regard to the transmethylation reactions performed, it was decided not to repeat all the reactions performed in Chapter 4 but rather to select a number of reactions which it was thought represented the range of reactions previously performed. The concentrations of reactants used were equal in each instance.

5.5. References

[1] Rosenkranz, B., Bettmer, J., Buscher, W., Breer, C. and Cammann, K., Appl. Organometal. Chem., 1997, 11, 721

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6.0 Conclusions

6.0 Conclusions

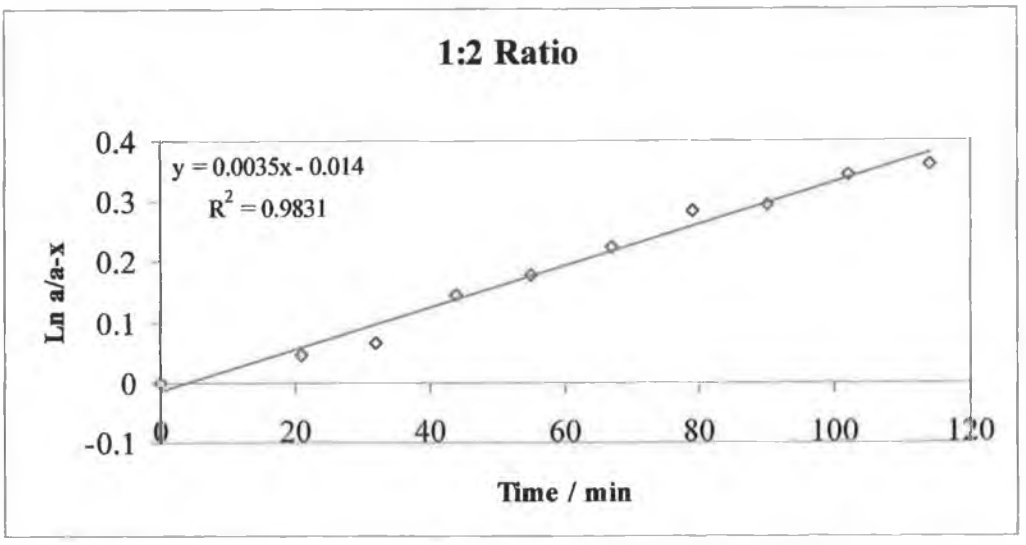
The transmethylation reaction of the methyltin chlorides with mercury chloride has been found to occur in the experiments conducted in the course of this work. The degree of transformation was found to depend on the concentrations of the relevant species present. When performed in a liquid medium the transfer of one alkyl group was favoured over the transfer of two alkyl groups which had been previously found to occur elsewhere.

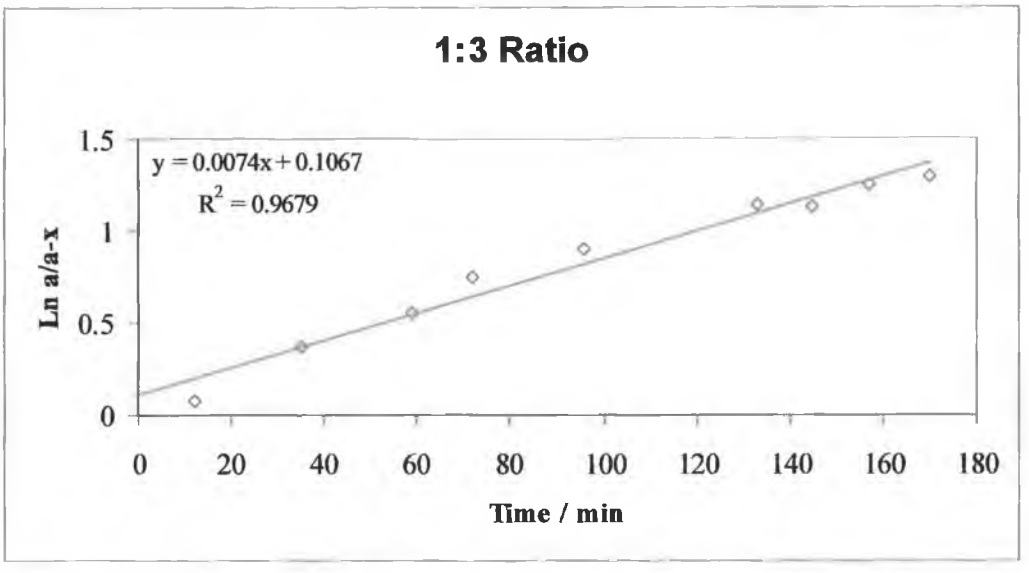
An investigation of the kinetics of the transmethylation reaction of trimethyltin chloride with mercury (II) chloride revealed that the reaction followed second order kinetics. Similar investigations of these reactions involving dimethyltin dichloride and methyltin trichloride determined that these reactions were also second order processes.

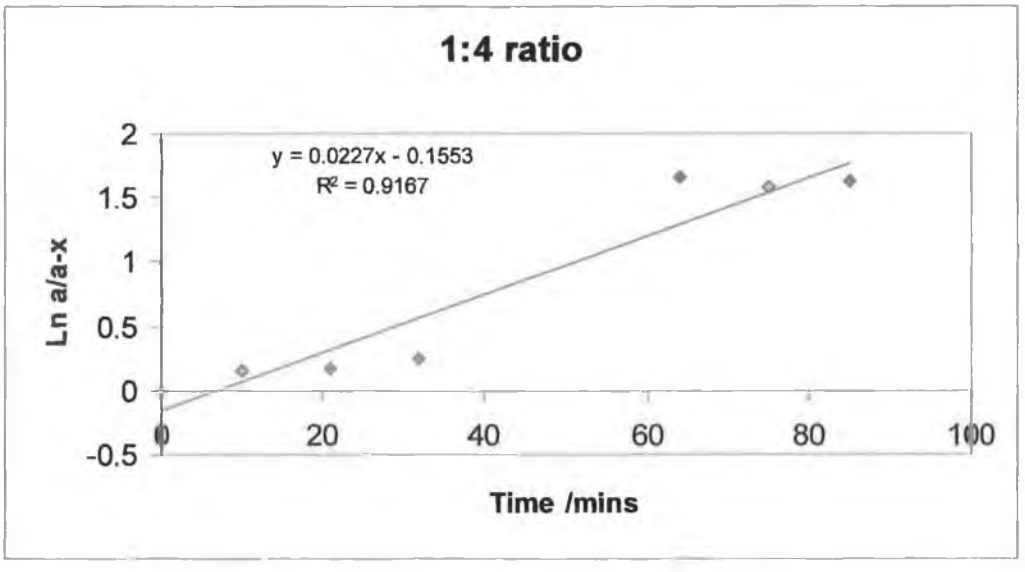
A number of these transmethylation reactions were repeated with the methyltin species bound to a solid support. For the reactions involving trimethyltin chloride it was found that at lower trimethyltin chloride concentrations the transmethylation reaction which occurs when the tin species is bound to the solid material occurs at a faster rate than when a similar reaction is performed in a fluid medium. Similar results were obtained for the reactions performed with dimethyltin dichloride.

Potentially, these results have widespread implications. The transmethylation reaction has been found to occur in a liquid environment – leading to the formation of more toxic products. However, should there be solid material present, to which the tin species may bind, the reaction is much more likely to occur. Although the reactions performed here were performed at substantially higher levels than would be found in the environment, it is clear that a trend is apparent and the levels of both the methyltin chlorides and the mercury chlorides in the environment should be carefully monitored in relation to each other species.

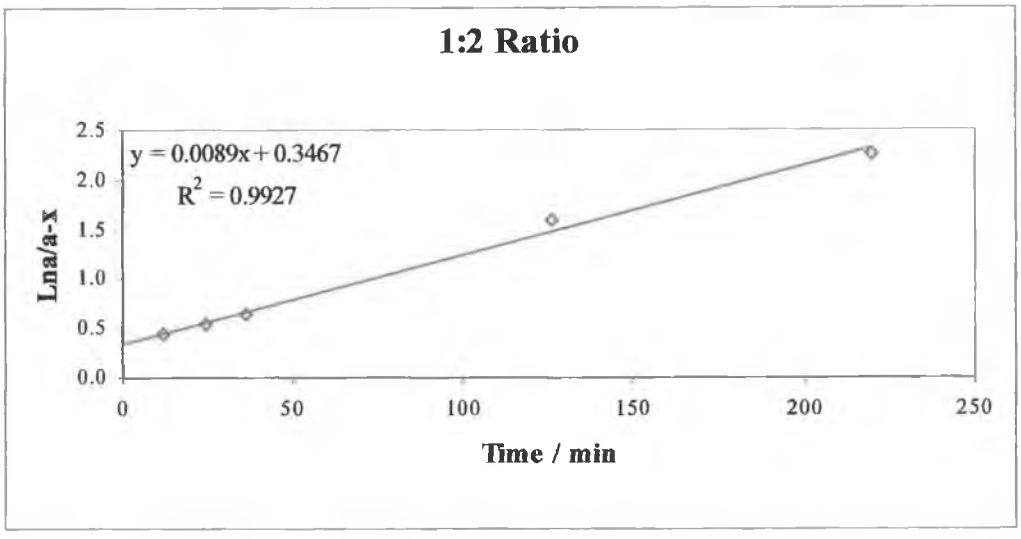
7.0 Appendix 1

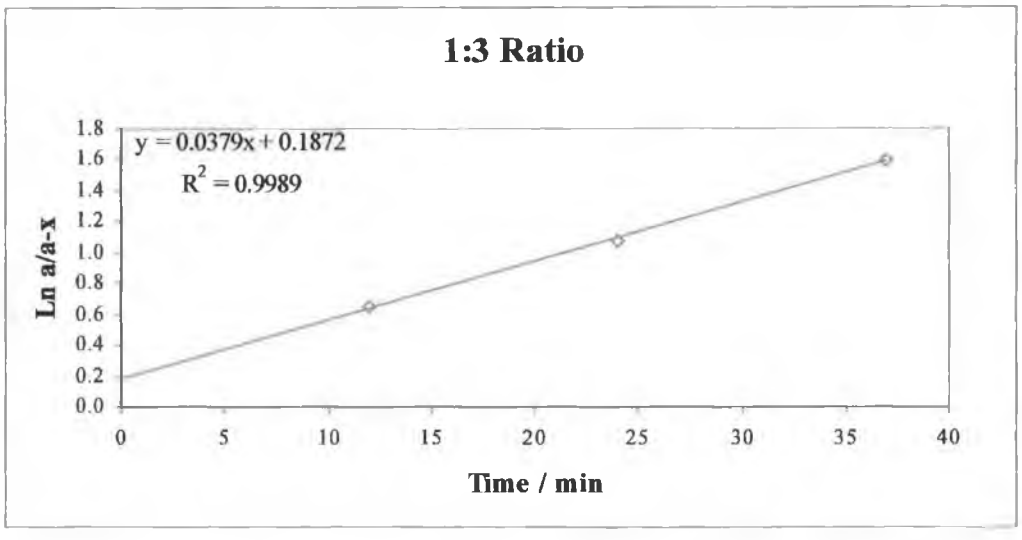


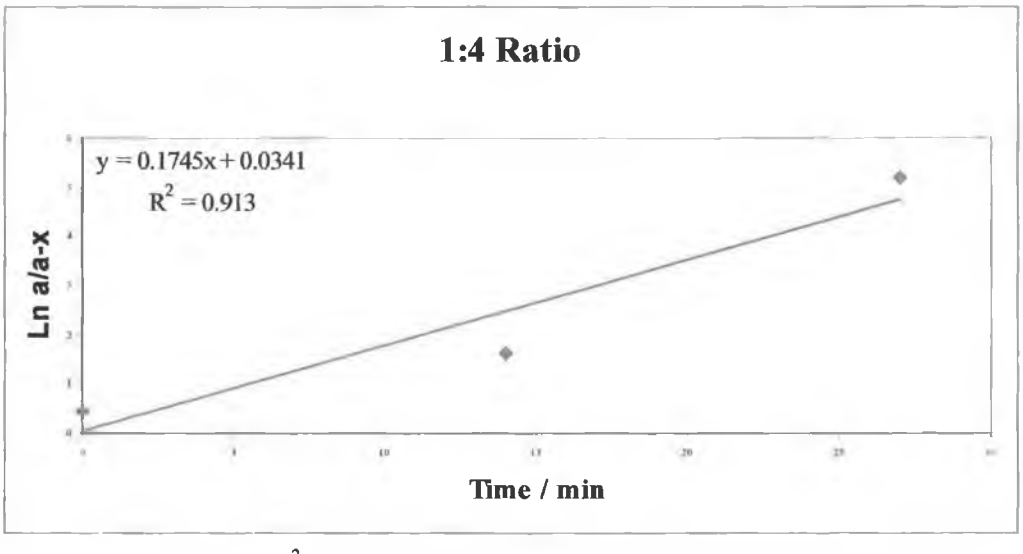




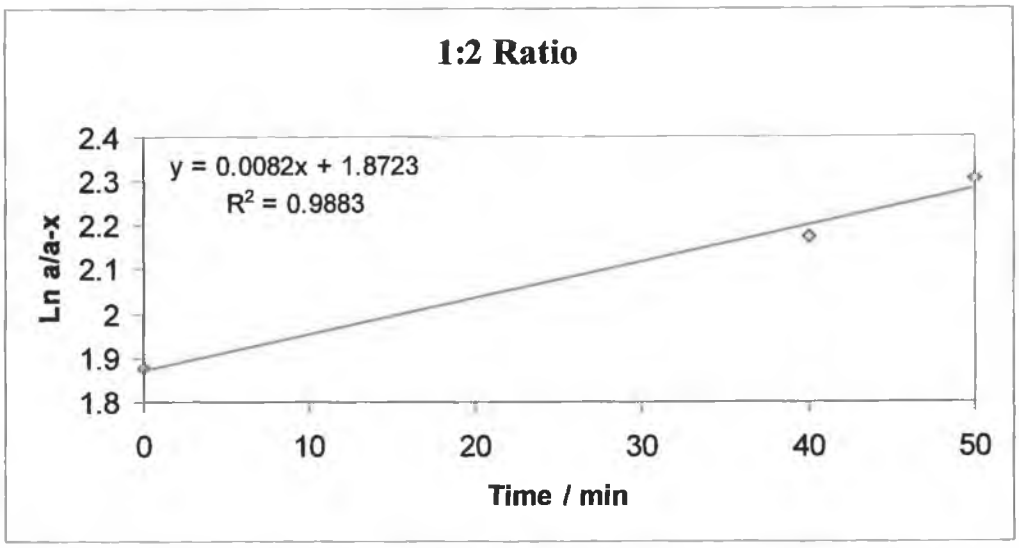
Appendix 1.1: 0.5×10^{-3} M Me_3SnCl reacted with (A) 1:2, (B) 1:3 and (C) 1:4 ratio with $HgCl_2$

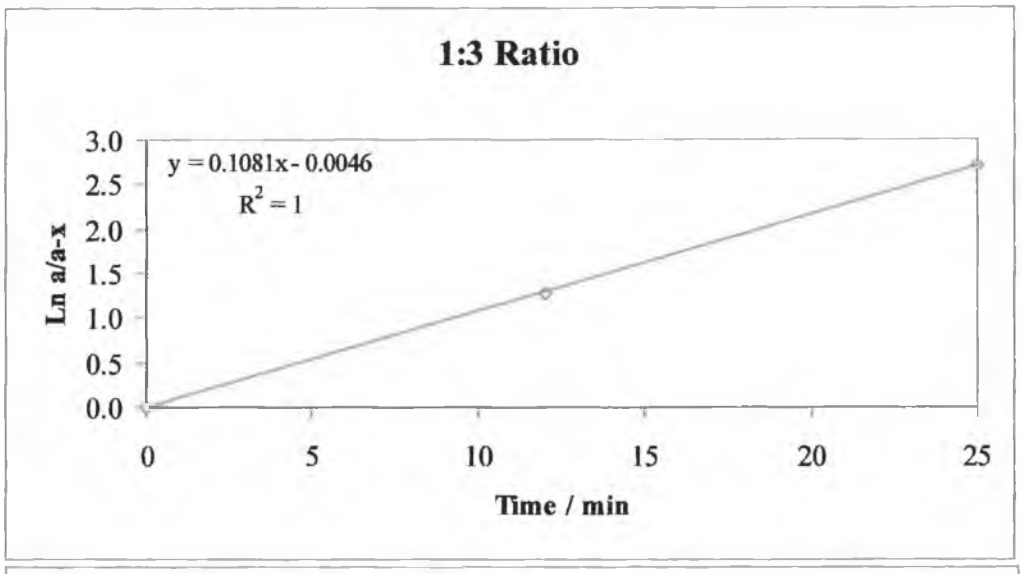


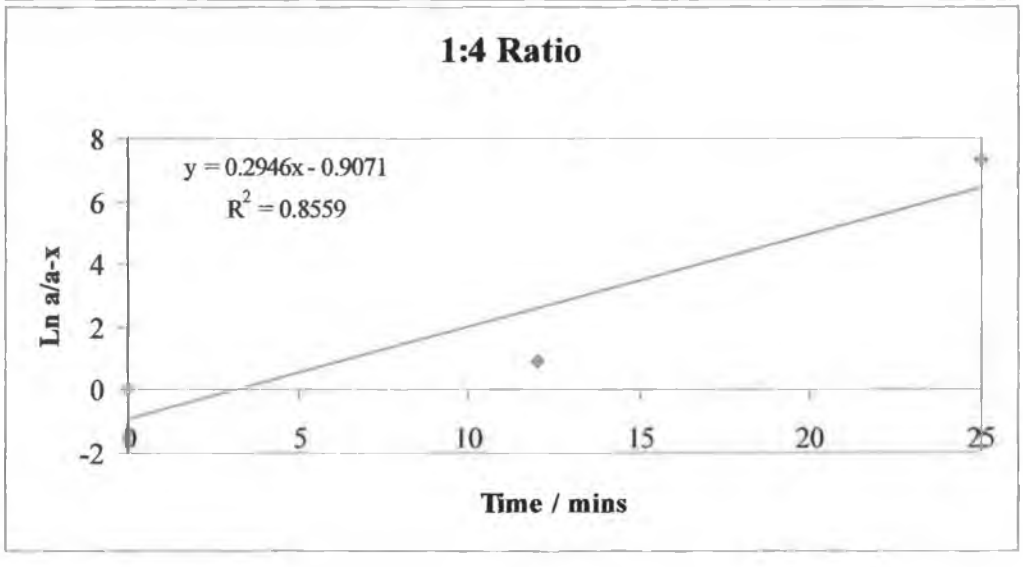




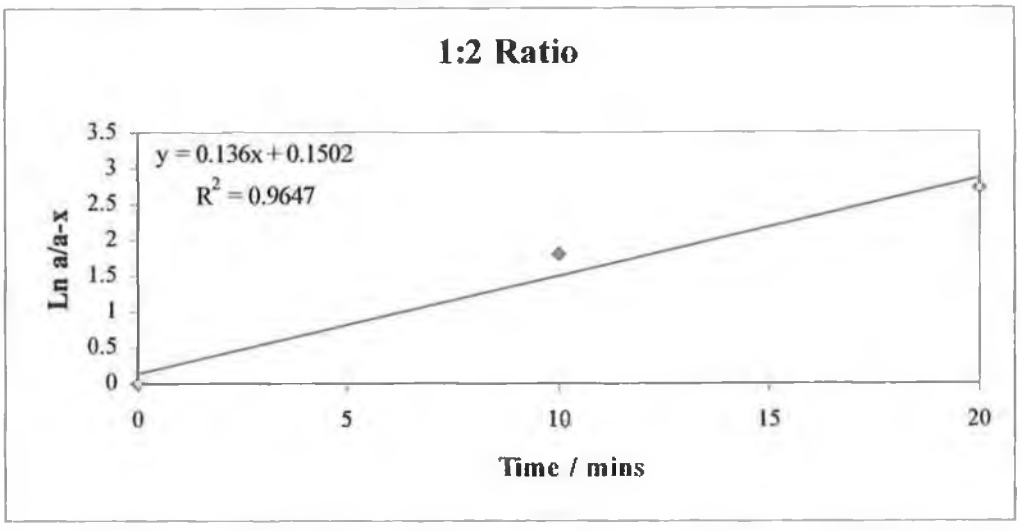
Appendix 1.2: 1.0×10^{-3} M Me_3SnCl reacted with (A) 1:2, (B) 1:3 and (C) 1:4 ratio with $HgCl_2$

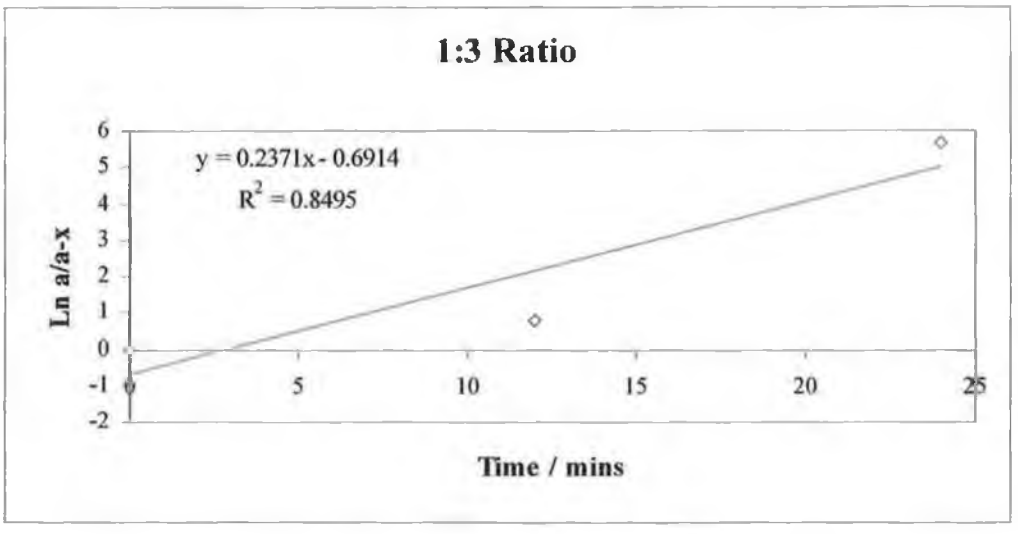




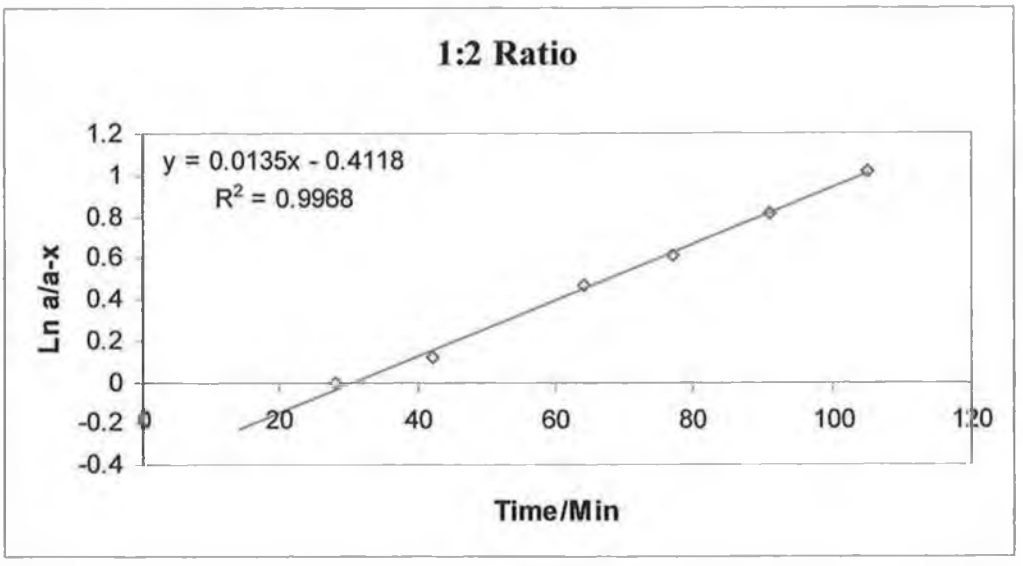


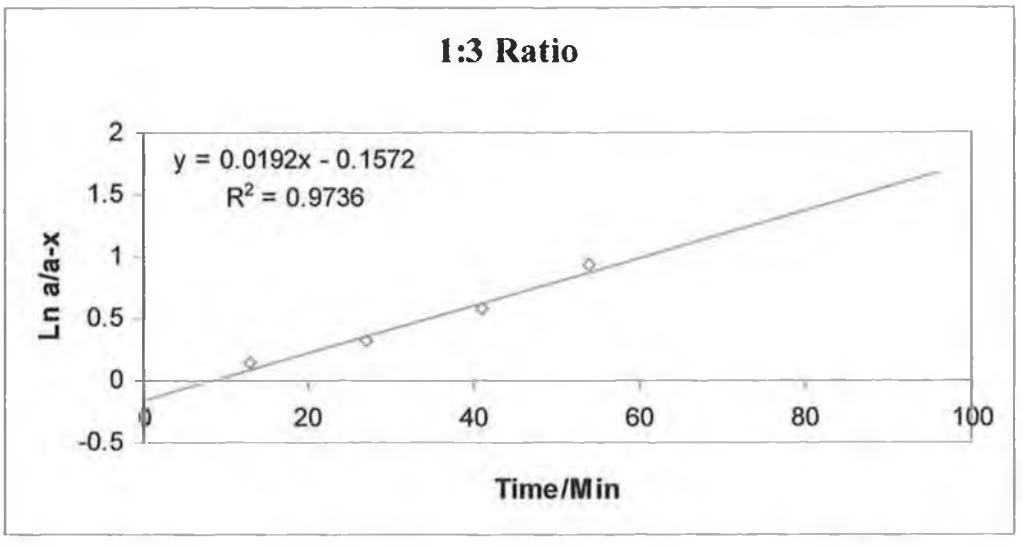
Appendix 1.3: 2.0×10^{-3} M Me_3SnCl reacted with (A) 1:2, (B) 1:3 and (C) 1:4 ratio with $HgCl_2$



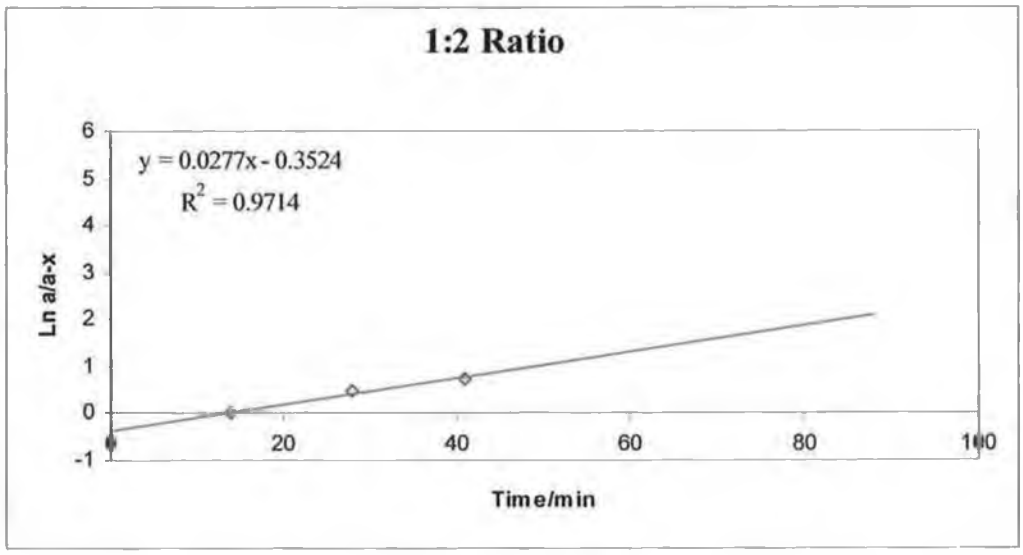


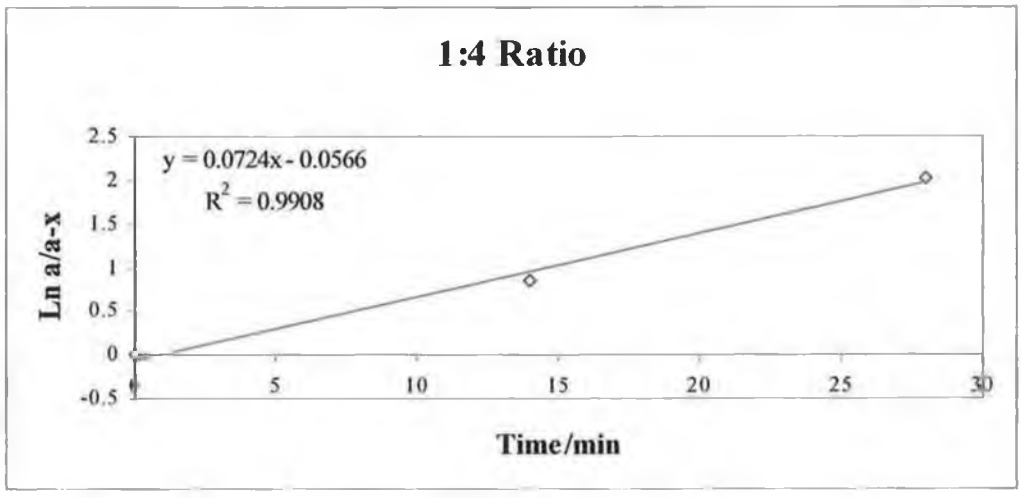
Appendix 1.4: 3.0×10^{-3} M Me_3SnCl reacted with (A) 1:2 and (B) 1:3 ratio with $HgCl_2$



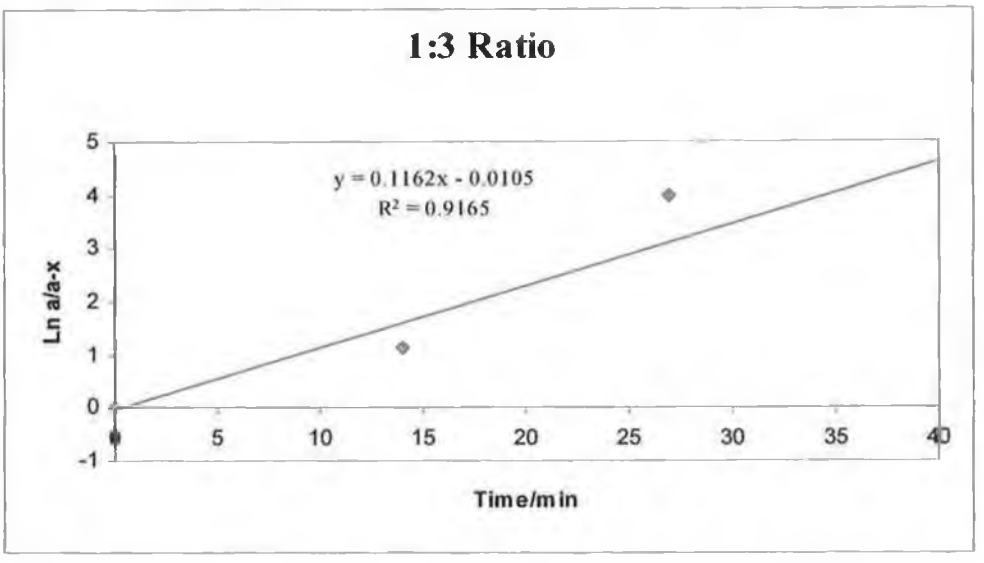


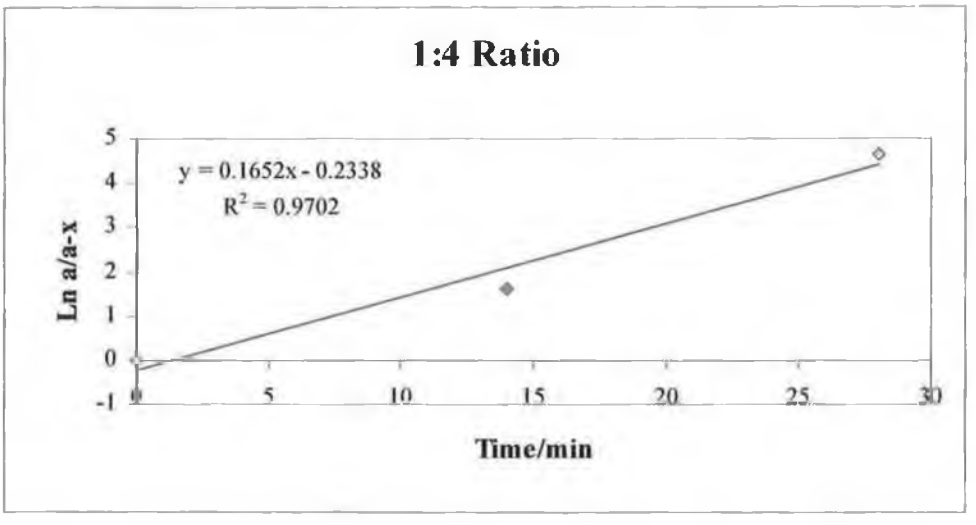
Appendix 1.5: 0.9×10^{-3} M Me_2SnCl_2 reacted with (A) 1:2 and (B) 1:3 ratio with $HgCl_2$



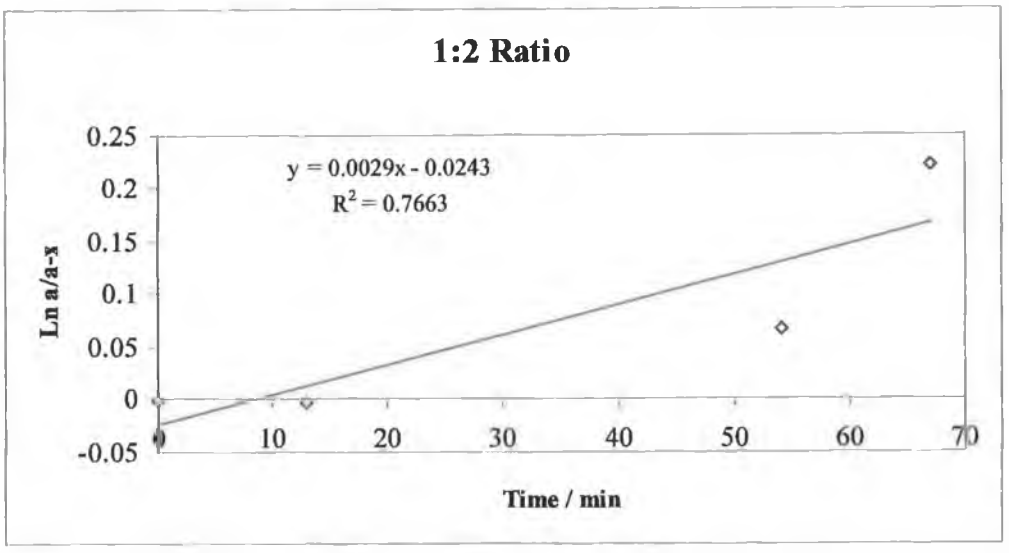


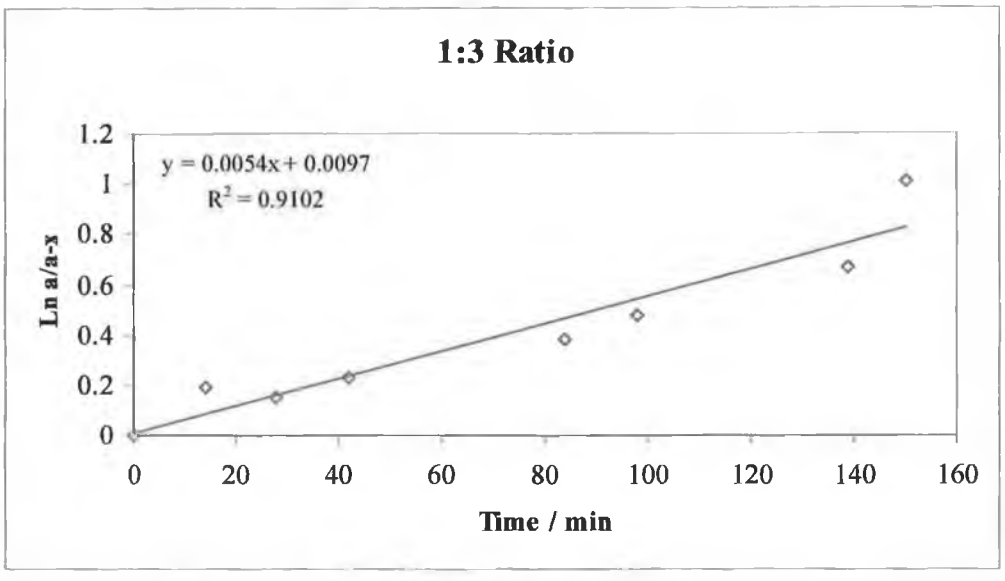
Appendix 1.6: 1.8×10^{-3} M Me_2SnCl_2 reacted with (A) 1:2 and (B) 1:4 ratio with $HgCl_2$

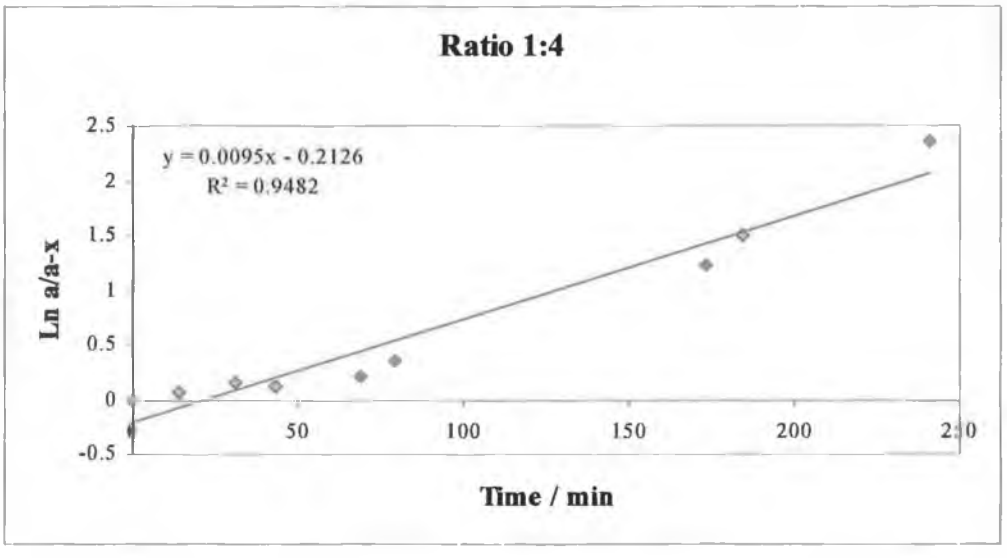




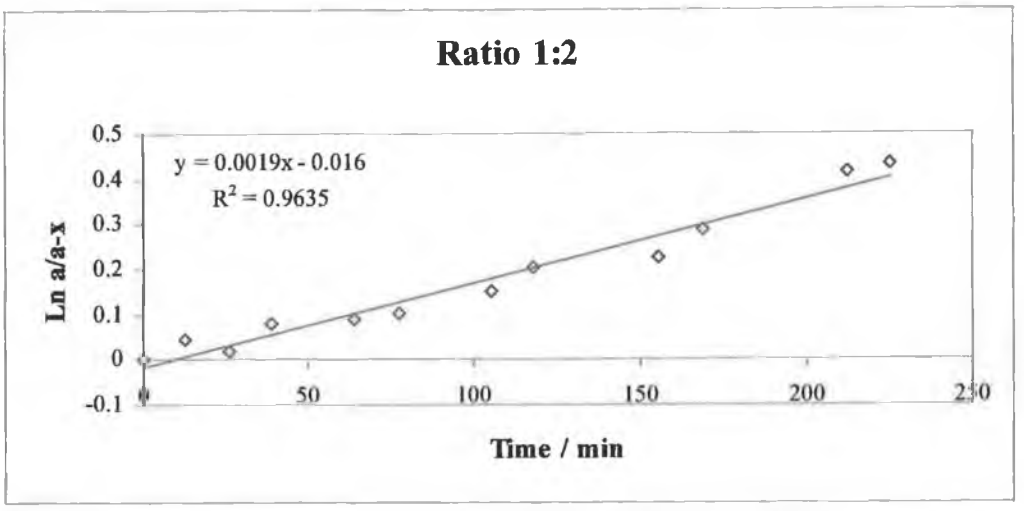
Appendix 1.7: 3.6×10^{-3} M Me_2SnCl_2 reacted with (A) 1:3 and (B) 1:4 ratio with $HgCl_2$

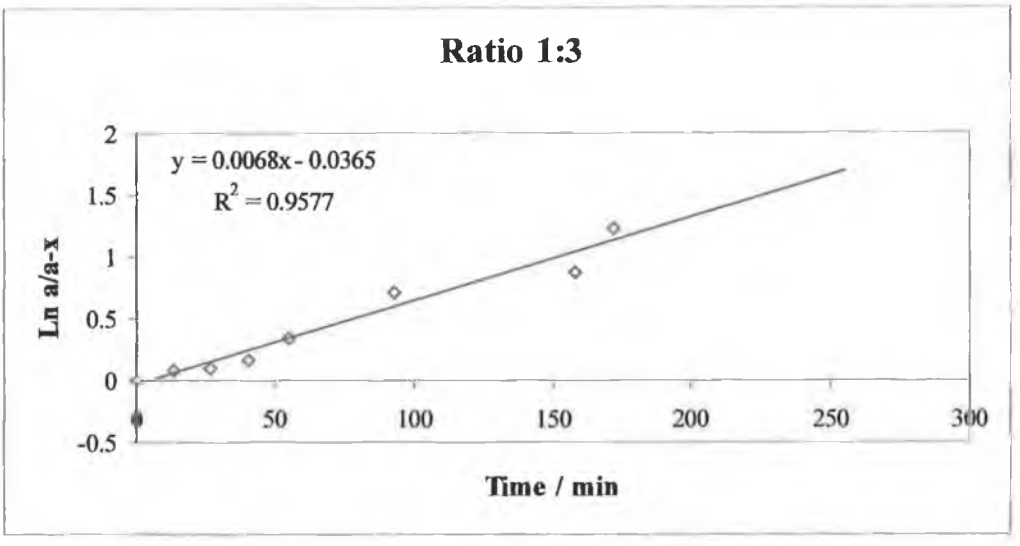


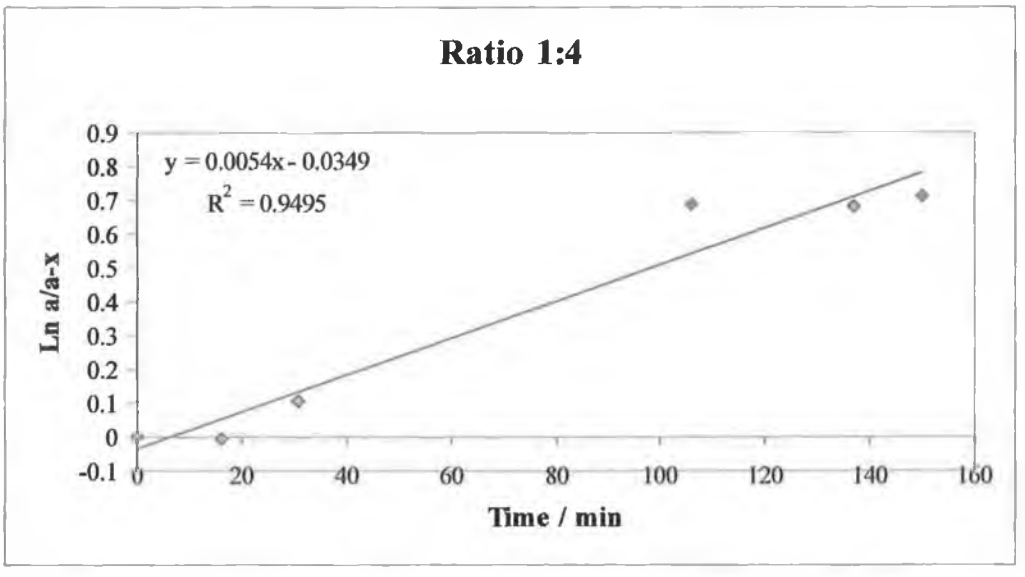




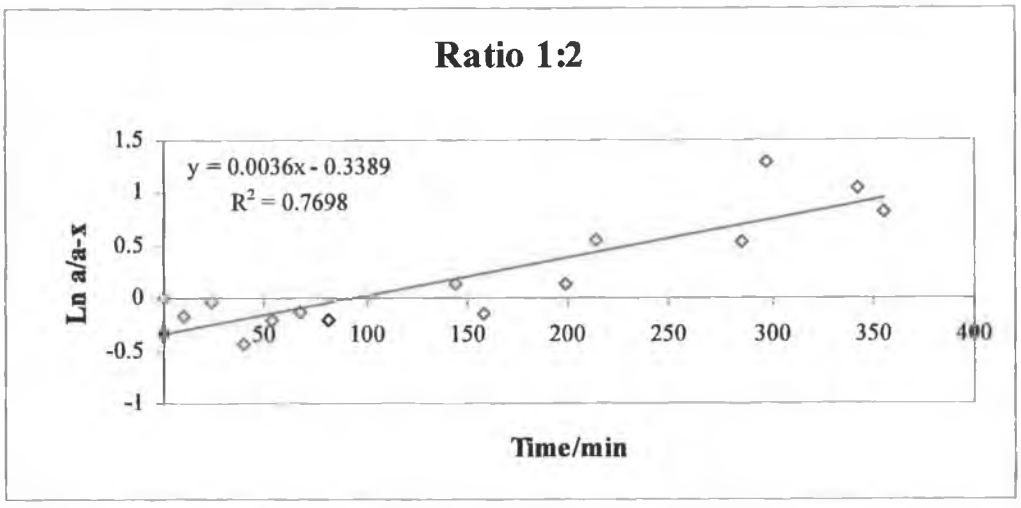
Appendix 1.8: 0.83×10^{-3} M $MeSnCl_3$ reacted with (A) 1:2, (B) 1:3 and (C) 1:4 ratio with $HgCl_2$

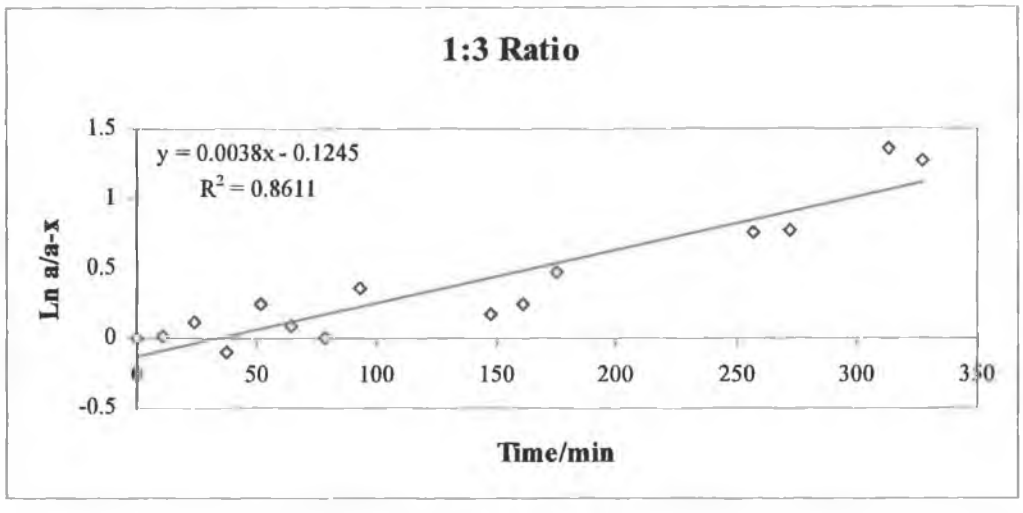


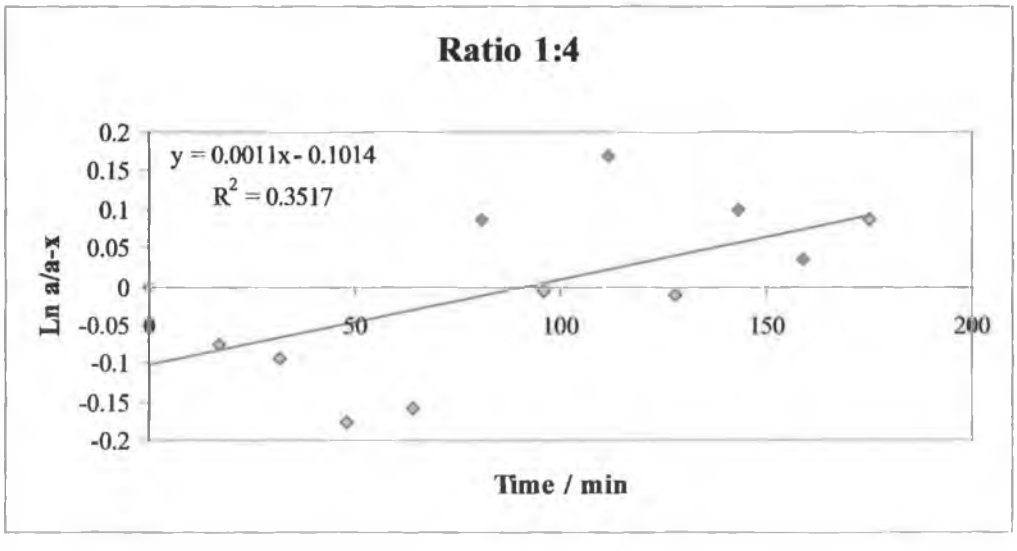




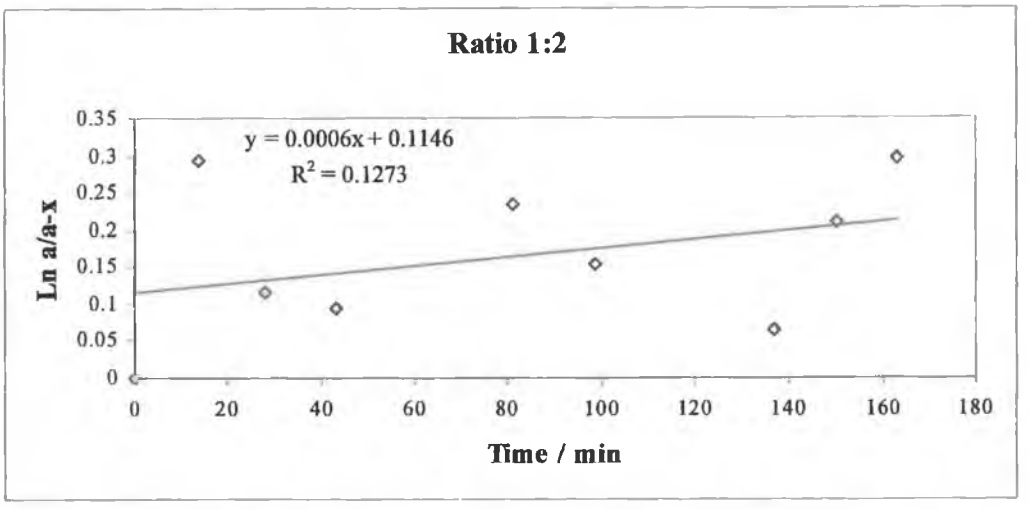
Appendix 1.9: 1.66×10^{-3} M $MeSnCl_3$ reacted with (A) 1:2, (B) 1:3 and (C) 1:4 ratio with $HgCl_2$

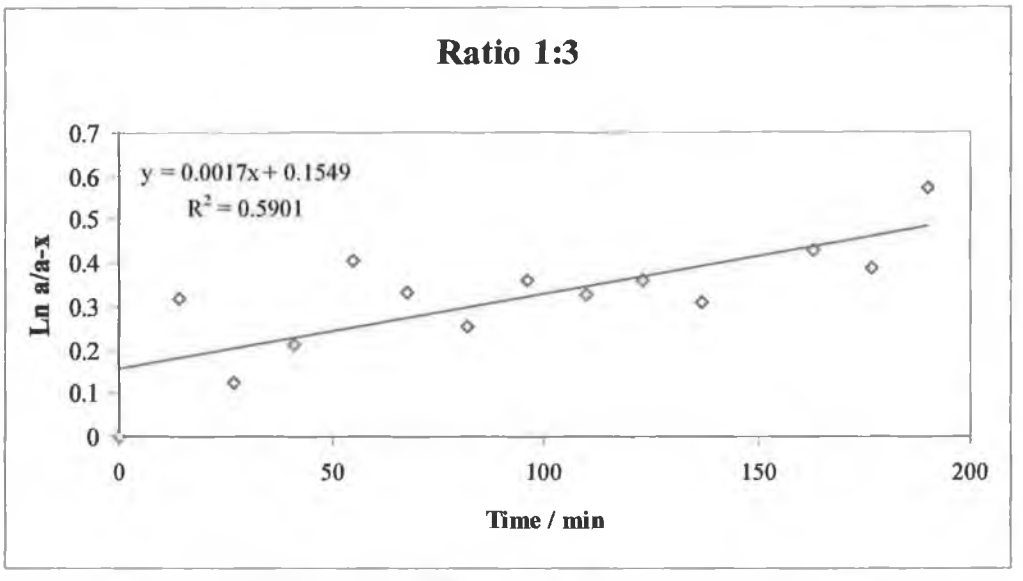


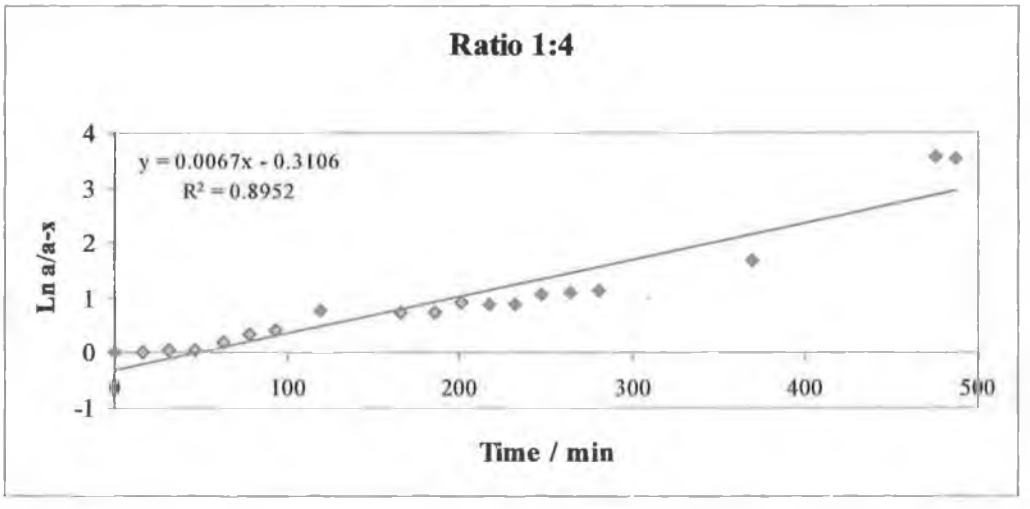




Appendix 1.10: 2.5×10^{-3} M $MeSnCl_3$ reacted with (A) 1:2, (B) 1:3 and (C) 1:4 ratio with $HgCl_2$







Appendix 1.11: $3.3x10^{-3}$ M $MeSnCl_3$ reacted with (A) 1:2, (B) 1:3 and (C) 1:4 ratio with $HgCl_2$