DUBLIN CITY UNIVERSITY

Ollscoil Chathair Bhaile Átha Cliath

Some Electroanalytical Investigations into the Cure Chemistry of Industrial Sealants

by

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A thesis submitted for the Degree

of

Doctor of Philosophy

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Dublin City University August 1996

DECLARATION

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy (PhD) 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: Declan Raftery Date: 23/9/96

ID No. 92700241

For my parents

Kevin and Noreen

Happy the man, and happy he alone,
He, who can call today his own,
He who, secure within, can say,
Tomorrow do thy worst, for I have lived today.

John Dryden 1631-1700

Acknowledgments

Firstly, I would like to thank Prof. Malcolm Smyth for his constant support throughout the course of the research and in particular during the preparation of this thesis. Also, for the many enjoyable games of golf and the in-depth discussions in the 19th thereafter. I am also grateful to Dr. Ray Leonard for affording me the opportunity to work with Loctite for the past four years and for his enthusiasm and assistance in that time. A word of thanks to the staff of Loctite (Irl) Ltd., in particular Dr. Martin Brennan.

In DCU, the assistance and expertise of the members of staff and technicians is very much appreciated. The 'J118 crew' past and present, Mikey, Mike, Karren, Teresa and Mary (all of whom provided a first class phone answering service!) and all the other postgrads in the chemistry department, thanks for making the years in college so enjoyable.

To some personal friends Dec B, Donagh, Alan, Aidan, Siobhan, Emer, Gillian and Mairead for the mid-week 'get togethers', memorable holidays.....and for your support and understanding of postgrad 'life' and your endless humour - thanks guys!!

And finally the Raftery family, without whom this thesis would never have seen the light of day, my parents Kevin and Noreen for their unwavering support over the last 25 years, a simple word of thanks could never express my gratitude - I love you both very much. And my 'little' brother, for his early morning humours and the free taxi service...thanks James.

CONTENTS

Chapter Two

INVESTIGATIONS INTO THE TRANSITION METAL BASED CURE CHEMISTRY OF ANAEROBIC ADHESIVES 28

Chapter Three

INVESTIGATIONS INTO THE METAL SUBSTRATE AND HYDROPEROXIDE BASED CURE CHEMISTRY OF ANAEROBIC ADHESIVES 67

Chapter Four

THE IDENTIFICATION OF HYDROGEN PEROXIDE AS THE AUTOXIDATIVE PRODUCT OF N-PHENYL-2-PROPYL-3,5-DIETHYL-1,2-DIHYDROPYRIDINE 94

ABSTRACT

This thesis represents a study of the cure chemistry of three contrasting adhesive technologies, applying a range of analytical approaches to gain further insight into the complex chemistry of adhesives. An introduction is given in chapter one into the general chemistry of adhesives and their analysis, with particular emphasis on anaerobic adhesives and the crucial role played by transition metals in the cure chemistry.

In order to elucidate the role played by tertiary amines and saccahrin in anaerobic adhesives, polarography was used to monitor the concentrations of various transition metal species in the presence of selected cure components. In addition, cyclic voltammetry was used to measure the oxidation potentials of anaerobic adhesive accelerators at a range of pH values.

A polarographic study of the reactions of elemental copper and iron in the presence of 1,2,3,4-tetrahydroquinoline based cure systems was carried out in chapter three. The ability of iron and copper ions to decompose cumene hydroperoxide, and the influence of anaerobic adhesive accelerators on these reactions, was also studied.

In chapter four, a brief introduction is given into the autoxidation of N-phenyl-2-propyl-3,5-diethyl-1,2-dihydropyridine (DHP) and its potential as an initiator in rapid curing, surface insensitive one-part adhesives. A variety of analytical techniques were then used, including spectrophotometry and an enzyme-based biosensor, to conclusively prove that hydrogen peroxide is generated in the autoxidation of DHP.

In chapter five, an investigation was made on the use of anion exchange chromatography coupled with conductivity detection, for the determination of inorganic anions and organic acid anions in cyanoacrylate adhesives.

A brief overview of the main findings of the thesis are given in chapter six, along with suggestions for future studies.

CHAPTER ONE

ADHESIVES AND THEIR ANALYSIS

1.1. INTRODUCTION

Adhesives are defined as 'substances that are capable of holding materials together by surface attachment'. Adhesives, often referred to as cements, glues or pastes, have been used since ancient times, with traces of glued articles having been found dating back to 3000 B.C. in Egypt. But only since the advent of modern polymer technology have they become significant items of commerce in manufacture and construction. Waxes, gums and natural resins have all been used to bond a variety of substances, but these materials have limited heat, moisture and biological resistance.

In the early part of this century, the only adhesives of major importance were the animal and vegetable glues, which had been in use for thousands of years. Casein glues were used as structural adhesives in World War I to construct the wooden main-frames of aircraft, but these were found to have limited resistance to moisture and mould growth. Such limitations with natural adhesives provided the stimulus for the rapid expansion, since the 1930s, in the development of new adhesives, based mainly on synthetic resins.

Phenol-formaldehyde was the first resin of importance, being mainly used for wood assembly and plywood manufacture. Later, demands of the aircraft industry for materials capable of metal bonding led to the employment of modified phenolic resins containing synthetic rubber, to produce adhesives displaying high shear and peel strength. The 1950's then saw the introduction of epoxy-resin based adhesives with the advantage of a 100% reactive solid system.

Today, the number and range of applications for adhesives is large, with modern industrial processes using adhesives in assembly lines which depend on the use of small quantities of adhesives for rapid bonding of components. Paper, packaging, footwear and woodworking still remain major outlets for adhesives, but usage has increased significantly in industrial equipment, building and construction, vehicle manufacture, instrumentation, electrical and optical assemblies and for military and space applications.

For the purpose of this introductory chapter, adhesives can be divided into three main categories: namely (a) Anaerobic adhesives, (b) Acrylic adhesives, (c) Cyanoacrylate adhesives

1.2. ANAEROBIC ADHESIVES

1.2.1 General Chemistry

Anaerobic adhesives derive their name from the characteristic of requiring a relatively oxygen-free environment for proper cure , such as that found in processes requiring closely bonding assemblies. Anaerobic sealants are typically single component acrylic adhesives which remain in liquid form in the presence of air, but when confined between two closely fitting metal parts, thereby excluding air, the monomeric constituents in the liquid polymerise or cure rapidly to form a tough heat and solvent resistant solid material [1]. Therefore, anaerobic sealants are predominantly used in the mechanical engineering industry, finding applications in the sealing, locking and retaining of metal objects.

Anaerobic adhesives find their origins in the 1950's when Burnett and Nordlander [2] of G.E.C. in the United States patented the use of anaerobic monomers, based essentially on polyethyleneglycol dimethacrylates, in a product called 'Permafill'. This formulation was, however, marketed with little success by G.E.C. Burnett and Nordlander discovered that the monomer when oxygenated by heating at 60°-80°C in the presence of bubbled air, remained as a liquid when cooled, as long as aeration was maintained. However, when the bubbling of air was discontinued, or when the liquid was pressed as a thin film between two glass plates, rapid cross-linking occurred, resulting in a solid polymeric material. In 1953, Krieble [3] of the Loctite Corporation, developed the first anaerobic sealant composed of methacrylate monomers, containing small amounts of cumene hydroperoxide; this formulation

being inherently more stable than the previous G.E.C. oxygenated materials. This combination of polyethyleneglycol dimethacrylate (typically triethyleneglycol dimethacrlyate) and organic hydroperoxide continues to form the basis for many of the more than 250 U.S. patents [4] issued since then. Other components are added to the formulations to improve both the cure rate and increase the shelf life of the products at ambient temperatures.

Anaerobic adhesives cure or polymerise by a metal catalysed redox-based system [5] (Figure 1.1). When the adhesive is confined between two closely fitting metal parts, air is excluded and the metal surface catalyses the decomposition of the peroxide component to form free radicals. The free radicals then react with one of the terminal double bonds of another monomer molecule in a chain propagation sequence. This process continues until termination occurs, most probably by disproportionation. The linear polymer chain can undergo further reaction with more radicals to produce a three dimensional cross-linked thermoset resin, possessing good heat stability and solvent resistance. In the presence of oxygen, any radicals present react with molecular oxygen to form an inactive peroxy radical which is incapable of initiating polymerization. The feature of anaerobic polymerization that sets it apart from other acrylic polymerizations is the initiation process. The balance between the initiator/accelerator system and the inhibitors present must be very carefully controlled to allow the confinement of the adhesive between metal surfaces to cause rapid cure.

An anaerobic adhesive containing only a methacrylate monomer and organic peroxide is strictly limited by its slow rate of polymerization, the sensitivity of the cure process to the substrate to which the adhesive is applied, and the stability of the product. Therefore, modern research has concentrated on the need to produce an adhesive or sealant formulation that contains a prudent balance between rapid cure speed and a stable packaged product with a long shelf life.

$$ROOH + M^{n+} + H^{+} \rightarrow RO^{\bullet} + M^{(n+1)+} + H_2O \text{ (fast)}$$

$$ROOH + M^{(n+1)+} \rightarrow ROO^{\bullet} + M^{n+} + H^{+} \text{ (slow)}$$

$$2ROO^{\bullet} \rightarrow 2RO^{\bullet} + O_2$$

$$RO^{\bullet} + H_2C{=}CH_2 \rightarrow ROCH_2CH_2^{\bullet} \text{ etc.}$$

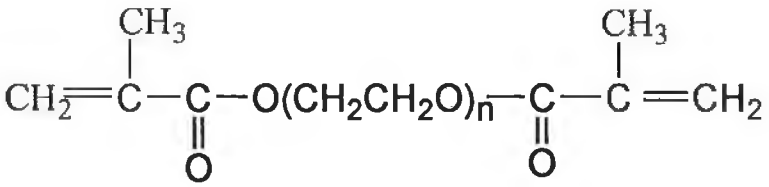
Figure 1.1 The reaction scheme of the redox-based cure chemistry of anaerobic adhesives, which highlights the key catalytic role played by the active metal surface.

In order to increase the cure rate at room temperature, cure promoters or accelerators, such as acids or bases, are incorporated into adhesive formulations. A variety of organic acids have been used, including p-toluene sulfonic acid, maleic acid and saccharin, while organic reducing agents have included aromatic amines such as N,N-dimethyl-p-toluidine, 1,2,3,4-tetrahydroquinoline and 1-acetyl-2-phenylhydrazine. Research has identified the accelerator combination of dialkylarylamines, such as N,N-dimethyl-p-toluidine and saccharin [6]; these compounds act as accelerators in their own right, but together they produce a synergistic increase in cure speed. No mechanistic details explaining the role of each compound has been published, and therefore the precise role each plays in the curing mechanism remains to be elucidated. Modern anaerobic adhesives therefore contain five basic components; namely a monomer, initiator, catalyst (accelerator), stabiliser(s) and modifier(s), all combining to yield formulations that maintain a prudent balance between rapid cure speed and a stable package with a long shelf-life.

1.2.2 Analysis of Anaerobic Adhesives

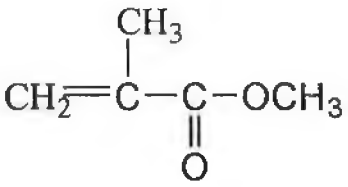
1.2.2.1 Analysis of Monomers

The polymerisable monomer is the major constituent in a commercial adhesive, and the performance of the cured adhesive is primarily due to the monomers employed. Methacrylates are the most common monomers used due to their ease of synthesis, performance, speed of cure and lower oxygen sensitivity. Although polyethyleneglycol dimethacrylate (I) continues to be the most widely used monomer because of its low cost, availability, low toxicity, high reactivity and good environmental resistance of the cured polymer, other methacrylate esters have also been used [7] (Figure 1.2).

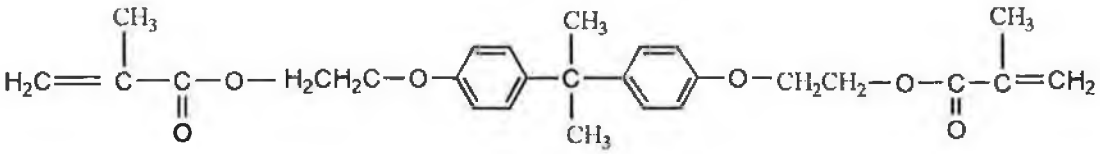


where n>2

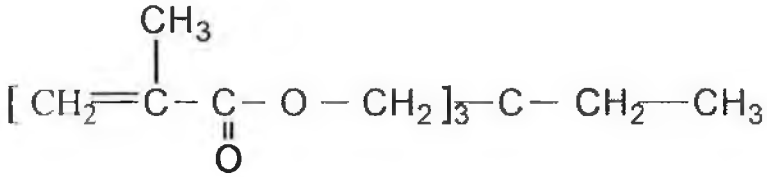
Polyethylene glycol dimethacrylate (I)



Methyl methacrylate (II)



Ethoxylated Bisphenol A dimethacrylate (III)



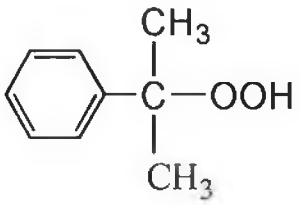
Trimethylolpropane trimethacrylate (IV)

Figure 1.2 Examples of monomers used in anerobic adhesives.

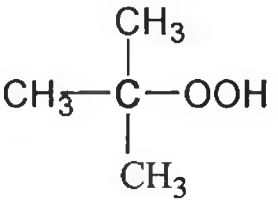
The presence and identity of monomers in an adhesive formulation is normally established using nuclear magnetic resonance (NMR) spectroscopy Their identities and concentrations can be further determined using either gas chromatography (GC) or high performance liquid chromatography (HPLC) with an internal standard. For GC analysis, OV1 (polydimethylsiloxane) or OV101 (polyphenylmethylsiloxane) are the preferred stationary phases, using either wide bore or capillary columns. A flame ionisation detector (FID) is usually sufficient for detection purposes, but mass spectrometry can be employed for unambiguous confirmation of peak identity. HPLC is the preferred method of analysis in the case of UV cured adhesives which use the higher molecular weight urethane-methacrylate resins. The relatively involatile monomers are normally separated using a reversed-phase C_{18} column with THF/H_2O mixtures as mobile phase with spectrophotometric detection [7].

1.2.2.2 Analysis of Accelerators

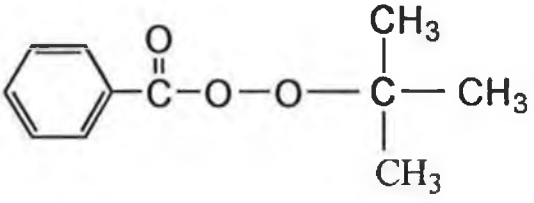
The performance of modern adhesive/sealant formulations is critically dependent on the cure system used. This must be judiciously balanced in order to give the desired performance, especially with respect to cure speed, together with a viable stability for the packaged product. Accelerators or cure promoters encompass a wide range of compounds, which when combined together result in the desired adhesive performance. The range of accelerators include an organic peroxide, an organic acid and an organic reducing agent; these additives not only increase the rate of curing but also the percentage of monomer polymerised. Typical peroxides used are alkyl hydroperoxides such as cumene hydroperoxide (V) and tertiary butylhydroperoxide (VI). Peresters and diacyl peroxides are also used occasionally, such as tertiary butylperbenzoate (VII) (Figure 1.3).



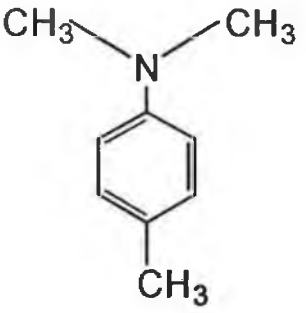
Cumene hydroperoxide (V)



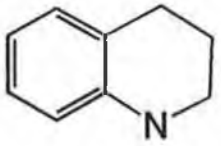
Tertiary butylhydroperoxide (VI)



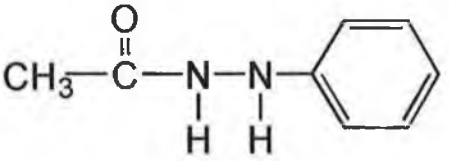
Tertiary butylperbenzoate (VII)



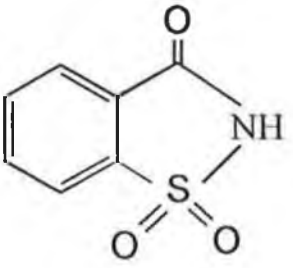
N,N-dimethyl-p-toluidine (VIII)



1,2,3,4-Tetrahydroquinoline (IX)



1-Acetyl-2-phenylhydrazine (X)



Saccharin (XI)

Figure 1.3 Examples of accelerators used in anaerobic adhesives.

Typical organic acids used in formulations are p-toluene sulphonic acid and maleic acid. Aromatic amines such as N-N-dimethyl-p-toluidine (VIII), 1,2,3,4-tetrahydroquinoline (IX), tributylamine or 1-acetyl-2-phenyl hydrazine (X) are employed as reducing agents in conjunction with saccharin. The cure accelerators are present at relatively low concentrations (approx. 1%); therefore, it is essential that appropriate analytical methods and techniques are available to identify and quantify these reactive compounds [7]. Thin layer chromatography has been found to be the most convenient procedure to confirm the presence of organic peroxides, organic acids and organic reducing agents in formulations. This simple inexpensive technique can separate the curative agents from their complex matrices and from each other. In the case of peroxy initiators, specific spray reagents can be used to visualise the compounds after separation on a silica plate with an appropriate solvent mixture (generally diethyl ether mixtures with petroleum spirit (40^{o}-60^{o})). An example of such a reagent is an aqueous methanolic solution of N,N-dimethyl-p-phenylenediamine dihydrochloride [8], which on heating gently with the peroxide will give rise to a typical purple response against a blue background. Other developing agents used in the detection of peroxides are acidified potassium iodide and p-aminodimethylaniline hydrochloride [9].

A dilute solution of 2,6 dichlorophenolindophenol, sodium salt, is used in the detection of organic acids on TLC plates [10]. Similarly, aromatic amines are detected using a dilute aqueous solution of iron(III) chloride and potassium hexacyanoferrate [11]. The peroxide content of adhesives can also be determined by iodometric titration, as peroxides react stoichiometrically with the iodide ion in acidic solution producing iodine which can then be titrated with a standard solution of sodium thiosulphate [12]. The end point of the titration can be detected potentiometrically using a platinum/reference electrode combination.

The acidic components of formulations are similarly quantified by titration using dilute caustic or tetrabutylammonium hydroxide as titrants in aqueous or non-aqueous media respectively, with potentiometric end point detection using a glass reference electrode combination. Aromatic amine content is normally determined by

non-aqueous titration with perchloric acid, using acetic acid as a solvent, and as before, the end point can be detected potentiometrically [7].

The peroxides present in adhesives react stoichiometrically with the iodide ion in acidic solution, producing iodine, which can be titrated with a standard solution of sodium thiosulphate [13 ,12], or estimated colorimetrically as tetra-n-butylammonium iodide [14]. The standard iodometric titration for the determination of peroxides is relatively time consuming, and alternative methods using either GC (OV1/FID detection) or HPLC (C_{18} column; acetonitrile/water mobile phase; UV detection at 254 nm) have been investigated. In general, chromatographic procedures involve a relatively short analysis time (5-6 min) as compared to the titrimetric assay (15 min). Peroxides are normally reduced to alcohols prior to GC analysis. The direct analysis of peroxides is somewhat rare owing to their sensitivities to heat and surfaces. However, this approach is favoured in Loctite, with thermal decomposition of the peroxide in the injection port being prevented by the use of an on column injection technique [7]. From studies carried out in Loctite it was concluded (i) that either GC or HPLC analysis may be substituted for iodometric analysis in the case of benzoyl peroxide and cumene hydroperoxide (CHP), without any apparent loss in either precision or accuracy; (ii) that GC analysis may be substituted for iodometric analysis in the case of t-butyl hydroperoxide without any loss in precision or accuracy (whilst there was some loss of accuracy in the GC analysis of di-t-butylperoxide, the precision of the assay was acceptable and thus prefered, in this case, to the tedious and problematic iodometric titration); (iii) the HPLC analysis may be substituted for iodometric analysis in the case of t-butylperbenzoate without any apparent loss in either precision or accuracy.

A much less popular, but nevertheless excellent, technique for the quantification of peroxides is polarography. The method has been described by Martin [15] in detail, who has provided tables of the half-wave potentials for hydroperoxides and other peroxide compounds. The disadvantage of this technique is that it cannot differentiate properly between individual peroxides in mixtures.

The polarographic reduction waves tend to be long and drawn out due to the irreversibility of the reduction process; a separation of about 0.40 V in half-wave potentials is necessary before discrete steps become apparent. The diffusion current is usually proportional to peroxide concentration over the $1x10^{-2}$ M-$1x10^{-6}$ M range, but since the proportionality constant varies with structure, individual calibration is necessary for accurate analytical work .

1.2.2.3 Analysis of Chelating Agents

Sequestering (or chelating) agents are frequently included in formulations to avoid premature polymerization initiated by trace levels of contaminating transition metal ions arising from raw materials, the manufacturing process or final packaging. The preferred chelating agents possess a combination of oxygen and nitrogen ligands such as sodium salts of ethylenediamine tetracetic acid [16] (EDTA) and analogous compounds. The stability of many adhesives is critically dependant on both the type and concentration of the free metal ion chelator. EDTA can be quantified by differential pulse polarography (DPP). Certain metal-EDTA complexes are reduced at more negative potentials than the metal ion itself. Therefore, if an excess of the metal ion such as copper(II) is added to a suitable supporting electrolyte (eg. 0.1 M acetate buffer, pH 5.0) containing a known amount of dissolved adhesive, then the voltammogram will exhibit two peaks. The peaks represent the free metal ion and the complexed ion respectively. The chelator can be quantified using a standard addition method, by the addition of aliquots of standard chelator of known concentration to the electrolytic cell, and measuring the subsequent current increase [7].

Due to the ability of trace metals to initiate polymerisation, it is necessary to screen both raw materials and finished products for trace metal ion content. This is normally carried out by atomic absorption spectroscopy (AAS) or direct current plasma emission spectroscopy [17] (DCP). These techniques only allow for the determination of total metal ion concentration, however, the feasibility of using ion

chromatography (IC) to distinguish between "free" and "chelated" metal ions, has been explored [18 ,19]. Another very promising approach appears to be the use of dynamically coated reversed-phase HPLC [20], combined with post-column derivatisation using 4-(-2-pyridylazo)-2-resorcinol (PAR) reagent and visible detection at 500 nm. This method has been applied to the simultaneous determination of six divalent metals ions in some anaerobic sealant formulations, with limits of detection as low as 30 ppb for certain metals. But polarography, either in the differential pulse mode or direct current mode, is the preferred method in the determination of multivalent metals such as chromium, which as a contaminant can occur as chromium(III) or chromium(II), or for the speciation between iron(III) and iron(II).

1.2.2.4 Analysis of Stabilisers/Inhibitors

Anaerobic adhesives require access to oxygen during storage, and for this reason they are packaged in low-density polyethylene containers. However, for commercial purposes, the dissolved oxygen content is insufficient, and to provide the required stabilization, stabilizers and inhibitors are employed to enhance shelf life. A distinction has to be made between a stabilizer and inhibitor. A stabilizer is an additive which reacts with chemical species that can lead to initiation, such as certain chelating agents which can sequester transition metals, preventing premature polymerization. Inhibitors are those additives that stop polymerization once it has begun, such as oxygen. Free radical inhibitors such as hydroquinone (XII), p-methoxyphenol (XIII) and 1,4-naphthoquinone are commonly found in anaerobic adhesive formulations [7] (Figure 1.4).

These inhibitors can be detected using TLC, by reaction with rhodamine and ammonia to give a coloured complex [21]. However, these stabilizers may achieve stability at the expense of performance, especially on non-ferrous metallic surfaces such as cadmium and zinc plates.

One way to overcome this problem is to fully oxygenate the formulation containing the methacrylate monomer and saccharin/N,N-dimethyl-p-toluidine at 25°C and then add the organic peroxide, such as CHP, prior to use. Alternatively, the problem can be overcome by treating the less active metallic surfaces with a suitable primer, such as a dilute solution of a transition metal salt (eg. copper naphthenate) in a suitable solvent [5].

1.2.2.5 Analysis of Modifiers

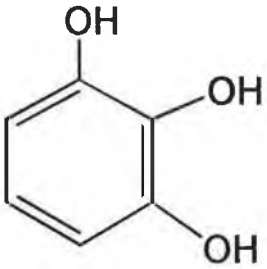
Modifiers are materials added to adhesive formulations that do not alter the cure chemistry. They include viscosity modifiers, inorganic fillers, silica-based thixotropic agents, pigments and dye stuffs, which are all incorporated in order to obtain the desired physical form and appearance. Examples of thickeners include poly(alkylmethacrylates) and polystyrene, while calcium carbonate is commonly employed as a filler.

Modifiers, such as thickeners, can be separated from the formulation matrix by precipitation of the thickener in a non-solvent (such as methanol) from an adhesive sample which is diluted initially in an appropriate solvent (e.g. chloroform). After removing all traces of solvent, the thickener can be identified by infra-red spectroscopy. Pyrolysis gas chromatography is another technique which can be used for the characterisation of polymer thickeners. In this analysis technique, complex involatile materials are broken down into smaller volatile components using high temperatures. A fingerprint characteristic of the sample is then obtained. In many instances a mass spectrometer can be coupled to the GC to facilitate the identification of the pyrolysis products [22].

Gel permeation chromatography (GPC) can also be used to determine the molecular weight range/distribution of the polymeric thickner. GPC separates molecules on the basis of their size and shape.

Hydroquinone (XII)

p-Methoxyphenol (XIII)



Pyrogallol (XIV)

Figure 1.4 Examples of inhibitors used in anaerobic adhesives.

Using a column packed with a highly crosslinked spherical polystyrene/divinylbenzene matrix with a tightly controlled pore diameter, the sample molecules will be eluted in order of decreasing molecular size. The larger molecules cannot penetrate the gel particles and therefore will pass straight through the spaces between the individual particles. The smaller molecules, however, will penetrate the gel particles depending on their size and their progress through the column will be retarded proportionally. In order to characterise the molecular weight distribution (MWD) of an unknown polymer, the GPC column can be calibrated with a series of narrow MWD standards of known molecular weight by plotting a graph of $\log_{10}$ molecular weight versus elution volume or time. The number of polymers for which suitable standards are available is limited, but polystyrene calibration standards are the most widely used and many polymers of different chemical structure and molecular weights are reported relative to polystyrene [23].

1.3. ACRYLIC ADHESIVES

1.3.1 Introduction

Anaerobic and acrylic adhesives have many chemical similarities, including common catalysts, initiators, and monomers. Because of these similarities, the two adhesives types are often confused. In most end use applications, acrylics bond metals, plastics and glass, in bonds that require flexibility as well as strength. Anaerobics, on the other hand, are used primarily as an interference adhesive or as a sealant. Chemically, acrylics are distinguished from anaerobics by their dependence on a curative, i.e. they are two-part adhesives, in which a primer component reacts on contact with the adhesive to initiate polymerization.

1.3.1.1 Monomers

A significant difference between anaerobic and acrylic monomers is the degree of acrylate functionality. Structural acrylic adhesives are typically formulated using mono-functional methacrylates resulting in lower crosslink density which is required for toughening of the acrylic matrix. Methyl methacrylate and methacrylic acid are examples of monomers commonly found in acrylic adhesive formulations [5].

1.3.1.2 Accelerators

The cure of acrylic adhesives is initiated by a two-part redox. system. One part is present in the base component of the adhesive, while the second part is present in the accelerator or curative component. The most widely used curative (catalyst) which has been used for many years in the rubber industry, is the condensation product of aniline and n-butyraldehyde, which is an oily dark amber liquid [5].

The condensation of aniline with n-butyraldehyde results in many products, but it appears that the major component and active ingredient is N-phenyl-2-propyl-3,5-diethyl-1,2-dihydropyridine[24] (DHP). The condensation product of aniline and butyraldehyde is commercially available as Vanax 808 or Du Pont 808. It can be applied neat or diluted in a suitable solvent, and once applied to a surface, the curative is effective for 4-8 hours, as the active ingredients are air oxidised. DHP reduces peroxides rapidly to produce free radicals, but the exact role that DHP and the other condensation products, numbering more than ten and accounting for up to 50% of the curative, play in the redox chemistry is not fully understood. The oxidant portion of the redox couple is typically based on a hydroperoxide such as cumene hydroperoxide. Aromatic peresters and soluble transition metal salts are often employed as initiators in low odour acrylic systems.

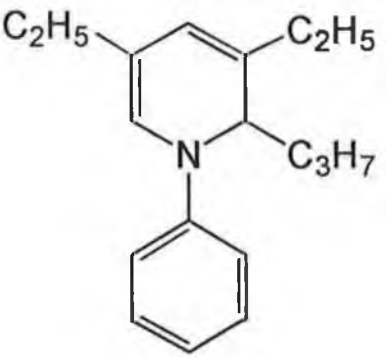
1.3.1.3 Toughеners

The ability to toughen the cured acrylic matrix is an essential part of acrylic technology. The acrylic-toughening literature deals mainly with the introduction of a 'rubbery phase' into the cured hard acrylic matrix, and this has become standard procedure in many brittle matrices, such as epoxies and cyanoacrylates. A very common rubber employed in acrylics is chlorosulfonated polyethylene, although butadiene and urethane based elastomers have also been employed.

1.3.1.4 Chemistry

The acrylic and anaerobic chemistries are very similar; the differences arise in the reactions that take place to initiate polymerisation. The common initiator/curative redox couple in structural acrylic technology, namely a hydroperoxide and the condensate of aniline and n-butyraldehyde, react to generate alkoxy radicals. The exact fate of the DHP in the redox reaction is not known; however, it is believed that the first step involves hydrogen abstraction [5] (Figure 1.5). The other major

catalyst system based on benzoyl peroxide and an aromatic amine redox couple has been well studied and the formation of the benzoyl radicals has been established. The cure of both acrylics and anaerobics are inhibited by the presence of oxygen. This sensitivity is attenuated in acrylic adhesives due to the higher catalyst concentrations, which generate radicals at a greater rate than in anaerobic systems.



N-Phenyl-2-propyl-3,5-diethyl-1,2-dihydropyridine (DHP)

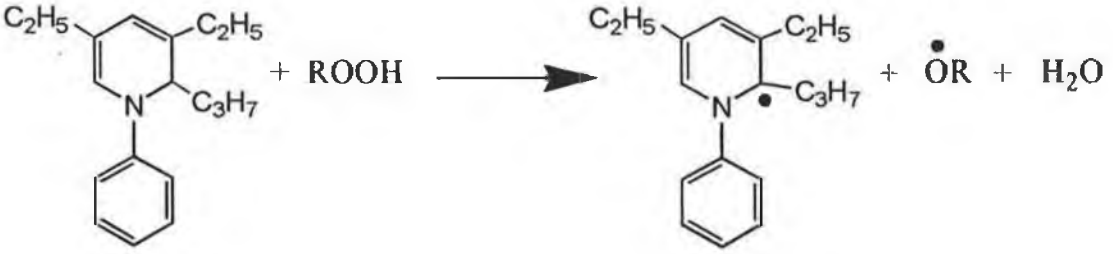


Figure 1.5 The structure of DHP, the condensation product of aniline and n-butyraldehyde. Also, the reaction of a hydroperoxide with DHP generating alkoxy free radicals, is illustrated.

1.4. CYANOACRYLATE ADHESIVES

1.4.1 Introduction

Alkyl cyanoacrylates were originally developed during World War II by Eastman Kodak, but the adhesive properties of these esters was not fully recognized, and it wasn't until several years later, in 1958, that the first cyanoacrylate adhesives were commercially available [25]. H.W. Coover, the chemist involved in the early development work, led a research team which was looking for acrylate polymers with improved properties. Ethyl cyanoacrylate was among the monomers under investigation. Coover was alerted to the unique properties of this monomer when the prisms of an Abbe refractometer were inadvertently bonded together. Several years later, in 1958, Eastman Kodak introduced the first cyanoacrylate, Eastman 910, into the industrial adhesive market [26].

The cyanoacrylates had limited success at first and were generally regarded as speciality industrial adhesives. This was due to two main factors; firstly, unpredictable stability problems were encountered in manufacturing, packaging, and shipping, and secondly, adhesive users were slow to recognise the distinctive properties of these adhesives. During the 1970's, the situation changed and rapid growth occurred. The stability and related manufacturing problems were overcome, and other manufacturers entered the market, notably Loctite and a number of Japanese companies.

Cyanoacrylate adhesives, or superglues, are unique among the many classes of adhesives, in that they are only single component, instant bonding adhesives that cure at ambient temperatures to form strong bonds between a wide variety of surfaces, without requiring an external energy source. The unique characteristics of cyanoacrylates virtually ensure their successful use over an extremely broad and diverse range of applications. Cyanoacrylates are widely used in the automotive industry in the assembly and repair of automotive components, in the manufacture of sporting goods, and in the assembly of cosmetic items such as lipstick tubes and mirrors. Cyanoacrylates are increasingly being used in the medical field. The strong

tissue bonding properties of the adhesives has resulted in their use as chemical sutures and haemostatic agents in many countries. Many lives were saved in the Vietnam War through the use of Eastman Kodak developed spray kits which enabled battlefield medics to stop massive blood loss, a prime cause of combat death [26]. With the incorporation of new surface-insensitive additives which allow rapid curing on acidic and porous substrates such as wood and paper, and the marketing of these versatile adhesives in the consumer sector, the number of applications where cyanoacrylates are used is limited only by the ingenuity of the user.

1.4.1.1 Chemistry

Adhesives based on cyanoacrylic acid esters differ significantly from most other reactive adhesives because they consist in the large part of pure monofunctional monomers. Cyanoacrylate adhesives rarely contain significant amounts of coreactants because they are able to homopolymerise rapidly at room temperature. Also, non-neutral additives drastically alter the cure rate and shelf life of the formulated adhesives. The reactivity of cyanoacrylates is due to the presence of two strong electron withdrawing groups, which make the double bond highly susceptible to attack by weak bases. Therefore, cyanoacrylates polymerize by an anionic mechanism, in the presence of a weak base, such as water, in a highly exothermic reaction. Typically this process is initiated by the anionic contaminants found on the surfaces being bonded [25,26] (Figure 1.6).

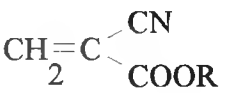


Figure 1.6 The structure of a cyanoacrylate, R= alkyl group such as methyl, ethyl or butyl.

Since the anionic cure of cyanoacrylates is as a result of base catalysis, acids are predominantly used as stabilizers and are essential to maintain a usable shelf-life. Acids of either the Lewis type or protonic have been used, such as SO_2, BF_3, aliphatic and aromatic sulfonic acids [27]. The acidic gases have the advantage of stabilising the monomer in both the liquid and gaseous phases, and these acidic gases also prevent the buildup of "popcorn" polymer in the headspace of partially filled user packages. Combinations of non-volatile sulfonic acids with gaseous stabilizers have been reported to offer synergistic effects [26]. Acid strength and level are crucial variables, as high levels of acids can over stabilize and contribute to a rapid deterioration in adhesive performance.

1.4.1.2 Monomers

The cyanoacrylate adhesives sold today are quite similar to the first product, Eastman 910. This is due to the fact that changes in the structure of the monomer have dramatic effects on adhesive properties. The most commonly used monomers are ethyl and methyl esters, while butyl esters have been used recently in the development of medical cyanoacrylates. At present, ethyl esters account for over 90% of worldwide production of cyanoacrylate adhesives.

The synthesis of alkyl-2-cyanoacrylates involves the Knoevenagel condensation reaction of an alkyl cyanoacetate with formaldehyde in the presence of a base, typically piperidine, to yield a poly(alkyl-2-cyanoacrylate). The polymerized cyanoacrylate is then heated to a temperature of 140-160°C, forcing the de-polymerization back to a cyanoacrylate ester. For this reaction to occur smoothly, the base catalyst from the initial step has to be neutralized by an acid such as phosphoric and p-toluene sulphonic acids [26].

1.4.1.3 Additives

Cyanoacrylates are susceptible to free radical polymerisation, although the predominant cure mechanism is anionic. Free radical stabilizers have only a minimal effect on the anionic polymerisation rate; hence the selection and concentration is not as critical as with the anionic stabilizers. The most common inhibitor used is hydroquinone, and since it effect on the anionic polymerisation rate is minimal, the level of inhibitor which may be added to stabilize against heat or UV induced initiation can be, and often is, relatively high, up to several thousand parts per million.

Cyanoacrylates typically have very low viscosities, which are generally too low to permit convenient use in many industrial applications. As a result, viscosity modification is required through the use of various soluble polymeric thickeners. Thickeners and fillers must be compatible with the cyanoacrylates and must not set off the easily triggered anionic cure. Polymers which have been used include polymethacrylates, polyacrylates, polycyanoacrylates and polyvinylacetates. More recently, elastomeric fillers have been incorporated to improve the flexibility and toughness of the basically brittle polymers. The sensitivity of the cure of cyanoacrylates to various substrates, in particular acidic surfaces which inhibit or slow the anionic cure, have been overcome by the use of various basic surface activators. Patents have been filed for the use of crown ethers [28], silacrowns [29] and calixarenes [30] as curing additives for use on wood and porous surfaces. The mechanism of action of these additives is not fully understood, but since crown compounds are phase transfer catalysts, some interaction with alkali metals in the surface seems likely [26].

Anyone who has used cyanoacrylates on a continuous basis in an area with poor ventilation, is familiar with the pungent, irritating, acrylic type odour. Difficulties with the odour and the precipitation of white vapours on surfaces adjacent to the bondline has lead to the development of alkoxyalkyl cyanoacrylates, which structurally are merely ethyl cyanoacrylates with a methoxy or ethoxy group

attached to the β-carbon of the ester side chain. These monomers are virtually odourless and have much lower vapour pressures, although performance is similar, but not equivalent to the lower methyl and ethyl esters.

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CHAPTER TWO

INVESTIGATIONS INTO THE TRANSITION METAL BASED CURE CHEMISTRY OF ANAEROBIC ADHESIVES

2.1. INTRODUCTION

2.1.1 General Chemistry

The redox catalysed decomposition of a hydroperoxide by metal ions at room temperature produces free radicals which, in the absence of oxygen, initiate the polymerisation of methacrylic monomers. The high efficiency of these systems, in conjunction with the high inhibiting power of oxygen in the radical polymerisations of methacrylates, made possible the development of anaerobic adhesives and sealants. Anaerobic adhesive formulations are single-component liquids that can be stored at room temperature and polymerise rapidly when confined between two metallic surfaces such as copper, brass, iron or steel, in the absence of air. Nowadays, industrial applications need materials having a long shelf life (at least one year) and high rates of polymerisation (half-time of polymerisation must not exceed a few minutes) [1 ,2].

The first requirement i.e. stability, is usually achieved by adding inhibitors, such as hydroquinone, and using hydroperoxides with a high stability at room temperature, such as cumene hydroperoxide. To increase the curing rate of anaerobic adhesives at room temperature, accelerators such as organic acids and organic bases are usually added. The desire to balance reactivity and stability of anaerobic adhesives has led to a continuing search for compounds that accelerate the polymerisation reaction without exerting a deleterious effect on product stability. Trialkyl amines were found to meet these criteria, as it was presumed that the amine acts by polarising the hydroperoxide ion. Further research identified a coaccelerator combination of saccharin and dialkylarylamines, which enhances cure speeds on all surfaces [1]. Although model studies showed that gradual autoxidation of dialkylarylamines occurs during prolonged storage, resulting a decrease in the cure speed and thereby, emphasizing the importance of the dialkylarylamines in the polymerisation process. The same studies indicated that the amine reacts extensively with hydroperoxides only at elevated temperatures, demonstrating that saccharin plays an equally vital role in the initiation chemistry [3] .

Preformed saccharin/amine salts have been used successfully to initiate acrylic polymerisations. Also, the presence of low levels of water appears to improve the performance of this cure system.

The metal substrate on which the anaerobic adhesive is applied plays a crucial role in initiation of polymerisation. The catalytic decomposition of the hydroperoxide generates free radicals. Equation (1) represents the fundamental step for the formation of primary radicals, while the second reaction (2) of metal ions in their higher oxidation state generates radicals which are incapable of initiating polymerisation, although it does regenerate the metal ion in its lower valent state. A stationary concentration of each ion would be established if the rates of equations (1) and (2) were equal; however this is rarely true and the contribution of equation (2) depends on the nature of the metal [4].

$$(1) \qquad M^{n+} + ROOH \rightarrow M^{(n+1)+} + RO^{\bullet} + OH^{-}$$

$$(2) \qquad M^{(n+1)+} + ROOH \rightarrow M^{n+} + ROO^{\bullet} + H^{+}$$

The range of compounds used as accelerators include accelerators based on trialkyl or triarylalkylamines, such as tributylamine (TBA), N,N-dimethyl-p-toluidine (DEPT), N,N-diethyl-p-toluidine (DMPT). More recently, further improvements in reactivity were obtained with the introduction of hydrazide accelerators such as 1-acetyl-2-phenylhydrazine (APH). However, the incorporation of more active cure systems was only made possible by concurrent advances in stabilisation chemistry. In combination with the various accelerators, saccharin, or in some cases maleic acid, have been employed.

Although anaerobic adhesives have now been used extensively for more than 20 years, there is hardly any scientific literature relevant to the cure chemistry, and the exact role played by each of the various cure components remains to be elucidated.

2.1.2 The Cure Chemistry

Until recently, there have been few reports in the literature which have attempted to postulate the cure mechanism of anaerobic adhesives. One of the first published studies was that of methyl methacrylate (MMA) polymerisation using various amine salts of saccharin [5]. The highest polymer conversion was obtained from the DMPT salts of saccharin. The rate of polymerisation was reported to be dependent on the square root of the amine salt concentration, and the activation energy was determined to be 79 KJ/mol. On the basis of these results, the authors speculated that the polymerisation proceeded by a radical polymerisation mechanism.

The above study was extended by investigating the kinetics of polymerisation with the addition of a peroxide, namely cumene hydroperoxide (CHP), to this cure system [6]. He found that although polymerisation took place in the absence of CHP, the polymerisation was approximately 20-50% faster in the presence of CHP, and that the rate of polymerisation was independent of the CHP concentration. The activation energy for this polymerisation system was determined to be 43.5 KJ/mol. Okamoto also investigated the structure-cure speed relationship and found that the cure speed actually decreased by addition of an electron donating group in saccharin and by any substituents on to the nitrogen atom in saccharin. The polymerisation speed was also decreased by open chain analogues of saccharin, such as N-benzenesulfonylbenzamide. The opposite was the effect with DMPT, the addition of electron-donating groups in the para position increased the cure speed but decreased the cure speed in the ortho position. Based on this kinetic study and the structure-cure speed study, he proposed that polymerisation proceeded by a redox radical mechanism and that the reducing agent of the redox system was a charge-transfer complex of DMPT and saccharin (Figure 2.1).

Okamoto further broadened his studies of cure mechanisms to include APH/saccharin/CHP based systems, including copper(II) with MMA [7].

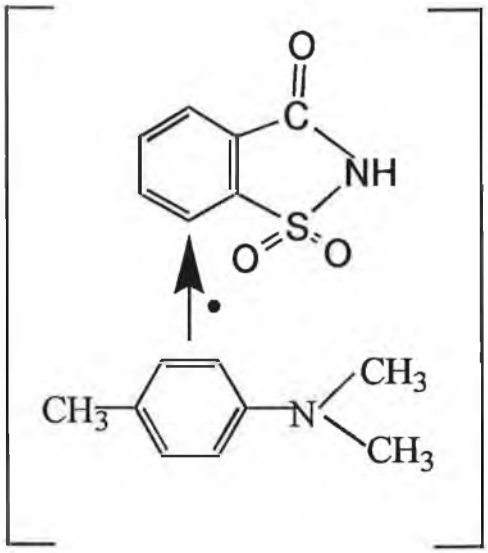
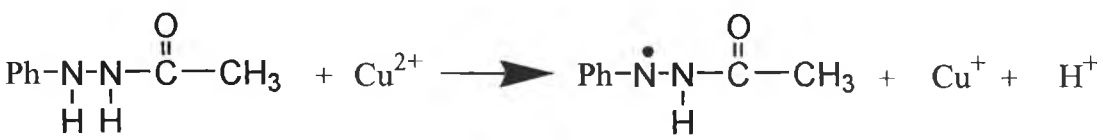
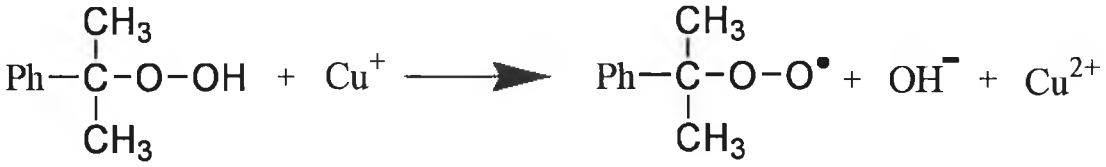


Figure 2.1 The proposed charge-transfer complex between DMPT and saccharin.

It was found that the APH-based system produced a slow cure with a long induction period. However, with the addition of 5 ppm copper(II), the induction period was reduced from 5 hr to 10-15 min. A slightly lower activation energy with the APH system than that obtained with the DMPT-based system was attributed to the presence of copper(II) as catalyst. The role of each organic cure component was then investigated by a series of kinetic studies on MMA polymerisation based on the APH cure system. An examination of the effect of saccharin indicated that it behaved as a catalyst to shorten the induction period from 30-40 min to 10 min. Although an interaction between CHP and APH was detected, the nature of the reaction was uncertain. Finally, a kinetic study monitoring the effect of MMA concentration on the rate of polymerisation suggested that the monomer does not participate in the initiation step of the redox polymerisation [7].

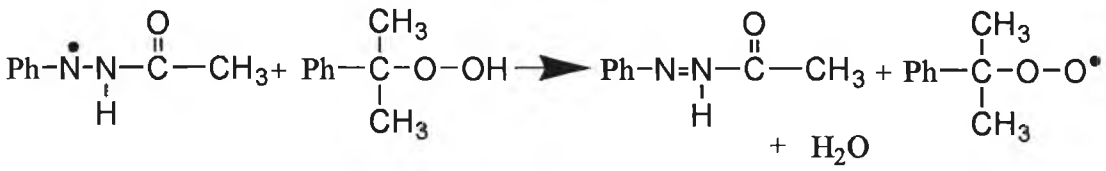
From these studies, Okamoto postulated the following initiation mechanism to explain the APH/CHP/saccharin metal catalysed redox radical polymerisation:



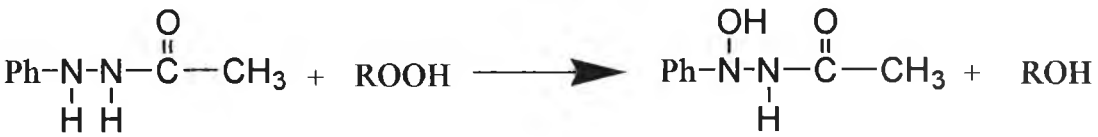


In the above mechanistic sequence, the postulated cumyloxy radical which is generated initiates the MMA polymerisation; APH and CHP behave as reducing and oxidising agent respectively, and copper acts as the catalyst.

Further studies at Loctite (Irl.) Ltd. have indicated that additional reactions between APH, CHP and copper in the initiation step of a redox polymerisation may take the following form [8]:



The long induction period encountered with the APH-CHP-saccharin systems (when copper is not present) could be due to the formation of a hydroxyamine in the following reaction [8];



The hydroxyamine, if in contact with a radical source, could generate a nitroxide radical, a paramagnetic species of exceptional inherent stability, and therefore, behave as a good radical inhibitor.

In order to complement and extend the investigation into the metal-catalysed APH-saccharin-CHP cure system, attention has focused on the reaction routes adopted by APH in the presence of maleic acid and the aforementioned organic cure components. Gas chromatography-mass spectrometry (GC-MS) and reversed-phase high performance liquid chromatography were employed to monitor the reaction routes and to identify the products originating from APH in these reactions [8].

The findings from these studies supported the postulated mechanism of Okamoto [7] and Leonard [8] for the reaction of APH in the presence of a peroxide, acid component and active metal. The acetylphenyldiazene (APD) which forms from the reaction between the APH radical and the peroxide, was found to undergo further metal-catalysed homolytic decomposition. One of the more important findings of this study was the different reaction rates and routes catalysed by copper in comparison to those catalysed by iron. Also, it was observed the difference between the acid components, saccharin and maleic acid in their overall contribution to reaction rates and routes.

Beaunez and co-workers carried out two detailed kinetic studies into the role of DMPT and saccharin in the radical polymerisation of MMA. The first study focused on a system based on a redox initiation with cumene hydroperoxide and copper saccharinate [9]. From this study a reaction scheme was proposed, which consisted of CHP rapidly reduces copper(II) to copper(I); a small of copper(I) is then complexed with DMPT; the complexed ions are strong reductants with respect to CHP whereas uncomplexed copper(I) ions are almost inactive; the decomposition of CHP is strongly catalysed by saccharin, with protonated CHP 13000 times more reactive than free CHP (based on quantum mechanical calculations). Therefore, it was proposed that DMPT has the role of complexing agent for copper(I) ions, where two DMPT ligands are complexed with one cuprous ion. Whereas, saccharin's

influence can be attributed to its acidic character, resulting in protonation of CHP thereby lowering the energy required to cleave the peroxide bond.

The second study focused on redox polymerisation initiated by cumene hydroperoxide and iron saccharinate [4]. Based on this kinetic study it was proposed that DMPT reduces iron(III) to iron(II); iron(II) ions complexed by two DMPT molecules are much more reactive than uncomplexed iron(II) with regard to CHP; and saccharin (as in the previous study) activates the decomposition of CHP by protonation of the peroxide bond and facilitating cleavage at room temperature. It is also proposed that uncomplexed iron(II) is unable to decompose CHP, regardless of the concentration of saccharin. The mechanism is very similar to that proposed in the case of copper saccharinate and goes a long way to explain the synergistic effect of both accelerators. In both systems the vital step involves the metal-catalysed decomposition of CHP to produce free radicals, the accelerators in different ways aid this reaction.

The curing mechanism of anaerobic adhesives based on DMPT-CHP-saccharin was investigated in model reactions by Wellmann and Brockmann [10]. They proposed that a new compound is formed by the interaction of saccharin and DMPT, which was isolated, and experiments showed that this compound is the key catalyst for the rapid curing of anaerobic adhesives. The new compound which was not previously described in the literature was called aminal (Figure 2.2), and a mechanism for the formation of aminal both aerobically and anaerobically was proposed. However, in real adhesive systems, aerobic formation of aminal is the main route to its formation i.e. during storage of the adhesive in the presence of air.

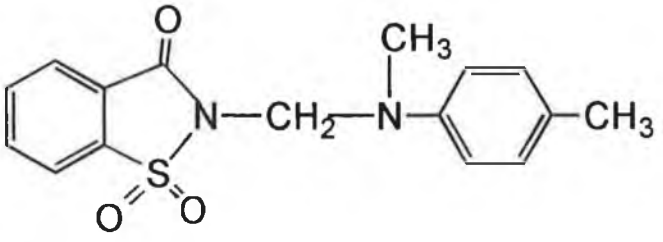


Figure 2.2 Structure of Aminal

Aminal has a number of functions in the complex cure chemistry; firstly it is a strong reducing agent capable of reducing metal ions from the higher to the lower oxidation state. Reducing agents are necessary for the system, because metal ions in the higher oxidation state only produce inactive free radical from the decomposition of CHP. It was found that aminal reduced copper(II) to copper(I) in the Fehling's reaction, decolourizing a $KMnO_4$ solution and producing metallic silver from a solution of a silver(I) salt, while all these tests were negative with DMPT and saccharin. Aminal is also able to form metal complexes and thus is able to provide the metal ions from the substrate surface required in the course of curing. The complex formation constant of aminal-metal complexes is larger than that of the corresponding saccharin-metal complexes. Okamoto's observations regarding the structure-cure speed relationship, such that the cure speed decreased when DMPT was replaced by secondary or tertiary amines or when replacing saccharin with open chain analogues, has given further weight in that in those circumstances aminal could not be formed.

However, Okamoto's theory [6] of a charge-transfer complex based on a number of observations including the development of coloration on contacting saccharin and DMPT, was discounted by Wellmann and Brockmann. In the presence of air, DMPT is oxidised to a radical cation (Figure 2.3) of the Wurster-type salt. The existence of this radical can be proved by trapping it with 2,2,6,6-tetramethylpiperidine-N-oxyl radical (TEMPO) (Figure 2.3). The formation of this radical is accompanied by an intense coloration of its solutions, which is contrary to the reported formation of a charge-transfer complex between DMPT and saccharin. Furthermore, the radical cation is stable in acid solution in very low concentrations, and in this case the stability is ensured by saccharin because of its acidic character.

From this study it was concluded that the cure mechanism of anaerobic adhesives depends on radicals which are quenched in the presence of air and generated by metal ions in the absence of air. Rapid and complete curing is ensured by combining the radical initiator, cumene hydroperoxide, with saccharin and DMPT. In the presence of air, saccharin and DMPT lead to the formation of a new compound, aminal, which forms metal chelates and is an excellent reducing agent. Thus,

saccharin and aminal provide soluble metal ions from the substrate surface, which are reduced by aminal to a lower oxidation state. These ions then generate active radicals from CHP. Therefore, the essential step in the anaerobic adhesive cure mechanism is the formation of aminal, which is spontaneous in the presence of air and guarantees rapid and complete curing.

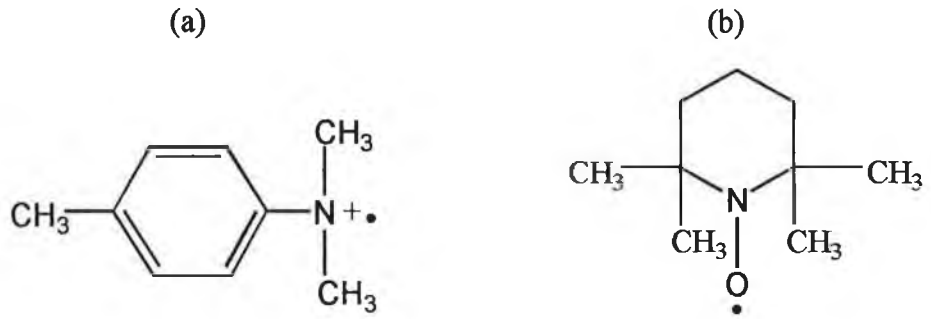


Figure 2.3 Structure of (a) the DMPT radical cation and (b) TEMPO trapping agent.

2.1.3 Polarography

2.1.3.1 Introduction

Voltammetry is the branch of electroanalytical chemistry that deals with the effect of the potential of an electrode in an electrolysis cell on the current that flows through it. The electrode whose potential is varied is called the indicator or working electrode. Voltammetric indicator electrodes may be made from any of a large number of materials, including mercury, platinum, gold, graphite and many others; they may have almost any shape, size, and construction; they may be stationary or in motion; and the solutions in which they are used may be stirred or quiet. Polarography is the branch of voltammetry in which a dropping mercury electrode is used as the indicator electrode.

Polarography has several advantages over conventional voltammetric techniques; firstly its surface area is reproducible with any given capillary; the constant renewal of the electrode surface eliminates passivity or poisoning effects which are a major problem with solid electrodes; the high overpotential of hydrogen on mercury renders the electrode useful for electroactive species with a reduction potential that is considerably more negative than the reversible potential for the discharge of hydrogen; mercury forms amalgams with many metals and thereby lowers their reduction potential; and the diffusion current assumes a steady value immediately and is reproducible. One disadvantage with polarography is that mercury is rather easily oxidised, so that very positive potentials cannot be attained. The dropping mercury electrode is useful over the range +0.3 V to -2.8 V vs the saturated calomel electrode in aqueous solutions [11].

Polarographic analysis can be used directly for the determination of any substance- solid, liquid or gas, organic or inorganic, ionic or molecular, that can be dissolved in a solvent of reasonable dielectric constant and either reduced or oxidised at a mercury electrode. This includes the ions of nearly all metals and many non-metals, and also organic compounds containing conjugated double or triple bonds, including polynuclear aromatic systems, as well as compounds like oximes, imines, ketones, aldehydes, peroxides and diazo compounds. To the practical analyst one of the most important advantages of polarography is that it enables one to determine two or more substances by obtaining a single current potential curve. For example, the simultaneous determination of cadmium and zinc or naphthalene and anthracene or even iron(II) and iron(III) is relatively easy and straight forward [11].

2.1.3.2 Polarographic determination of iron

Although there are numerous publications on the polarographic determination of iron(II), iron(III) and total iron, there are few papers that simultaneously speciate the valence states of iron in one experiment. Initial investigations into the electrochemical behavior of iron(II) date back to 1931, when Prajzler [12] found

that the half-wave potential of the iron(II) ion was -1.3 V vs SCE in barium chloride. In 1 M ammonium perchlorate as supporting electrolyte, where complexation of the iron(II) ion is minimal, it was reported [13] that the half-wave potential was even more negative at -1.46 V vs SCE.

The hydrogen ion discharge easily masks the ferrous ion wave, since the iron(II) wave is virtually coincident with that of the hydrogen ion. Therefore the supporting electrolyte pH must be above 5 and below approximately 7, at which point precipitation of iron(II) hydroxide occurs. The pH of the electrolyte cannot be regulated with buffers, because the acid constituents of buffers can show a hydrogen wave. Lingane [14] studied systematically the polarography of iron(II) and iron(III) in tartrate, citrate and oxalate media. The reduction of iron(III) complexes to iron(II) complexes in these electrolytes produces well formed waves at pH values smaller than about 6. In all three media, the subsequent reduction of iron(II) complexed to the metal is masked by the hydrogen ion or alkali metal ion discharge, with the exception of strongly alkali citrate and ammonical tartrate supporting electrolytes where the reduction wave with a half-wave potential of about -1.53 V vs SCE is clearly seen.

The iron(III)-iron(II) complex couples behave reversibly at pH values less than about 7 in all three media i.e. the half-wave potentials of the anodic waves of the iron(II) complexes are identical with that of the cathodic half-wave potentials of the iron(III) complexes. With the exception of oxalate media, the iron(III)-iron(II) complexes behave irreversibly in alkaline tartrate and citrate electrolytes; the anodic iron(II) waves being at much more positive potential than the cathodic iron(III) waves. At pH values greater than 7.9 in oxalate media, the hydrous iron(II) oxide precipitates.

The optimum pH for the determination of iron(III) in 0.5 M sodium citrate is about 10 with a half-wave potential of -0.85 V vs SCE, whereas a pH of 6 or 7 is more appropriate for the determination of iron(II) complexes [14]. In tartrate media, pH 6.0, containing 0.005% gelatin, the half-wave potential of the cathodic iron(III) to iron(II) wave occurs at -0.19 V vs SCE and the iron(II) to metal wave at -1.52 V vs

SCE. A significant shift in the half-wave potentials of the iron(III) to iron(II) couple, to -1.21 V vs SCE was observed when the pH of the tartrate media was adjusted to 9.0, and the half-wave potential of the second reduction process was seen at -1.73 V vs SCE. Lingane proposed that the considerably more positive half-wave potentials observed in the oxalate medium for the reversible iron(III)-iron(II) complex couples as compared with citrate and tartrate media, was due to the oxalate complex of the iron(III) ion being weaker or that the oxalate complex of iron(II) ion is stronger, or both, than the corresponding citrate and tartrate complexes [14].

Voriskova [15] reported a well defined reduction wave for the iron(II)-ammonia complex, with a half-wave potential of -1.48 V vs SCE in a supporting electrolyte containing 1 M ammonia-ammonium chloride with 0.005% gelatin.

Iron yields two waves in a supporting electrolyte of 3 M potassium hydroxide that contains 3% mannitol, with which iron(II) and iron(III) ions form soluble complexes. The first wave, with a half-wave potential of -1.12 V vs SCE, corresponds to the reduction of the iron(III)-mannitol complex to the iron(II) state, and the second wave, with a half-wave potential of about -1.74 V vs SCE, is due to the reduction of the iron(II)-mannitol complex to the metal [16].

Contradictory results were obtained by different research groups when the polarography of iron in fluoride media was investigated. Von Stackelberg and von Freyhold [17] reported that iron(III) produces a reduction wave in potassium fluoride with a half-wave potential of about -1.36 V vs SCE. According to Heyrovsky [18], the iron(II)-iron(III) couple does not behave reversibly in fluoride media; the anodic wave corresponding to the oxidation of the iron(II) ion to the iron(III)-fluoride complex (which was not observed by von Stackelberg and von Freyhold) is at a considerably more positive potential than the reduction wave of the iron(III)-fluoride complex. However, West and Dean [19], using a 1 M sodium fluoride supporting electrolyte of pH 5.8 containing 0.004% gelatin, claimed that they did not observe the reduction wave for the iron(III)-fluoride complex in this media as reported by von Stackelberg and von Freyhold and by Heyrovsky. The

reason behind this contradictory result is not immediately evident from the literature, but gelatin may have obliterated the wave, or the pH of the supporting electrolytes (which was not reported by the latter two research groups, but was probably acidic) was significantly different from that used by West and Dean.

Iron(III)-iron(II) mixtures have been determined in oxalate media by direct current polarography, while total iron in the same electrolyte was determined by alternating current polarography [20]. Detection limits of approximately 100 μM were reported with this procedure. As the redox process is reversible in this media, the half-wave potentials for the reduction of iron(III) and the oxidation of iron(II) are the same. Consequently, this procedure could not be adopted to differential pulse polarography, as it is not possible to differentiate between the two oxidation states of iron with this polarographic mode.

In pyrophosphate media of pH 8.0, the iron(III)-iron(II) redox couple is not reversible. According to Parry and Anderson [21], this characteristic is one of the most important requirements in determining polarographically a redox couple in solution. Among other factors that they listed as important in selecting a suitable electrolyte included; (1) all waves observed in the electrolyte should be cathodic of mercury oxidation; (2) the waves should be sufficiently separated to allow for individual measurement; (3) the waves should be well defined and it would be desirable that the limiting currents be proportional to the respective concentrations; and (4) there should be no interaction of a given ion on the other waves. If there is such interaction, it should be small and easily correctable. Parry and Anderson, using normal pulse polarography, reported the half-wave potentials of iron(II) and iron(III) at -0.38 V vs SCE and -1.03 V vs SCE respectively in pyrophosphate media, pH 8.0.

Kennedy [22] investigated the possibility of determining iron(III)-iron(II) mixtures by differential pulse polarography using a supporting electrolyte based on ammonium tartrate buffered by a suitable buffer e.g. 2-(N-morpholino)ethanesulphonic acid (MES), 3-(N-morpholino)-1-propanesulphonic acid (MOPS), or N-(2-hydroxyethyl) piperazine-N-(2-ethanesulphonic acid) (HEPES). He reported that at a low pH a high proportion of iron(III) was reduced to iron(II), while at high pH, trace amounts of oxidants converted the iron(II) to iron(III). However, by buffering the electrolyte with such a buffer at a pH of 7.4, reasonably stable, well separated peaks corresponding to the iron(II) oxidation and iron(III) reduction were produced.

2.1.3.3 Polarographic determination of copper

Due to the instability of the lower valence state of copper, it is difficult to determine copper(I) polarographically. There are a limited number of supporting electrolytes which form more stable complexes with copper(I) than with copper(II), thereby stabilising copper(I) sufficiently to permit the step-wise reduction of copper(II) complexes. Thanheiser and Maasen [23] were the first to report that in the presence of electrolytes containing high concentrations of ammonia or chloride ions, a polarogram consisting of a double wave, whose two parts were of equal height, was obtained. This corresponded to the anodic wave resulting from the oxidation of the copper(I) complex to the copper(II) state and a cathodic wave resulting from the reduction of the copper(II) complex to the metal.

The step-wise reduction of copper(II) also occurs in thiocyanate or pyridine supporting electrolytes. Lingane and Kerlinger [24] reported that in 0.1 M potassium thiocyanate the half-wave potentials were -0.02 V and -0.39 V, and in 0.1 M pyridine-pyridinium chloride +0.05 V and -0.25 V vs SCE. Because the first wave starts so close to the oxidation of mercury, a blank experiment with the supporting electrolyte is normally required to determine the conversion of copper(II) to copper(I). Such background correction is not necessary with a supporting

electrolyte of 1 M ammonia-ammonium chloride, as the half-wave potentials for the two processes are -0.22 V and -0.50 V vs SCE respectively [23].

Meites investigated thoroughly the influence of pH, the effect of total tartrate concentration, and the effect of gelatin on the polarographic characteristics of copper in tartrate media [25]. At pH values less than 6, only a single wave was observed whose half-wave potential varies linearly with pH. From a pH of about 6 to 13.5, the wave divides into two parts accompanied by a significant negative shift of the half-wave potentials. According to Meites, this doublet arises from the reduction of separate tartrate complexes, the identity of which was not confirmed. Meites also found that gelatin causes distortion of the waves when present in concentrations greater than 0.001%.

In addition to his investigation with tartrate media, Meites also studied the polarography of copper in citrate, oxalate and carbonate supporting electrolytes. The polarographic behavior of copper in citrate is similar to that in tartrate media, with a comparable significant effect of pH on the half-wave potential being observed [26]. In 0.5 M potassium citrate, the half-wave potential shifts from -0.02 V at pH 3.0 to -0.22 V vs SCE at pH 7.5. Between pH 7.5 and 8.5, an abrupt shift of half-wave potential to -0.38 V vs SCE was seen, which remains constant up to a pH of about 12.0, when it then increased up to -0.50 v vs SCE at pH 14.0. Meites found that, in his studies with copper on 0.1 M potassium oxalate, the half-wave potential shifts from -0.03 V vs SCE at pH 1.0 to -0.20 V vs SCE at pH 5.7, and remains constant at the latter value up to a pH of about 10 [27].

2.1.4 Cyclic Voltammetry

2.1.4.1 Introduction

Cyclic voltammetry has been a popular tool for many years now for studying electrochemical reactions. Organic chemists have applied the technique to the study of biosynthetic pathways and to studies of electrochemically generated free radicals. An increasing number of inorganic chemists have been using cyclic voltammetry to evaluate the effects of ligands on the oxidation/reduction potential of the central metal ion in complexes and multinuclear clusters. This type of information plays an integral part in many of the approaches towards solar energy conversion and in model studies of enzymatic catalysis [28].

Cyclic voltammetry consists of cycling the potential of a stationary electrode immersed in a quiescent solution and measuring the resulting current. The current of the stationary working electrode is measured under diffusion-controlled, mass-transfer conditions. Although the potential scan is frequently terminated at the end of the first cycle, it can be continued for any number of scans. Most modern equipment uses a three-electrode cell in which a counter or auxiliary electrode provides the current that is needed at the working electrode. Therefore, virtually no current flows through the reference electrode, typically a saturated calomel electrode (SCE) or a silver/silver chloride electrode, and its potential remains constant. Mercury can be used as the working electrode but it is limited to the negative potential range. Platinum and various carbon electrodes are popular for performing oxidations. However, solid electrodes are susceptible to adsorption, or surface fouling, and surface oxidation [28].

2.1.4.2 Cyclic voltammetry of accelerators

Voltammetric studies of compounds employed as anaerobic adhesive accelerators has been limited primarily due to the nature of the compounds involved and their

almost exclusive use in the adhesive industry. The electrochemical oxidation of DMPT has been studied in both aqueous buffer and acetonitrile [29 ,30]. It was observed that DMPT behaved identical in each environment, although much better definition was obtained in the non-aqueous medium [29]. One of the studies was prompted by the inherent stability of the radical cation of DMPT, which was inconsistent with the stability of other para- substituted N,N-dimethylanilines [30]. In acetonitrile, DMPT is oxidised at a potential of +0.7 V vs SCE; the initial anodic peak is then followed by a smaller wave at a slightly more positive potential. It was found that the primary wave exhibited very little reversibility unless, the scan direction is reversed just after the primary wave, resulting in a great deal of reverse current.

The anodic oxidations of seventeen aliphatic amines has been examined by cycilc voltammetry in aqueous alkaline solution with a glassy carbon electrode [31]. It was found that all the amines are irreversibly oxidised, with most tertiary amines showing two waves with the peak potentials of the first waves less positive than those of secondary amines. Mann also studied the voltammetric oxidation of aliphatic amines, including tributylamine, and observed, that in general, the order of increasing difficulty of oxidation of amines is tertiary, secondary and primary [32]. Experiments were carried out in acetonitrile with 0.1 M sodium perchlorate as supporting electrolyte. In summary, cyclic voltammetry is a convenient tool for obtaining qualitative information about electron transfer processes and it is a rapid method for obtaining good estimates of formal oxidation-reduction potentials.

2.1.5 Aims of research

Because of the limited understanding of the anaerobic cure mechanism in terms of the role of the various components and the differing theories that abound, it was decided to investigate the interactions of the various amines with regard to copper and iron in the presence and absence of saccharin, to gain further insight in to the complex cure chemistry. To date, any significant published research has concentrated on elucidating the cure mechanism from kinetic polymerisation studies as distinct from empirical analytical results. Therefore, this study concentrated on monitoring the changes in oxidation states of iron and copper using polarography, and determining what influence, if any, the various amines and aminal have in conjunction with other cure components.

2.2. EXPERIMENTAL

2.2.1 Reagents and Apparatus

All reagents were of Analar grade, unless otherwise stated. All salts, organic acids and anaerobic adhesive accelerators were supplied by Loctite (Irl.) Ltd with the exception of APH which was supplied by Eastman Kodak. Polarographic analysis of iron(II) and iron(III) was carried out in 0.1 M pyrophosphate buffer (pH 8.0), while copper(II) was determined in 2.0 M ammonium acetate buffer (pH 3.0).

The following buffers were used in the cyclic voltammetry studies : 0.1 M HCl (pH 1.0); 0.1 M citrate (pH 4.0 and 5.0); 0.5 M phosphate (pH 7.0); 0.05 M carbonate (pH 9.8); 0.2 M KCl/0.2 M NaOH (pH 12.2).

Aminal was synthesized according to the method of Wellmann and Brockmann [10], by initially reacting CHP with DMPT in the absence of air to yield the N-oxide. The N-oxide was then reacted with saccharin in the presence of a catalytic amount of copper(I) chloride to yield aminal. The product was purified by recrystallisation from chloroform/ petroleum ether and was characterised by ^{1}H and ^{13}C NMR.

All polarographic determinations were carried out using a Princeton Applied Research (Princeton, N.J.) EG&G PARC Model 303 SMDE linked to an EG&G Model 384 Polarographic analyser and a Houston Omniscribe chart recorder. Cyclic voltammetric experiments were carried out using a BAS CV-50 voltammetric analyser, using a conventional three electrode system, with glassy carbon, Ag/AgCl and platinum as working, reference and counter electrodes respectively.

2.2.2 Procedures

Solutions of 8000 ppm of either iron(III) or copper(II) were made up in methanol in 100 cm^3 acid washed conical flasks, from the nitrate and acetate salts respectively. To this solution was added 0.2 g (1% w/v) of accelerator and the solutions were monitored over time for generation of lower oxidation states of the respective metals; in the case of copper the decrease in copper(II) concentration was monitored over time. These experiments were then repeated but in the second instance, saccharin was added at a concentration of 2% w/v and the solutions were analysed using polarography for generation of lower oxidation states of the respective metals. When other organic acids were used in place of saccharin they were used at 2% w/v concentrations, except maleic acid which was used at 1% w/v. The concentration of iron(II) produced in the solutions containing the iron(III) salt and various accelerators was monitored with time by sampled direct current (DC) polarography. This determination was carried out by initially recording a sampled DC polarogram of the supporting electrolyte (10 cm^3 of 0.1 M sodium pyrophosphate, pH 8.0) following deaeration for about 2 min in a stream of oxygen free nitrogen. Depending on the concentration of iron(II) generated, an aliquot of 10 μL or 50 μL of the reaction mixture was added; after deaeration and stirring for 30 seconds and an equilibration step of 15 seconds, a sampled DC polarogram of the sample was recorded. By means of a standard addition procedure, the concentration of iron(II) was quantified by three successive aliquots each of 50 μL or 100 μL of 1000 ppm iron(II), depending on iron(II) concentration, and a sampled DC polarogram obtained after each addition.

Differential pulse polarography was used to monitor for decreases in copper(II) concentrations produced with time in the reaction mixtures. In 2 M ammonium acetate electrolyte, and using a similar procedure outlined above for iron(II) determination, the concentration of copper(II) was quantified by a standard addition method; the only variation was that three successive 50 μL aliquots of 500 ppm copper(II) were added to the cell.

The experiments involving aminal were carried out as detailed previously for the accelerators, with the exception that 0.1 g aminal was used in 20 cm^3 ethanol (0.5% w/v).

Cyclic voltammetry studies were carried out with accelerators present at a concentration of $1x10^{-3}$ M in buffer in a voltammetric cell employing a scan rate of 10 mV/sec. The oxidation potentials of the accelerators APH, THQ, TBA, DMPT and DEPT were measured at pH 1.0, 4.0, 5.0, 7.0, 9.8, and 12.2.

2.3. RESULTS AND DISCUSSION

The experiments were designed to examine the ability, if any, of certain anaerobic adhesive accelerators to act as reducing agents with respect to iron(III) and copper(II) and also to determine the influence, if any, of saccharin on these interactions. A number of authors have speculated about the role of these accelerators as reducing agents, but there has been no empirical analytical evidence of this. The experiments concentrated on iron and copper ions as these constitute the vast majority of metals encountered in anaerobic adhesives applications and they are also the most active metals in terms of catalytic decomposition of hydroperoxides. Although some other metals demonstrate activity, most notably cobalt, primers are usually employed to increase the cure speed on these substrates. Primers usually consist of dilute solutions of organic soluble salts of either copper or iron.

Polarography was selected as the analytical technique of choice to monitor for changes in the concentration of lower oxidation state ions of copper and iron in the presence of various anaerobic adhesive cure components. Among the reasons for this selection was the ability of polarography to speciate between the different valence states of the metals, good sensitivity, no sample preparation required, and most importantly the speed in carrying out the determination of the metal species after sampling the reaction system.

2.3.1 Iron(III) Reduction Study

The polarographic procedure for the determination of iron(II) in reactions containing anaerobic adhesive accelerators, was based on work by Parry and Anderson [21], except that sampled direct current polarography was used rather than normal pulse polarography for detection. This mode of current sampling was selected in preference to that of normal pulse or differential pulse polarography because the polarographic trace of iron(II) and incidentally iron(III) obtained in the presence of

the adhesive accelerators, gave rise to two well defined waves that could be easily measured. Good stability of the iron(II) and iron(III) responses were detected, and contrary to the report of Parry and Anderson, no significant reduction of iron(III) to iron(II) in the pyrophosphate supporting electrolyte was detected. This stability was attributed in part to using a less sensitive polarographic detection mode and a shorter analysis time to that used by Parry and Anderson [21].

An investigation into the effect of anaerobic accelerators on the polarographic determination of iron(II) was initially made. No competing electrochemical processes were observed with APH, THQ, DMPT, DEPT or TBA in the pyrophosphate supporting medium in the potential range of -0.1 V to -1.3 V vs Ag/AgCl. In the case of both the iron and copper experiments, the reaction mixtures were monitored over a three hour time period for generation of lower oxidation states of the respective metals. Modern anaerobic adhesives cure rapidly with short induction times; therefore, any reactions in terms of reduction of metal ions outside this time period is of no practical importance.

In the iron(III) experiments it was observed that APH rapidly reduced the 8000 ppm of iron(III) to iron(II) within 5 min, with THQ being only slightly slower. Both DMPT and DEPT demonstrated a more gradual reduction profile, with TBA being found to be inactive, with no reduction observed (Figure 2.4). The addition of saccharin to these reaction mixtures did not significantly affect the reducing abilities of the accelerators. From these results it is apparent that with the exception of TBA, all the other accelerators are capable of acting as reducing agents with regard to iron(III). The development of accelerators can be traced back to the 1960s, when initially trialkylamines, such as TBA, were introduced. Since then, the triarylalkylamines, such as DMPT and DEPT, were introduced, followed in recent years by THQ and APH. The development of more active accelerators such as APH was only made possible by simultaneous advances in stabilisation technology, such as the use of EDTA and analogues of EDTA as transition metal sequestering agents in adhesive formulations.

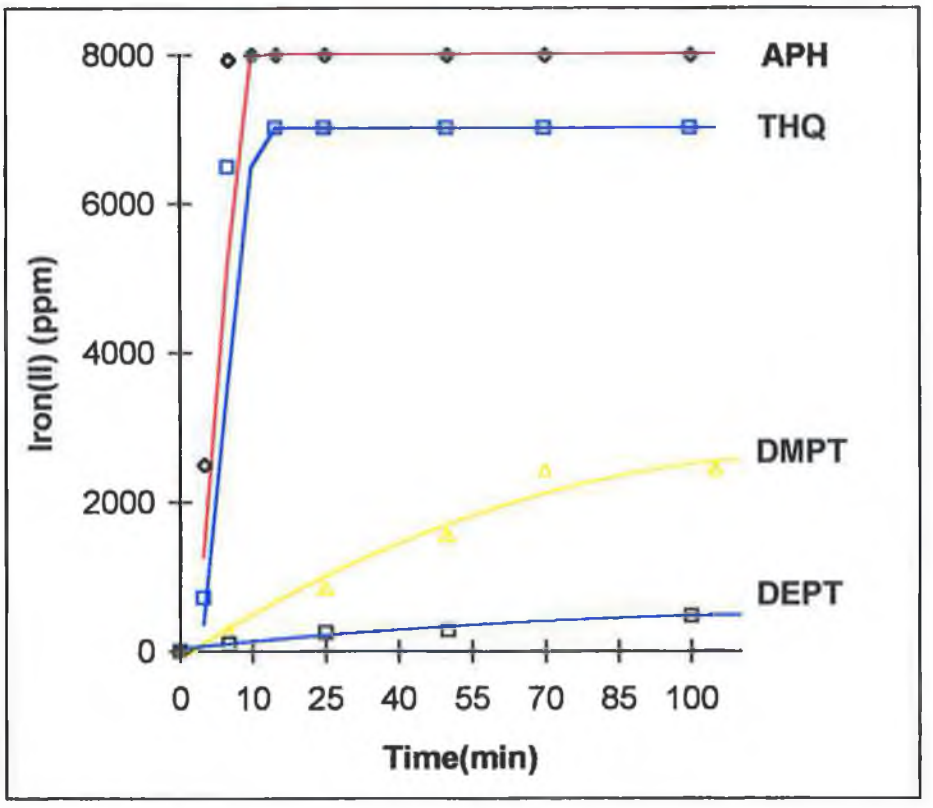


Figure 2.4 Results of the iron(III) reduction experiments for the anaerobic adhesive accelerators.

It was also obvious from the experiments with iron, the extreme reactivity of both APH and THQ to iron(III), and this further emphasizes the requirement for highly efficient means of adhesive stabilisation, especially from any trace metal contamination.

2.3.2 Copper(II) Reduction Study

The polarographic determination of copper(II) was carried out in 2 M ammonium acetate (pH 3.0) and no competing electrochemical processes were observed for any of the accelerators in the supporting electrolyte. The polarographic determination of copper(I) is a difficult procedure involving the analysis of reaction samples in two separate electrolytes and determining the copper(I) concentration from a difference calculation, in which the copper(II) concentration is subtracted from the total copper concentration obtained by differential pulse polarography in an ammonia-ammonium chloride supporting electrolyte [11]. For the sake of simplicity and time. in these experiments the decrease in copper(II) concentration was monitored over time as direct indicator of the generation of copper(I).

The results obtained in the copper(II) experiments shown in Figure 2.5, indicated that, as in the previous experiments, that APH and THQ are the most active accelerators. However, it was observed that in the absence of saccharin no reduction of copper(II) was detected in any instance. While in the presence of saccharin (2% w/v) APH and THQ reduced approximately 6000 ppm and 3500 ppm copper(II) respectively, while the other accelerators, DMPT, DEPT and TBA were inactive in the time frame 0-3 hours.

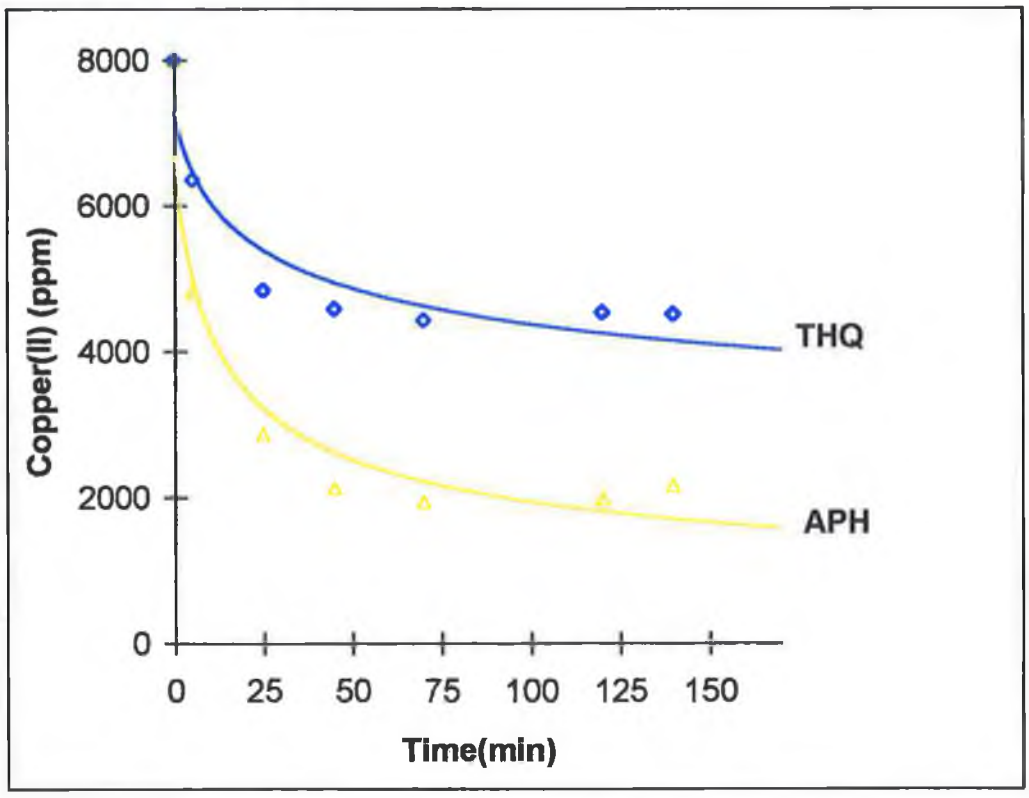


Figure 2.5 The results of copper(II) reduction studies, where reduction was only observed for APH and THQ in the presence of saccharin.

2.3.3 Organic Acid Study

This of course raises the question, what exactly is the role of saccharin? Saccharin is a weak acid; therefore, it is possible that the influence of saccharin is due to its acidic character. To investigate this, the two sets of experiments were repeated, but in this case saccharin was replaced by other organic acids. The organic acids chosen for the study were benzoic acid, glacial acetic acid and maleic acid, with pK_a values of 4.19, 4.75 and 1.92 respectively. The pK_a of saccharin value is more difficult to determine due to its limited solubility in water, but has been estimated to be between 2-3 [8]. In the iron experiments, the presence of the acids did not have any effect on APH; however, with THQ, its reducing power was affected, as shown in Figure 2.6. The presence of saccahrin, however, did not affect its reducing ability, unlike benzoic acid, acetic acid and maleic acid in particular. A similar situation was obtained in the case of DMPT, in which saccharin reduced the amount of iron(III) reduced to iron(II) by over 60%. But with either benzoic acid, acetic acid or maleic acid present, in the case of DMPT no reduction was observed (Figure 2.7). This trend was continued with DEPT as shown in Figure 2.8, where, with the exception of saccharin, the other acids decrease the amount of reduction observed, while in the case of maleic acid, no reduction was detected. The results are not as clear with DEPT, as the concentration levels of iron(II) are considerably lower in comparison to the other accelerators, and therefore the degree of error is higher (Figure 2.8). TBA was not investigated as it showed no activity in the experiments carried out in the absence of the acids.

APH was the only accelerator which did not show any reduction inhibition in the presence of the organic acids. In all other instances, the most dramatic drop in reduction ability was seen in reaction mixtures containing maleic acid, which it should be noted was present at only 1% w/v concentrations. In the presence of the other two acids, benzoic acid and acetic acid, the decrease in reduction ability was not as dramatic. DMPT in particular seems to be very sensitive to the presence of acidic species with the exception of saccharin, resulting in complete elimination of its reducing ability.

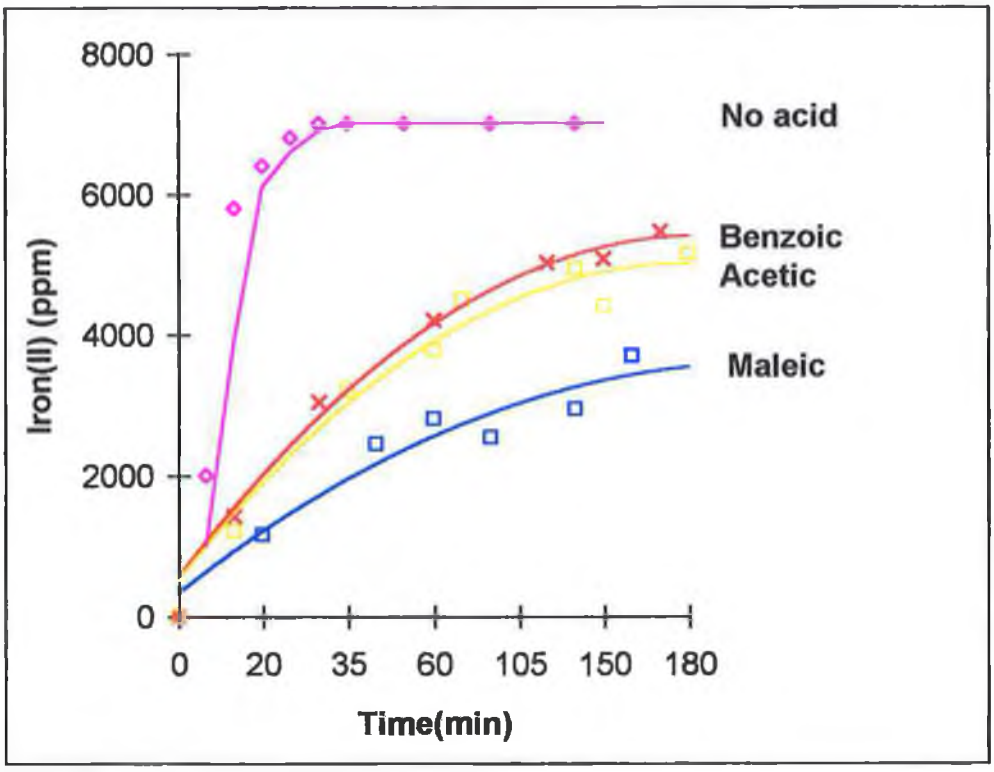


Figure 2.6 Results of THQ/iron(III) reduction experiments in the presence of a range of organic acids.

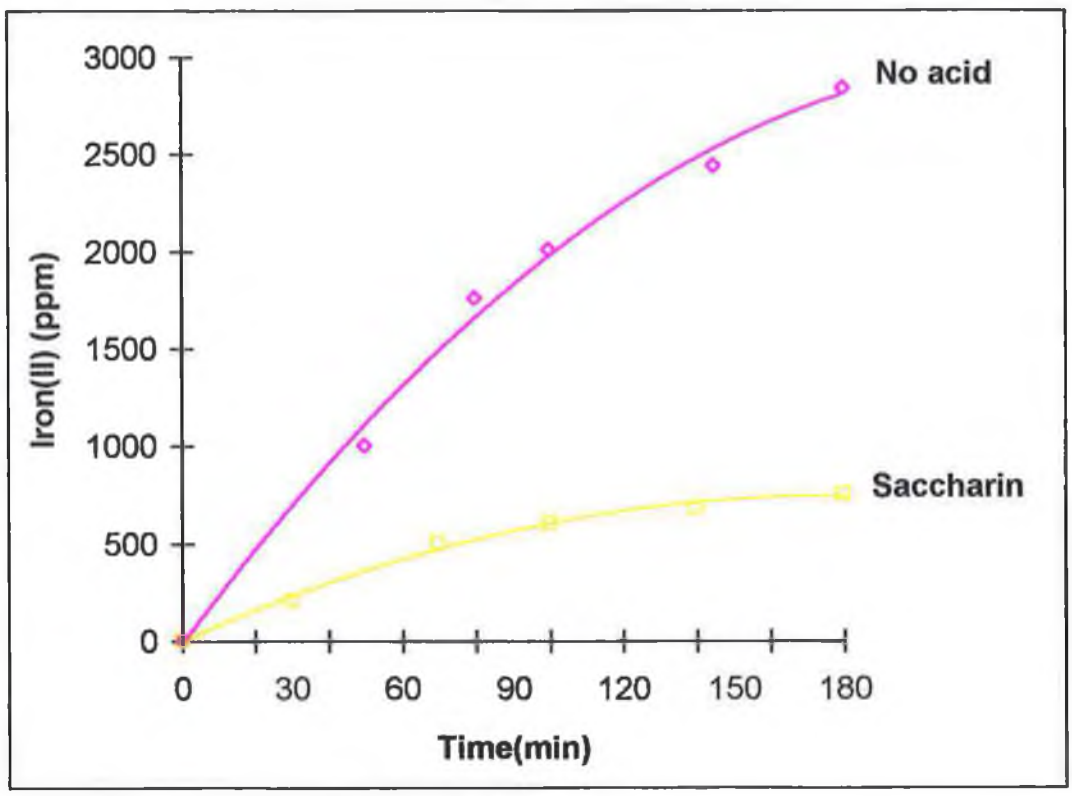


Figure 2.7 Results of DMPT/iron(III) reduction study in the presence of organic acids.

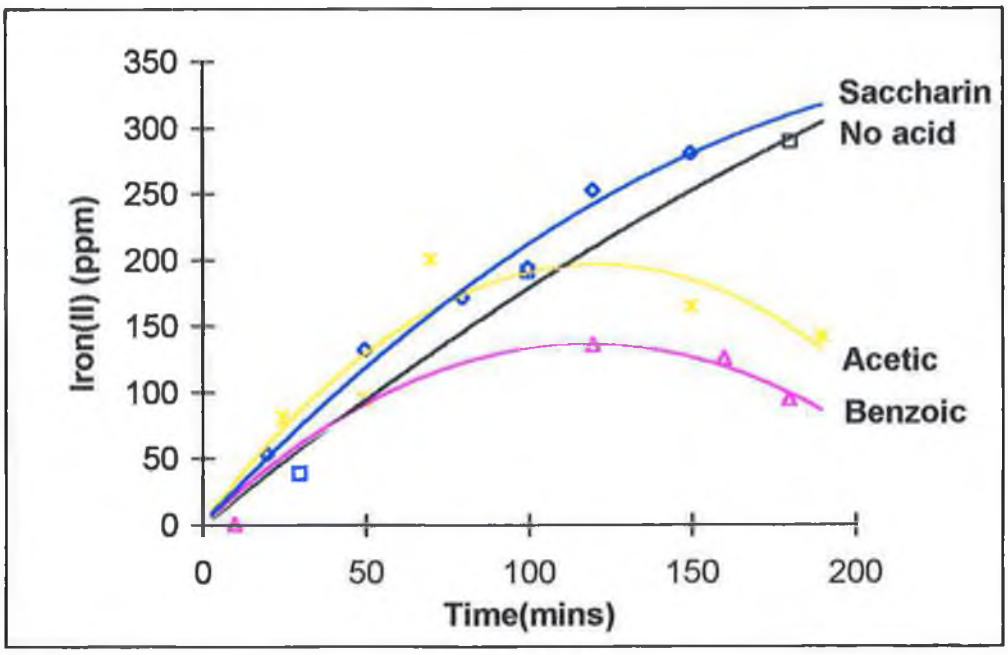


Figure 2.8 Results of DEPT/iron(III) reduction study in the presence of organic acids.

Dealing with these three acids in isolation, there is a trend being followed, in terms of the strength of the acids. Benzoic and acetic acids, are weak acids having very similar pK_a values and consequently the inhibition exhibited by these two acids is practically identical. While maleic acid is a considerably stronger acid and was present in the reaction mixtures at 50% the concentration of the other acids, it exhibited the greatest degree of inhibition. Maleic acid is used in certain anaerobic adhesive formulations at concentrations considerably lower than that of saccharin, and this was reflected in using only 1% w/v maleic acid in these experiments.

In all cases, saccharin did not inhibit the reducing ability of the accelerators to the same degree as the other compounds, and certainly not as much as would have been expected from its pK_a value. The initial copper experiments indicated that copper(II) is reduced by both APH and THQ, only in the presence of saccharin. However, when saccharin is replaced by the alternative organic acids, no reduction is observed. Thus, it can be concluded that the influence of saccharin in copper(II) reduction is not due to its acidic character.

2.3.4 Cyclic Voltammetry

Cyclic voltammetry was employed to determine the oxidation potentials of the various accelerators and to assess if there was any relationship between activity and oxidation potentials for these reducing agents. Figure 2.9 shows the oxidation potentials of the accelerators over a wide pH range as determined using cyclic voltammetry. It is obvious from a first look at this graph that TBA exhibits the highest oxidation potential across the pH range, and is also the least active accelerator, while APH has the lowest oxidation potential, and coincidentally is the most active of all the compounds. TBA oxidation potential below pH 7.0 could not be determined as it was outside the practical working range of the glassy carbon electrode. The decreasing oxidation potential starting with TBA through to APH follows the development of accelerators over the years, and also reflects their increasing reduction activity with regard to iron(III).

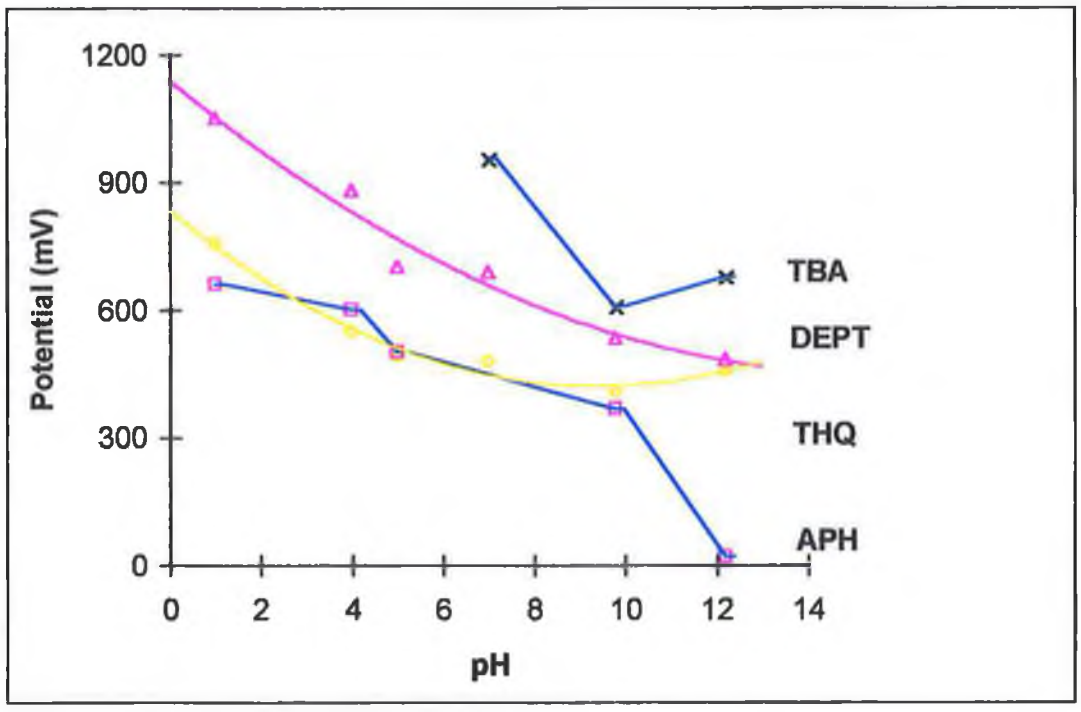


Figure 2.9 Cyclic voltammetric studies to determine the oxidation potential of the accelerators over the pH range 1.0-12.3.

The cyclic voltammetric experiments may help to explain the inhibition in reducing ability observed with the accelerators in the presence of organic acids. As the pH decreases, there is an increase, in all cases, in the oxidation potential of the accelerators. Although it is a generalisation, it would indicate that as the oxidation potential increases, the accelerator's ability to act as efficient reducing agents would decrease and this is reflected in the results of the reduction of iron(III) in the presence of the organic acids.

The overall findings in this study agree with previously published studies and observations, such as that of Beaunez and co-workers [4], who proposed that DMPT acts as a reducing agent with regard to iron(III) saccharinate but not in the case of copper(II) saccharinate. Maleic acid is used, usually in conjunction with saccharin, in certain APH-based adhesive formulations [8]. Observations from this study illustrate clearly that maleic acid can be used with APH without any decrease in reducing ability; however, if maleic acid was used with the other accelerators, a dramatic decrease in reducing ability would be observed and presumably, as a consequence, a dramatic loss in adhesive performance. The observation that DMPT can act as a reducing agent in reducing iron(III), casts further doubt on the charge-transfer theory proposed by Okamoto [6]. A previous study by Loctite, highlighted the different reaction rates and pathways in APH-based cure chemistry, depending not only on the metal present but also on the acid, i.e. saccharin or maleic acid [8]. This difference was highlighted in this study also, as saccharin was required for the reduction of copper(II) but not in the reduction of iron(III). It further emphasizes the point that the reaction of transition metals ions with cure accelerators can not be approached in a general fashion, and that in fact completely separate chemistry prevails in each instance.

Anaerobic adhesives cure in the absence of air; therefore, all of the above reduction studies were repeated, but the reaction mixtures were kept in an argon atmosphere, to determine if oxygen or lack of it had any influence on the activity of the accelerators. Although oxygen inhibits the free radical poymerisation, it did not

affect the observed reducing ability of the accelerator in either the copper(II) or iron(III) studies.

2.3.5 Aminal study

Aminal was synthesized and a polarographic study undertaken to determine if aminal is a strong reducing agent, as proposed by Wellmann and Brockmann [10]. Firstly, it was observed that iron(III) is not reduced to iron(II) by aminal, either on its own or in the presence of saccharin. However, aminal does act as a strong reducing agent with regard to copper(II) as shown in Figure 2.10, and the presence of saccharin does not effect this reaction. This confirms to a certain degree the observations of Wellmann and Brockmann [10], that aminal is a strong reducing agent, but only in terms of copper(II). However, in iron based systems, DMPT acts as the reducing agent, as shown by the previous experiments. This further emphasizes the point mentioned earlier, that the reactions of transition metals with cure accelerators is complex chemistry in which the reaction routes and rates differ depending on the metal involved.

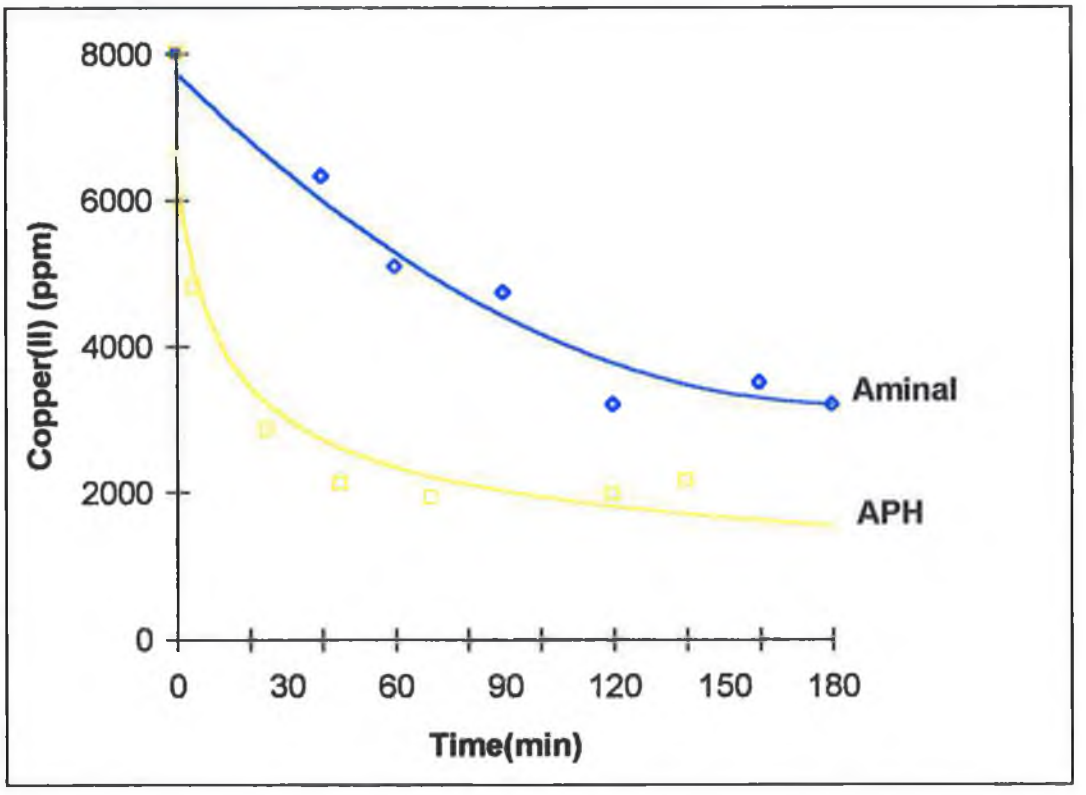


Figure 2.10 Results of aminal/copper(II) reduction study, in comparison to APH/saccharin reduction of copper(II). Aminal and APH were used at concentrations of 0.5% w/v and 1.0% w/v respectively.

2.4. CONCLUSIONS

The accelerators used in anaerobic adhesives, with the exception of TBA, are capable of reducing iron(III) to iron(II). APH and THQ in particular are very effective reducing agents. However, in the case of copper(II), only APH and THQ can reduce the metal to its lower oxidation state and this only occurs in the presence of saccharin. This apparent unique role of saccharin in copper(II) reduction is not due to its acidic character, as the replacement of saccharin with other organic acids proved. The acids also inhibited iron(III) reduction to varying extents, depending on the accelerator. Saccharin did not inhibit the reduction as much as would have been expected according to its pK_a value in comparison to the other acids, yet again identifying an apparent unique property of saccharin.

Cyclic voltammetric determination of the oxidation potentials of the accelerators at a variety of pH values confirmed that on decreasing pH, the oxidation potentials increase, thus, explaining the decrease in reduction ability in the presence of the organic acids. The oxidation potentials , in general, decreased in the series: TBA; DEPT; DMPT; THQ; APH; thereby charting the development of more active accelerators over the years.

Although, DMPT did not reduce copper(II), in the time period studied, a new compound, aminal, formed from the reaction of saccharin and DMPT, is a very efficient reducing agent of copper(II). The question of whether aminal is formed and to what levels in adhesive formulations, remains to be investigated.

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CHAPTER THREE

INVESTIGATIONS INTO THE METAL SUBSTRATE AND HYDROPEROXIDE BASED CURE CHEMISTRY OF ANAEROBIC ADHESIVES

3.1. INTRODUCTION

3.1.1 General Chemistry

Anaerobic adhesives are single component acrylic adhesives which remain stable in liquid form for long periods of time at room temperature. However, in the absence of air, typically when confined between two closely fitting surfaces, the monomeric constituents in the liquid polymerise rapidly to form a tough solvent and heat resistant material. Anaerobic adhesives are predominantly used in the mechanical engineering industry, finding applications in the sealing, locking and retaining of metallic objects. Anaerobic adhesives consist mainly of ethyleneglycol dimethacrylate-based monomers and an organic hydroperoxide based initiator, typically cumene hydroperoxide (CHP). They are used in combination with an organic acid and a tertiary amine which are employed as cure promoters or accelerators. A variety of organic acids are used, including maleic acid and o-benzoic sulphimide (saccharin), while tertiary amines in use include N,N-dimethyl-p-toluidine (DMPT), 1,2,3,4-tetrahydroquinoline (THQ), and more recently 1-acetyl-2-phenylhydrazine (APH). The cure chemistry has been discussed with in detail in chapter two.

3.1.2 Metal Substrate

Anaerobic adhesives are predominantly used for bonding metallic substrates. Transition metal ions on the substrate surface play a key role in the cure chemistry, reacting with the hydroperoxide to generate free radicals. Anaerobic adhesives are sensitive to the nature of the substrate requiring confinement on an active metal surface. For example, they cure rapidly on copper- and iron-rich surfaces, but cure slowly or not at all on cobalt-, cadmium- or zinc-plated metals [1]. This sensitivity is due to differing reactivities of the various metals with hydroperoxides. In the case of substrates such as plastics and cadmium etc., primer compositions are sprayed or brushed on to the substrate before bonding. The active ingredients in the primer

provide the surface chemistry that initiates polymerisation. Solvent-soluble metal salts are often sufficient, such as copper napthenate. The role of the acids present in formulations i.e. maleic acid and saccharin, is to provide soluble metal ions from the substrate surface which can subsequently decompose the hydroperoxide. The polarographic determination of copper and iron has been discussed in detail in chapter two.

3.1.3 Role of Hydroperoxide

The redox decomposition of hydroperoxides by metal ions at room temperature produces free radicals which, in the absence of oxygen, initiate polymerisation. This is the principle of operation of anaerobic adhesives [1]. Although a wide range of free radical initiators have been used in anaerobic adhesives, hydroperoxides have been the most important. They have the advantages of being easy to handle and afford stable compositions with methacrylate monomers. Many patents describe the incorporation of hydroperoxides by bubbling oxygen through the composition. Oxygen forms hydroperoxides by reacting with certain active hydrogens in the monomer. However, cumene hydroperoxide and tert-butyl hydroperoxide are most frequently added as initiators. Studies by Humphreys [2] have illustrated the stability of hydroperoxides in adhesives formulations, as even at 100°C the reaction of CHP with other cure components was found to be relatively slow.

Two kinetic studies were carried out by Beaunez and co-workers on the radical polymerisation of methyl methacrylate initiated by a redox system consisting of CHP/metal salts of saccharin/DMPT [3 ,4]. The first study was based on copper saccharinate and it was proposed that CHP rapidly reduces copper(II) to copper(I). A small fraction of the copper(I) is then complexed with DMPT in a 1:2 complex and the complexed ions are strong reductants with respect to CHP, whereas uncomplexed copper(I) is inactive. The decomposition of CHP is strongly catalysed by saccharin, with saccharin protonating the oxygen atom linked to the hydrogen atom.

The average rate constant for the saccharin catalysed decomposition of CHP was found to be 13000 times higher than the rate constant for the uncatalysed reaction. In the second study based on iron saccharinate it was proposed that iron(II) is complexed by two DMPT molecules and is thus much more reactive than uncomplexed iron(II) with regard to decomposition of CHP, which again is activated by saccharin [4].

There are several methods available for the determination of hydroperoxides, e.g. classical titration methods and polarography. Kuta and Quackenbush [5] used polarography for the determination of 22 organic peroxide compounds such as hydroperoxides and diacyl peroxides. The supporting electrolyte consisted of 0.3 M lithium chloride in an equal volume mixture of methanol and benzene. However, replacement of the benzene with other aromatic solvents such as toluene or xylene, gave essentially the same result. The 22 different organic peroxides were compared polarographically and divided into six groups based on their structures and behaviour in the polarographic cell. Hydroperoxides were found to be reduced at potentials around -1.0 V, with half-wave reduction potentials for cumene hydroperoxide and tert-butyl hydroperoxide of -1.08 V and -1.15 V vs SCE respectively. Willitis and co-workers studied the polarographic determination of oxygen-containing organic compounds with 0.3 M lithium chloride in methanol-benzene [6]. The half-wave reduction potentials of cumene hydroperoxide and tert butyl hydroperoxide in that instance were reported as -0.68 V and -0.96 V vs SCE respectively. An identical supporting electrolyte has been used for the polarographic determination of hydroperoxides in gasolines [7].

Fordham and Williams studied the decomposition of cumene hydroperoxide by metal salts and complexes in styrene solution [8]. It was noted that both the metal and the non-metal portion appeared to influence the decomposition rate. The decomposition rate of metal phthalocyanines decreases in the following order: iron(II) > iron(III) > colbalt > chromium. While it was found that copper(I) chloride in the presence of o-phenanthroline monohydrate decomposed 43% of the CHP after 60 min, while the equivalent copper(II) solution only decomposed 4.5%.

3.1.4 Aims of Research

The first aim of this study was to investigate the reactions of elemental copper and iron in the presence of 1,2,3,4-tetrahydroquinoline based cure systems in order to further elucidate the cure mechanism of anaerobic adhesives and to determine the influence of various cure components on the generation of lower oxidation states of copper and iron.

Secondly, the catalytic decomposition of cumene hydroperoxide by copper and iron ions in the presence of various cure components was studied, although it is generally accepted that the lower oxidation states of both iron and copper are the most active in terms of generation of active radicals from the decomposition of cumene hydroperoxide, it has been propsed from kinetic studies that the accelerators, and DMPT in particular, catalyse the reaction of iron(II) and copper(I) with cumene hydroperoxide and also, that saccharin protonates the CHP, thereby activating the decomposition. Polarography was used to monitor for changes in concentration of CHP in the presence of various cure components.

3.2. EXPERIMENTAL

3.2.1 Apparatus

Polarographic measurements were obtained using an EG&G PARC (Princeton Applied Research, Princeton, NJ) Model 264A polarographic analyser in conjunction with an EG&G PARC Model 303A static mercury drop electrode. A small mercury drop size was used routinely throughout this study with a drop time of 0.5 seconds, pulse height of 50 mV and an equilibration time of 15 seconds prior to commencing the polarographic scan.

3.2.2 Materials

All experiments were carried out at room temperature and all materials were of Analar grade unless otherwise stated. Both the raw materials used in anaerobic adhesives formulations and the iron filings were supplied by Loctite (Irl) Ltd., with the exception of APH which was obtained from Eastman Kodak. The copper powder was obtained from BDH Chemicals. Deionised water which was obtained by passing distilled water through a Milli-Q water purification system (Millipore, Milford, MA), was used in all preparations. Solutions of copper(I) and copper(II) were made up from the chloride and acetate salts respectively, while iron(II) and iron(III) were made up from the ammonium sulphate and nitrate salts respectively.

In the polarographic study of the reactions of elemental forms of copper and iron the following supporting electrolytes were used : (A) 1 M ammonia-ammonium chloride in the determination of copper(I), (B) 2 M ammonium acetate, pH 3.0, in the determination of copper(II), and (C) 0.1 M sodium pyrophosphate, pH 8.0, in the determination of both iron(II) and iron(III). For this series of experiments, all reaction mixtures were made up in methanol.

Cumene hydroperoxide was determined in as supporting electrolyte consisting of 0.3 M lithium chloride in a 1:1 mixture of methanol/toluene. The iron(III) and copper(II) salts were dissolved in methanol, while due to solubility difficulties in methanol, ammonium iron(II) sulphate was made up in water and copper(I) chloride was dissolved in ammonium hydroxide/water.

3.2.3 Procedures

3.2.3.1 Reactions with elemental copper and iron experiments

The reaction mixtures which contained the accelerator 1,2,3,4-tetrahydroquinoline (THQ) in the presence of iron filings or copper powder and other constituents found in anaerobic sealant formulations were prepared in the following manner: All reactions took place in acid washed 100 cm^3 conical flasks. In a step by step approach, which initially started with a reaction mixture of metal filings or powder in methanol containing dissolved accelerator (1% w/v), further reaction mixtures were prepared which contained various combinations of organic cure components at concentrations at which they are normally present in anaerobic adhesives. Both maleic acid and cumene hydroperoxide (CHP) were present at 1% w/v, whereas saccharin was present at 2% w/v concentrations.

Reaction mixtures: All solutions contained either copper powder or iron filings at a concentration of 2% w/v, with the following additives:

1. accelerator
2. accelerator + saccharin
3. accelerator + maleic acid
4. accelerator + cumene hydroperoxide
5. accelerator + saccharin + cumene hydroperoxide
6. accelerator + maleic acid + cumene hydroperoxide

The concentration of the iron or copper ions generated was monitored with time using sampled DC and differential pulse polarography, respectively. An aliquot of sample was analysed at regular intervals using polarography and the concentration of copper or iron species present was determined by means of standard addition procedure, in which three successive aliquots of standard solution were added to the polarographic cell containing the sample.

3.2.3.2 Cumene hydroperoxide decomposition study

All reactions took place in acid washed 100 cm^3 conical flasks. In a step by step approach, CHP ($1x10^{-3}$ M) was dissolved in 10 cm^3 methanol, and an aliquot , typically 100 µl, was removed and added to 10 cm^3 of lithium chloride electrolyte and the concentration of CHP determined polarographically. The CHP concentration was determined using differential pulse polarography by scanning in the range -0.50 V to -1.10 V vs Ag/AgCl after purging with oxygen free nitrogen for thirty seconds. Then the various metals salts and cure components were added to the CHP solution (in molar-molar quantities) and the concentration of CHP was monitored with time, by removal of 100 µl aliquots of the reaction mixture and subsequent polarographic analysis.

Reaction mixtures: All solution contained CHP and iron or copper salts at a concentration of $1x10^{-3}$ M, with the following reaction mixtures:

1. CHP
2. CHP + metal salt
4. CHP + metal salt + acid (1:1 and 2:1 ratio)
5. CHP + metal salt + accelerator (1:1 and 2:1 ratio)
6. CHP + metal salt + accelerator + acid (1:1 and 2:1 ratio)

The 'acid' component refers to saccharin and maleic acid, while the accelerators used were APH, DMPT, THQ and DEPT.

3.3. RESULTS AND DISCUSSION

3.3.1 Elemental Iron and Copper Study

The polarographic determination of both iron and copper has been covered in detail in chapter two. An investigation on the effect of the organic cure components on the polarographic determination of iron(III) and iron(II) ions was initially made. No competing electrochemical process was observed with THQ, saccharin or maleic acid in the pyrophosphate medium in the potential range -0.1 V to -1.3 V vs Ag/AgCl. However, CHP interfered in the polarographic determination of both iron species. The potential range, size and duration of the interference depended upon the combination of cure components present with the hydroperoxide in the reaction system. In reaction systems containing only THQ with cumene hydroperoxide, no iron(III) or iron(II) was detected due to the excessively high background current produced by the hydroperoxide in the supporting electrolyte. As CHP reacts poorly with THQ in such catalytic systems, little decomposition of the hydroperoxide occurred during the experimental period, with the result being that the interference did not decrease with time. Although interference from the hydroperoxide was present in other solutions, in most cases sufficient cumene hydroperoxide had reacted within an hour of starting the reactions to decrease the background polarographic response, permitting the estimation of both iron species in the respective reaction systems.

Both copper(I) and copper(II) ions were determined by DPP using ammonia-ammonium chloride and ammonium acetate, pH 3.0, as supporting electrolytes receptively. In the presence of weak chelating agents such as acetate, copper(II) forms more stable complexes than copper(I) ions, and the polarogram consists of one peak corresponding to the direct reduction of copper(II) to the metal. On the other hand, electrolytes containing high concentrations of ammonia or chloride stabilise the copper(I) ion sufficiently so that stepwise reduction to the metal occurs. The polarogram obtained in such electrolytes consists of a double wave, where the first wave corresponds to the reduction of copper(II) to copper(I) and the second

wave to the reduction of copper(I) to the metal. As the separation of the two half-wave potentials is about 0.27 V, the two distinct electrochemical processes are not resolvable using DPP and only one broad peak was obtained. In order to determine the concentration of copper(I) ions generated in reaction mixtures of copper powder and cure components, a difference calculation was made whereby the copper(II) concentration was subtracted from the total copper(II) and copper(I) concentration obtained in the ammonia-ammonium chloride electrolyte [9 ,10]. The polarographic determination of the two copper species was not subject to the degree of matrix interference encountered with the iron experiments. Initial interference from CHP disappeared within five hours of starting the experiment, permitting the detection of both copper species in the reaction mixtures.

It was found that significantly higher levels of copper(II) was generated with THQ/ maleic acid compared to THQ/BS based systems when contacted with elemental copper as shown in Figure 3.1. Similar behaviour can be observed in Figure 3.2 with the iron catalysed systems, where THQ with maleic acid produce higher levels of iron(III) with time than corresponding systems based on THQ and saccharin.

However, this situation was reversed in the case of the lower valence state of both metals, where relatively insignificant levels of iron(II) ions were detected in solutions containing maleic acid compared to those containing saccharin, shown in Figure 3.3. This trend is continued in Figure 3.4, where THQ in combination with saccharin generates higher levels of copper(I) with time compared to the maleic acid based systems. It should be noted that in solutions containing only THQ in contact with the metal, only trace levels of metal ions were detected, clearly demonstrating the key role played by saccharin and maleic acid in liberating metal ions from the substrate surface.

When these reactions were carried out in the presence of CHP, THQ/saccharin was still observed to generate significantly higher levels of iron(II) than the corresponding maleic acid solutions, although there was a decline in the overall levels of iron(II).

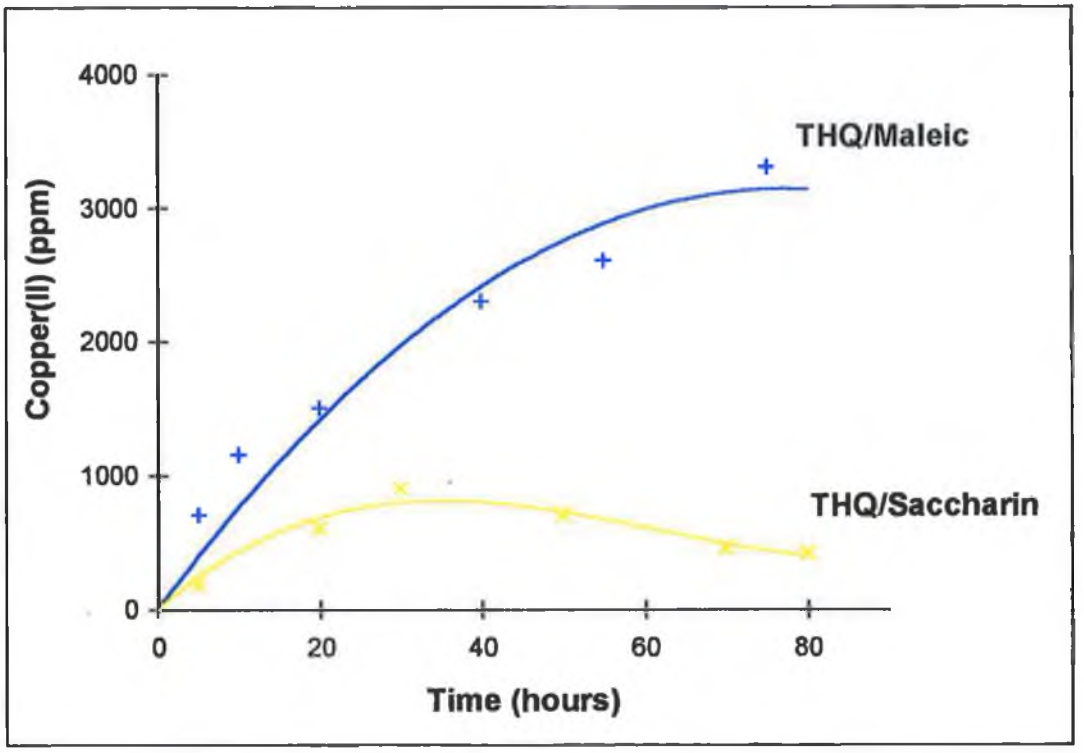

Figure 3.1 Results for the generation of copper(II) from elemental copper in the presence of THQ based cure components.

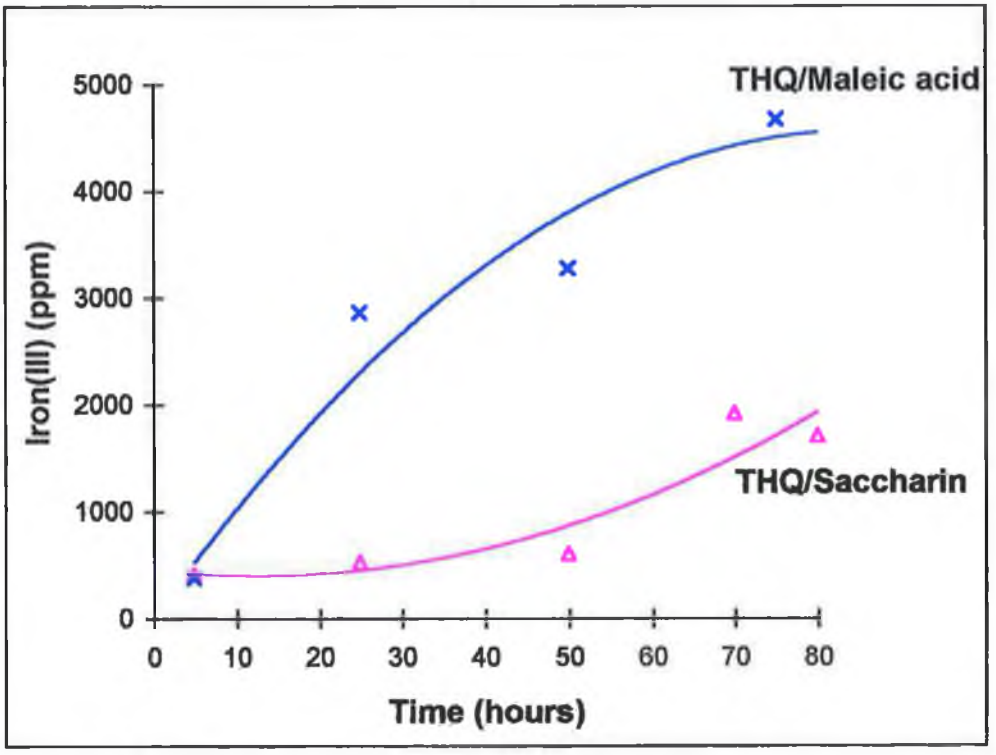

Figure 3.2 Results for the generation of iron(III) from elemental iron in the presence of THQ based cure components.

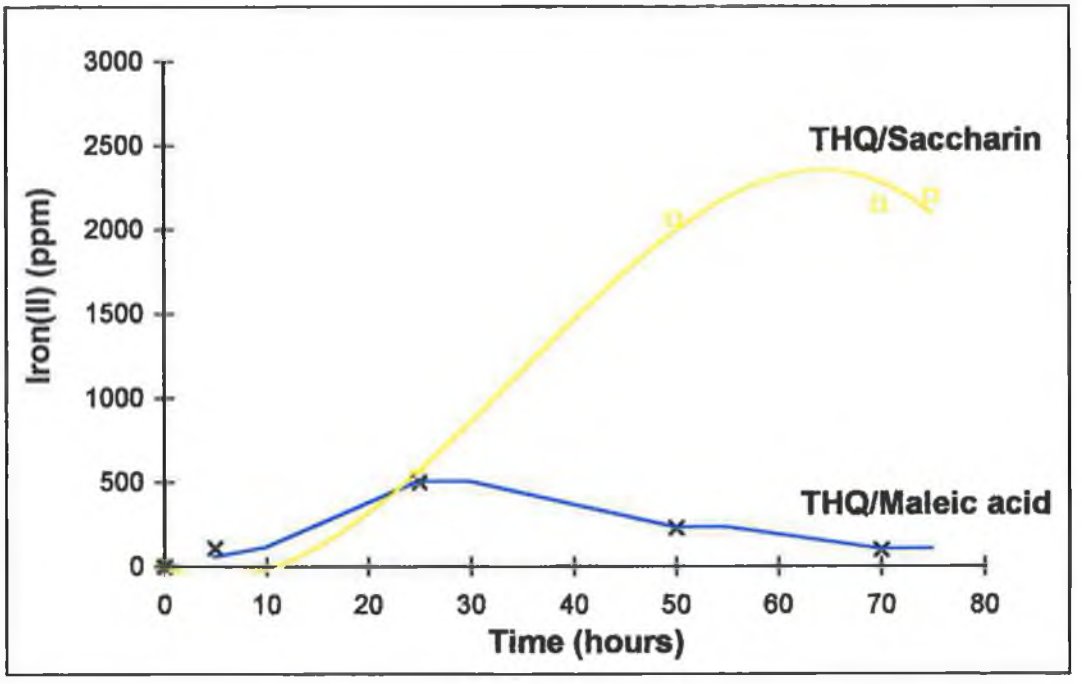

Figure 3.3 Results from the polarographic study of the generation of iron(II) from elemental iron in the presence of THQ based cure components.

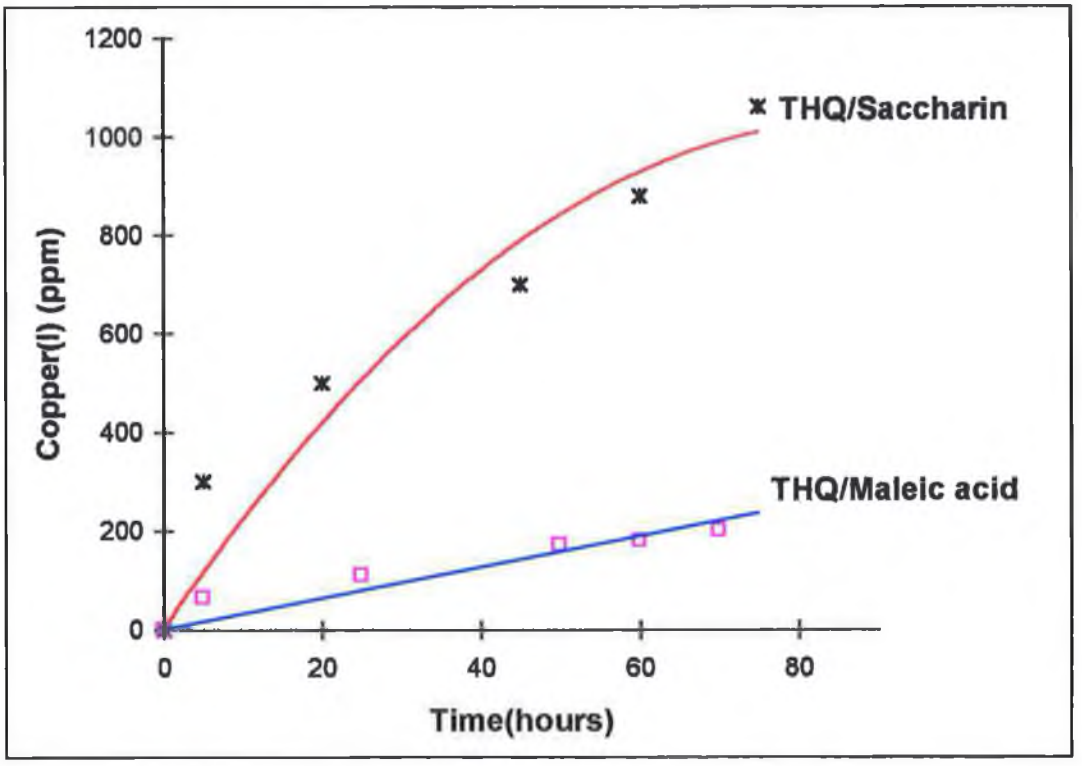

Figure 3.4 Copper(I) generated with time from elemental copper in THQ based cure systems.

From Figure 3.5 it can be seen that the addition of CHP did not affect the THQ/maleic acid solution, which produced higher levels of iron(III) than the saccharin containing solution. Also there was a decrease in the relative levels of iron(II), due to the reaction of the iron(II) with the hydroperoxide.

In the copper based experiments, the addition of CHP resulted in a decrease in the copper(I) levels in both THQ solutions. The reduction in the detectable levels of copper(I) would support the hypothesis that copper becomes an intrinsic part of the catalytic cycle, whereby, as copper(II) is produced, it is immediately reduced by THQ to copper(I), which in turn catalyses the decomposition of the hydroperoxide.

Again this experimental sequence also demonstrates very clearly the differences between cure systems based on saccharin and those based on maleic acid with regard to the oxidation states of the metal ions liberated from the substrate. If, as is widely accepted, the lower valence forms of these metals are the most active with regard to the homolysis of the organic hydroperoxide, then saccharin should on this basis (and all other things being equal) be a more effective promoter of the redox based curing reaction of anaerobic adhesives than maleic acid. A possible explanation for the different behaviour of saccharin and maleic acid may be related to the fact that maleic acid being a stronger acid, has a stronger tendency to protonate THQ to a greater extent and thus interfere with its reducing ability. This proposal is supported by measurements of the oxidation potentials of the various accelerators favoured in anaerobic sealant technology by cyclic voltammetry as shown previously in Figure 2.9.

The results obtained in this study complement a previous study carried out in to the reactions of elemental copper and iron in the presence of 1-acetyl-2-phenylhydrazine (APH) and 1-acetyl-2-phenyldiazene (APD), the initial oxidation product of APH, based cure systems [11]. In the case of APH the presence of either maleic acid or saccharin influenced the production of lower valence state ions of iron and copper, as in the THQ study discussed previously.

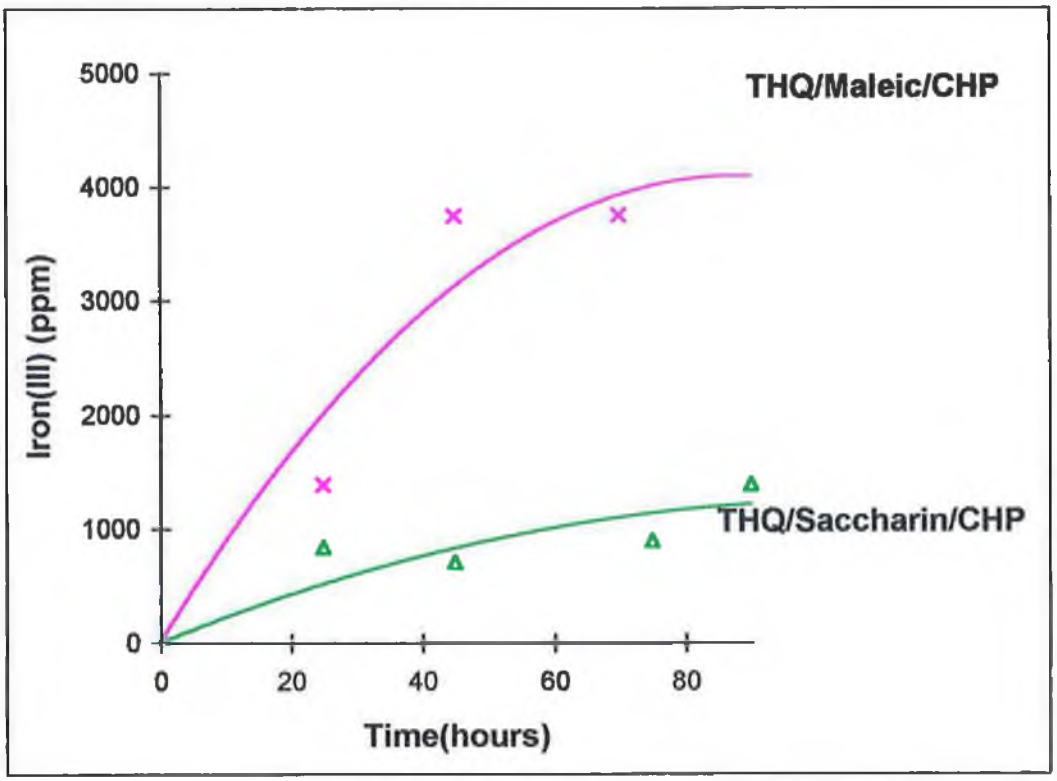

Figure 3.5 Iron(III) generated with time from elemental iron with CHP in THQ based cure systems.

However, the strength or nature of the acid, in the case of APD, did not influence the production of copper(II) and to a lesser extent the production of copper(I). These findings complement the hypothesis made in the case of both APH and THQ, whereby the protonated versions of APH and THQ retards the conversion of higher states of the metal to the lower state. But since this situation does not arise with APD, the copper(II) that is generated is readily reduced to copper(I) by APD.

The results discussed in chapter two have further emphasised the difference between the two acids. Thus, whereas polarographic studies have demonstrated that APH and THQ readily facilitate the reduction of iron(III) to iron(II), it has been proven that the presence of saccharin exerts very little influence on this reaction. By contrast the comparable reduction of copper(II) to copper(I) by APH is relatively inefficient unless saccharin is included in the reaction mixture. Moreover, maleic acid cannot effectively be substituted for saccharin in this latter reduction.

These experiments were designed to help gain greater insight and understanding of the reactions occuring at the substrate-adhesive interface, which are critical in the cure chemistry. These results would suggest that anaerobic adhesive formulations should take into account the type of metal expected to catalyse the redox radical polymerisation, as one formula that possesses good reactivity and stability properties when catalysed by a particular metal such as copper may not be appropriate when it is catalysed by another metal such as iron, for example.

3.3.2 Cumene Hydroperoxide Decomposition Study

It was invisaged that this investigation would determine conclusively the reactions of certain transition metal ions with cumene hydroperoxide and also examine the influence of acidic cure components as well as accelerators on these reactions. Polarography offered a rapid means of monitoring for changes in CHP concentration with no sample preparation required. CHP in the lithium chloride electrolyte gave rise to a broad well defined peak, characteristic of hydroperoxides, with a half-wave reduction potential of -0.80 V [5]. None of the anaerobic adhesive components or the transition metals interfered in the polarographic determination of CHP.

The results of the decomposition of CHP by copper and iron ions is shown in Figure 3.6. Within two minutes 98% of CHP had been decomposed by iron(II), while after 40 minutes 90% of CHP had been decomposed by iron(III). However, copper(II) demonstrated a very slow decomposition profile with only 10% decomposed after 70 minutes, and copper(I) was inactive. The addition of saccharin or maleic acid to the reactions involving copper, resulted in no detectable increase in the reaction rate.

The subsequent experiments concentrated on the influence of cure components on the copper(I) catalysed decomposition of CHP, as it is generally assumed that the primary influence of transition metals comes from the lower oxidation state. The inclusion of either APH or DMPT in a 1:1 ratio to copper(I) did not have any influence on the reaction. However, when these accelerators were added in a 2:1 ratio there was a dramatic increase in activity (Figure 3.7). In the case of THQ, the presence of the accelerator whether in a 1:1 or 2:1 ratio increases the reaction rate, as shown in Figure 3.8. In the presence of DEPT a reaction was observed between CHP and copper(I), but when the concentration of DEPT was increased to a level of a 2:1 ratio to the copper(I), then the reaction was actually retarded somewhat (Figure 3.8).

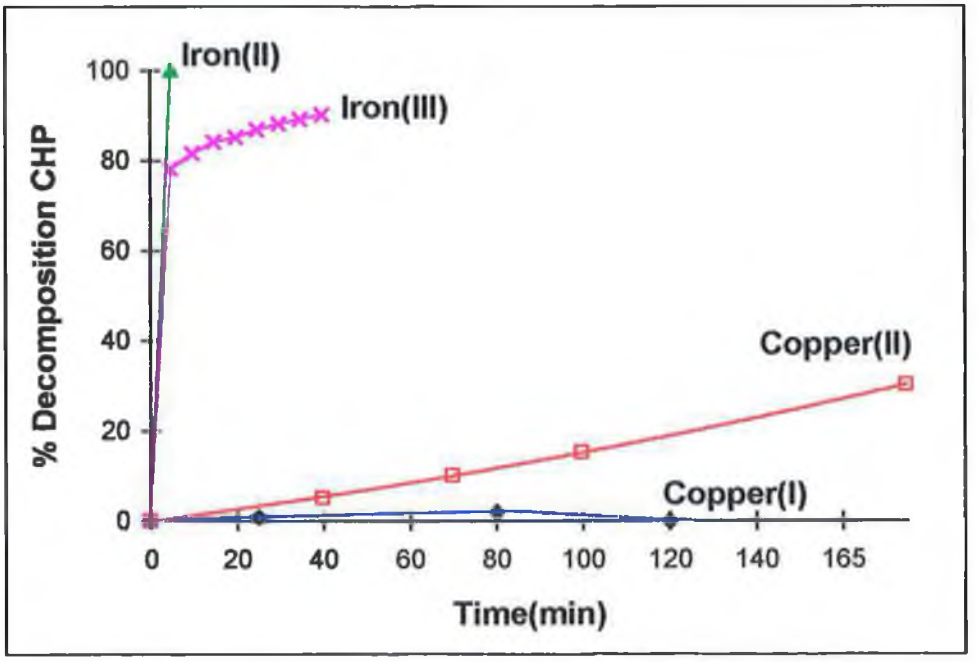

Figure 3.6 Results from the polarography based study on the decomposition of CHP by transition metals ions.

The addition of saccharin to the APH-copper(I) reaction mixtures did not affect the reaction rates, even when present in a 2:1 molar ratio to the copper(I). But the addition of maleic acid in a 2:1 ratio increased the reaction rate significantly, while it should be noted that the reaction rate did not increase noticeably with maleic acid present in a 1:1 molar ratio (Figure 3.9). The addition of saccharin in the DMPT experiments had no effect in a 1:1 ratio but when present in a 2:1 ratio the reaction was actually inhibited. In the presence of maleic acid no reaction was observed. (Figure 3.9). In the THQ based reaction mixtures the addition of either acidic species had a detrimental affect on the rate of decomposition (Figure 3.10), as was the case in the DEPT based reaction mixtures.

It has been shown that although both iron(II) and iron(III) are relatively efficient at decomposing CHP, copper(II) is considerably slower and copper(I) was inactive. Even with the addition of maleic acid or saccharin the copper species remain very inefficient at decomposing the hydroperoxide. Therefore, the initial experiments would indicate that the acids were not actually activating the decomposition by protonation of the hydroperoxide as had been proposed [3,4].

It has been proposed that uncomplexed iron(II) is ineffective at decomposing CHP; however, in this study it has been shown conclusively that iron(II) is very efficient at decomposing CHP in comparison to the other metal ions. Copper(I) is not capable of decomposing CHP unless complexed with acclerators. In the case of APH and DMPT the experimental evidence suggests a 2:1 complex is formed between the accelerators and copper(I). This agrees with the observations of Fordham and Williams [8], as only in the presence of complexing agents such as o-phenanthroline monohydrate and 2,2-bipyridyl was CHP decomposed by copper(I). THQ whether present in a 1:1 or 2:1 ratio, increased the decomposition rate similarly. However, with DEPT the effect was not as clear cut. DEPT when present in a 1:1 ratio had a positive effect on the decomposition rate, but in a 2:1 ratio there was a dramatic drop in decomposition rate.

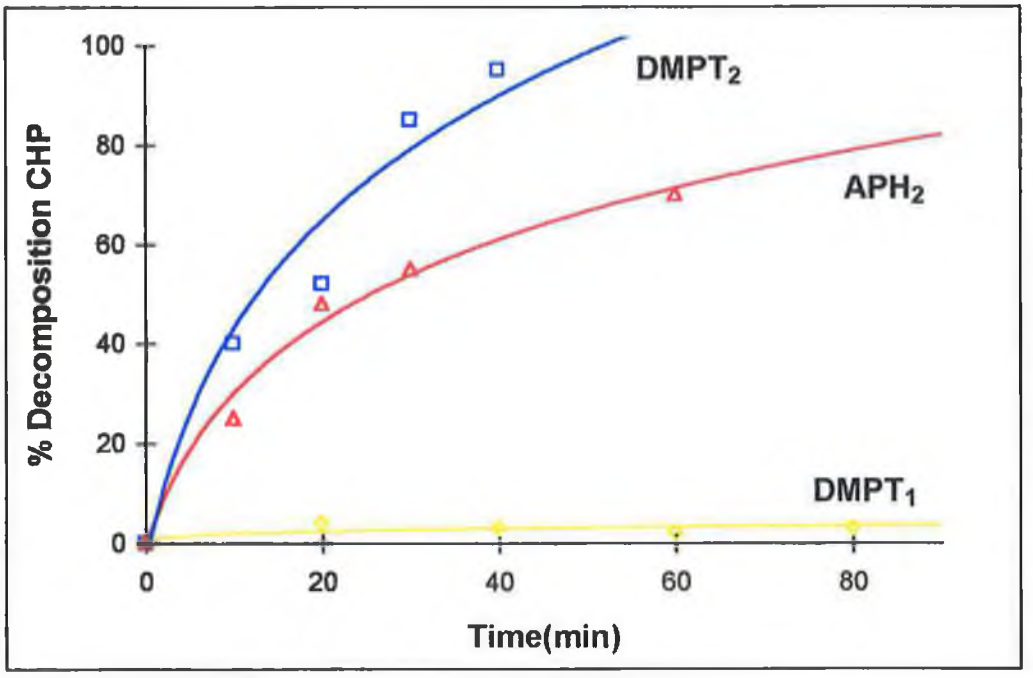

Figure 3.7 Results showing the effect of APH and DMPT on copper(I) catalysed decomposition of CHP. Subscripts indicate molar ratio of accelerator to copper(I).

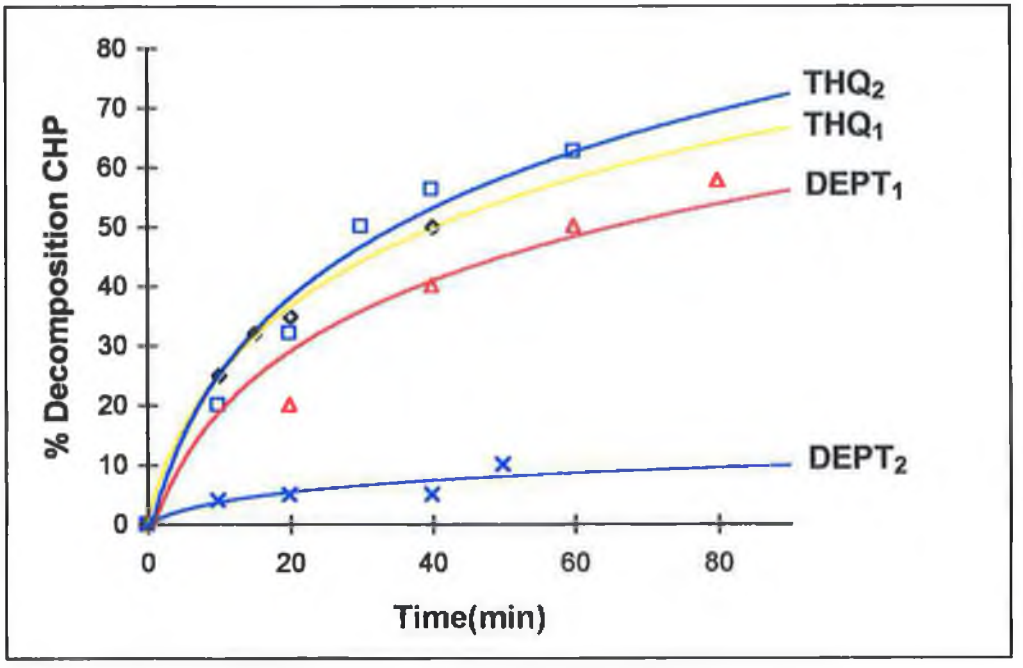

Figure 3.8 Copper(I) catalysed decomposition of CHP in the presence of THQ and DEPT. Subscripts indicate molar ratio of accelerator to copper(I).

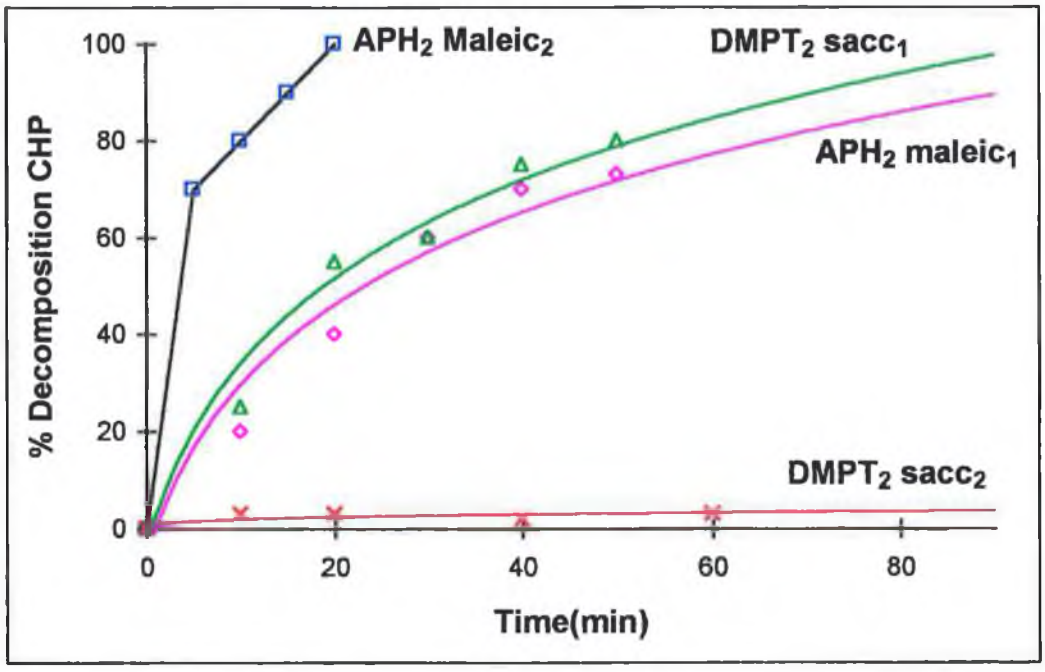

Figure 3.9 The effect of maleic acid and saccharin on APH and DMPT in copper(I) based decompositions of CHP. Subscripts indicate molar ratio relative to copper(I).

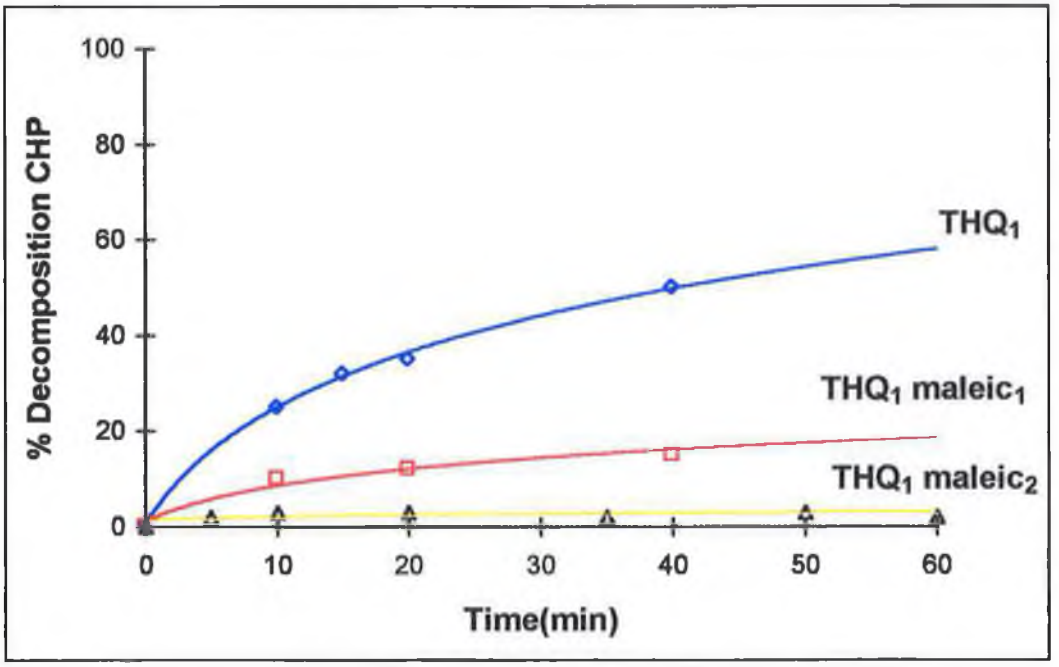

Figure 3.10 Influence of maleic acid and saccharin on the copper(I) catalysed decomposition of CHP in the presence of THQ.

The inclusion of maleic acid and saccharin was to determine if these acids could activate or catalyse the decomposition of CHP. Only in the case of APH, with a 2:1 ratio of acid to CHP, was there an increase in reaction rate, while the weaker acid, saccharin, did not affect the decomposition rate. In all other instances, the addition of the acids inhibited the decomposition to varying degrees depending on the accelerator. Although the acids may be protonating CHP to a certain degree, as shown in chapter two, acids affect the reducing ability of accelerators and it would seem plausible that the acids could affect the ability of the accelerators to form complexes with copper(I), thereby inhibiting the decomposition of CHP.

The reducing ability of APH was uneffected by organic acids, and similarly in this experiment, the presence of maleic acid in the APH reaction mixtures actually increased the decomposition rate. This provides tentative evidence of protonation of the hydroperoxide, protonation maybe occuring with the other accelerators, but this is outweighed by the affect of saccharin or maleic acid on the accelerator and its complexing ability.

These experiments have highlighted the complex nature of anaerobic adhesive chemistry, as accelerators act firstly not only as reducing agents for transition metal ions but also as complexing agents with copper(I) to catalyse the decomposition of cumene hydroperoxide. Acidic species, such as saccharin and maleic acid are essential for the liberation of transition metal ions from the substrate surface but they also inhibit to varying the degrees the formation of copper(I) complexes which are essential for the rapid decomposition of hydroperoxides.

3.4. CONCLUSION

Anaerobic adhesive accelerators in combination with either maleic acid or saccharin play a key role in liberating transition metal ions from the substrate surface. The resulting concentration and oxidation state of these metal ions, which is of vital importance, is inherently dependent on the acid used. Based on this study it would be expected that the fastest cure would be obtained using saccharin as distinct from maleic acid in formulations based on 1,2,3,4-tetrahydroquinoline.

The reaction of transition metal ions in the lower oxidation state with cumene hydroperoxide generates free radicals which can initiate the polymerisation of methacrylate monomers. Iron(II) readily decomposes cumene hydroperoxide, however, copper(I) is inactive. Anaerobic adhesive accelerators, APH and DMPT in particular, can form complexes with copper(I) which can readily decompose cumene hydroperoxide. In general, both saccharin and maleic acid reduces the rate of decomposition of cumene hydroperoxide by these accelerator-copper(I) complexes. The only evidence that saccharin or maleic acid actually catalyse the decomposition by protonating the hydroperoxide, was found in the case of APH with excess maleic acid present.

3.5. BIBLIOGRAPHY

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CHAPTER FOUR

THE IDENTIFICATION OF HYDROGEN PEROXIDE AS THE AUTOXIDATIVE PRODUCT OF N-PHENYL-2-PROPYL-3,5-DIETHYL-1,2-DIHYDROPYRIDINE

4.1. INTRODUCTION

4.1.1 Development of Air Activated Adhesive Technology

Since 1987, Loctite have been pursuing a research project with the specific aim of developing one-part rapid cure adhesive chemistry. During the course of the original project many approaches to the development of a one-part, room temperature cure chemistry were examined. The possibility of employing atmospheric oxygen to bring about a polymerisation reaction appeared particularly promising (Figure 4.1). In theory, the initiating species could be a radical, a cation or an anion. A number of autoxidation systems were identified by Loctite which were capable of producing a free radical polymerisation of acrylic monomers on exposure to air. Given the promising results obtained with these systems, it was decided to focus on the concept of "air-activated" polymerisation [1].

A wide number of compounds were evaluated for their autoxidative capability. It was felt that the presence of a reducing agent would enhance the activity of any peroxides produced by autoxidation, as it was well known in redox adhesive technology that the condensate of aniline and n-butyraldehyde was particularly efficient at decomposing peroxides. Therefore, after further experiments and close observation of control experiments, it was discovered that the material undergoing autoxidation was in fact the aniline-butyraldehyde condensate or 1,2-dihydropyridine, referred to by Loctite as DHP. It was discovered that in fact DHP underwent rapid autoxidation in the presence of an acid such as glacial acetic acid. It was identified that DHP in combination with an acid and a reactive monomer yielded a very fast curing, surface insensitive one-part air-activated adhesive [2]. This cure system based on DHP was found to be the most active of all the autoxidative systems examined by Loctite.

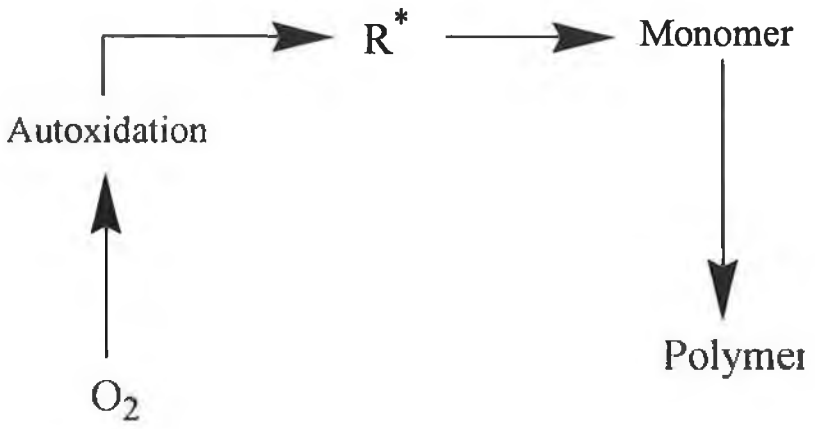


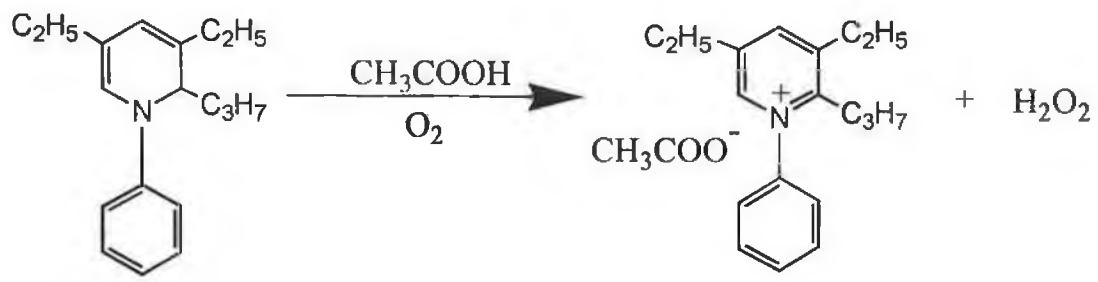
Figure 4.1 The initial concept for development of air activated adhesives was based on the identification of a material or mixture of materials which react with molecular oxygen to produce a species capable of undergoing further reaction to give an initiating species R*. On interaction with a suitable monomer polymerisation results.

The proposed reaction scheme consisted of DHP in the presence of an acid undergoing autoxidation to produce a pyridinium salt and hydrogen peroxide. It was also proposed that further DHP reacted with the generated hydrogen peroxide to produce free radicals, which were capable of initiating polymerisation (Figure 4.2). Research by Loctite also demonstrated that no polymerisation took place in the absence of the acid, and also that no reaction took place between DHP and hydrogen peroxide in the absence of the acid. However, experiments carried out by Loctite found that mixtures of DHP/acid/monomer were highly unstable even under an argon atmosphere.

As a general rule, vigorous steps are taken to avoid all forms of trace metal contamination in adhesive formulations, as it gives rise to a premature cure and severe loss in stability. But in the case of DHP chemistry, it was found by inclusion of trace amounts of transition metals such as iron, copper or cobalt at a concentration of about 50 ppm, great improvements in stability were observed. But when metal concentrations in the region of 500 ppm were added, this yielded infinitely stable, practically unreactive formulations. It is unlikely that the autoxidation reaction is free radical in nature, as experiments have shown it to be light insensitive and unaffected by radical scavengers. The pyridinium salt has been isolated and characterised by NMR; in addition, oxygen uptake experiments have indicated that half a mole of oxygen is consumed per mole of DHP. This is consistent with a reaction where one mole of oxygen consumes one mole of DHP and the reaction product consumes a further mole of DHP.

The initial interest and subsequent research into autoxidation based on DHP was based on work carried out by Cilento and da Silva Araujo [3]. Their research found that protonated 4-amino-2,6-di-iodophenol catalyses the autoxidation of 1-benzyl-1,4-dihydronicotinamide to a pyridinium cation by hydrogen anion abstraction. Starch iodide tests for hydrogen peroxide were positive, and also a radical mechanism is unlikely as the reaction is light insensitive and only slightly inhibited by 9,10-dihydroanthracene, a good radical scavenger.

(a)



(b)

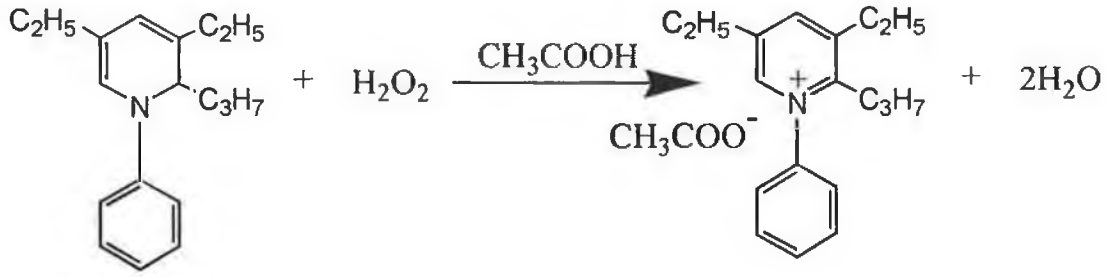


Figure 4.2 Proposed DHP-based aerobic reaction mechanism. (a) In the first reaction, the autoxidation of DHP occurs in the presence of glacial acetic acid leading to the formation of hydrogen peroxide. (b) This is then followed then by the secondary reaction of hydrogen peroxide with DHP.

In the course of the reaction the theoretical amount of oxygen, for hydrogen peroxide formation, is taken up, while the dihydronicotinamide completely disappears. The reaction scheme therefore consisted of the autoxidation of 1-benzyl-1,4-dihydronicotinamide, catalysed by protonated 4-amino-2,6-di-iodophenol, to yield a pyridinium cation and hydrogen peroxide. This reaction scheme closely mirrors that of the DHP-based autoxidation scheme and was the basis for developing the initial theory that hydrogen peroxide was generated in the autoxidation of DHP.

4.1.2 Autoxidation

Few reactions are of greater importance, or have received more attention than autoxidation, which normally refers to the reactions of substances with molecular oxygen under ambient conditions without the intervention of a flame [4]. Its action may be beneficial or deleterious depending on the conditions and circumstances under which it occurs. Light, heat, concentration of oxygen, moisture and the presence of catalysts or inhibitors affect the reaction rates between oxygen and organic compounds, often with seemingly different results [5]. Many types of molecules undergo autoxidation, the rate varying widely with the structure. The process of autoxidation has been studied extensively over the last century and many excellent reviews published reflecting the universal importance of the subject [6 ,7]. The process of film formation in applied protective coatings, for example, is essentially an oxidative process, while the ultimate failure of these protective coatings is a result of prolonged and excessive oxidation. Rancidification and other forms of oxidative deterioration of many fats and fat-containing materials are likewise due to autoxidation. Other important groups of organic materials, such as rubber, plastics and fuel oil, also undergo autoxidative deterioration unless precautions are taken to control it.

It is now generally accepted that most autoxidation reactions involve a free radical chain process and these reactions are readily catalysed by substances that yield free radicals upon decomposition, such as peroxides and hydroperoxides. Since hydroperoxides can be both catalysts and reaction products, many autoxidations are autocatalytic in nature. Autoxidation in general involves the incorporation of molecular oxygen into an unsaturated molecule, forming a hydroperoxide, which is broken down in subsequent reactions. Underlying all investigations of autoxidation is the desire to determine the nature of the products formed, and to understand the primary reactions involved, since only by controlling these reactions can the desired products be produced successfully.

4.1.3 Dihydropyridines

Dihydropyridine chemistry can trace its roots back to 1882 when Hantzsch [8] published the synthesis that now bears his name. The discovery in the 1930's that the "hydrogen transfer enzyme", NADH, was a reduced nicotinamide derivative, stimulated work on model dihydropyridines. It was not until many years later, however, that it was proved unambiguously that NADH was a 1,4-dihydronicotinamide. Model dihydropyridines have been used extensively to elucidate the mode of action of the co-enzyme. Dihydropyridine derivatives have found a wide variety of practical applications from pharmaceuticals and photographic materials to rocket fuels. A number of excellent reviews of dihydropyridine chemistry are available, in particular two by Kuthan [9 ,10]. Early dihydropyridine chemistry is dominated by the distinction between 1,2- and 1,4-isomers, a distinction which is now most conveniently made by modern physical methods. The predominant reaction of dihydropyridines is oxidation to the corresponding more stable pyridines which occurs by loss of a hydride ion from the dihydropyridine or by the transfer of two single-electrons and one proton.

Convincing evidence exists for both mechanisms, but the latter mechanism receives the broadest acceptance. Therefore, oxidation with chemical reagents such as organic peroxides, is probably the most universal procedure for aromatisation of dihydropyridines.

From the adhesives perspective, any interaction between DHP and transition metals, which may be present as contaminants in formulations or on the surface of a substrate, is significant. But also, as mentioned previously, transition metals were found to have a stabilising effect on DHP-based formulations. Treatment of dihydropyridines with metals usually results in electron transfer, leading to radical intermediates, which does not help to explain the stabilising effect of the metals on DHP-based formulations.

The oxidative and thermal instability of dihydropyridines is well known, thus most studies were conducted on N- substituted 1,4- dihydro derivatives with electron withdrawing substituents on the ring. The less stable 1,2- derivatives received much less attention, and are only stable when steric hinderance is evident. Kosower and Sorensen [11] discussed the synthesis and properties of some simple 1,4 dihydropyridines as a means to elucidate the mode of action of the co-enzyme NADH. Although one of the best known 1,2-dihydropyridines, there have been few specific reports in the literature on the chemistry of N-phenyl-2-propyl-3,5-diethyl-1,2-dihydropyridine (DHP). The compound was first prepared by Craig et al. [12] when they discussed the isolation of a weak organic base from the reaction of butyraldehyde and aniline in the presence of acetic acid. This weak base underwent oxidation in acidic conditions in the presence of an appropriate anion to yield quaternary salts. This weak base, yellow in colour, was found to have the empirical formula, $C_{18}H_{25}N$, and it was subsequently determined incorrectly to be the 1,4-dihydropyridine instead of the 1,2-derivative. This compound has found use in recent years as a reducing agent within the polymer and rubber industries where it is the active component within the crude condensation product of aniline and butyraldehyde. Both the crude condensate and a purified distillate have been patented for use within reactive adhesives [13].

4.1.4 Hydrogen Peroxide Analysis

4.1.4.1 Introduction

Hydrogen peroxide is an extremely versatile compound, which has been the subject of extensive investigation since its discovery by Louis-Jacques Thenard in 1818. The chemical has found a wide variety of uses from industrial, as an oxidising agent and a bleaching agent, to military applications such as a propellant for rockets and torpedoes. The interactions of transition metal ions with hydrogen peroxide are crucial in developing a detailed understanding of the DHP-based aerobic cure chemistry.

Over 100 years ago, Fenton [14] reported that iron(II) ions strongly promoted the oxidation of malic acid by hydrogen peroxide. Subsequent work has shown that the combination of hydrogen peroxide and an iron(II) salt, 'Fenton's reagent', is an effective oxidant of a wide variety of organic substances. In 1934, Haber and Weiss [15] proposed that the hydroxyl radical is the actual oxidant in such systems, with iron(II) catalysing the decomposition of the hydrogen peroxide.

$$H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^- + OH^{\bullet} \quad \text{(fast)}$$

$$H_2O_2 + 2Fe^{3+} \longrightarrow 2Fe^{2+} + OH^{\bullet} \quad \text{(slow)}$$

It is now well recognised that the decomposition of hydrogen peroxide and peroxides in general is catalysed by transition metals. Induced decomposition of peroxides by transition metals is fairly well understood. A number of metals, and transition metals in particular, which possess two or more valence states with a suitable oxidation-reduction potential between them, can react with peroxides to produce free radicals. The metallic ion can act as a reductant or oxidant. The rate of decomposition varies between the metallic species according to the series [16]:

$$Fe^{2+} > Fe^{3+} >> Cu^{2+} >> Co^{2+} > Ce^{2+} > Th^{4+}$$

Iron(III) and iron(II) ions are therefore the most efficient at decomposing hydrogen peroxide, with copper(II) and cobalt(II) ions trailing far behind. From the autoxidation perspective, transition metals function as pro-oxidants [6].

4.1.4.2 Titration-based methods

Traditionally the determination of hydrogen peroxide in is carried out by redox titration with permanganate, cerate, iodide/thiosulphate, or titanous solution. The determination of not only hydrogen peroxide but peroxides in general is based on the method of Lea [17] and that of Wheeler [18]. Both methods are based on the assumption that sodium iodide (or any iodine salt) and glacial acetic acid, when contacted with a peroxide, liberate iodine quantitatively, which can then be quantified by titration with standardised sodium thiosulphate. Dissolved oxygen and moisture have marked effects on the results obtained, but the interference from oxygen can be minimised by efficient purging of solutions and displacement of the head space air over the sample with an inert gas. Although iodometric titration lacks the sensitivity of some methods, other more sensitive, less time-consuming methods, such as gas chromatography and high performance liquid chromatography, are not employed to the same extent due to the sensitivity of peroxides to heat and surfaces. Potentiometric end-point detection has lowered the detection limits and improved the accuracy of this technique compared to visual detection of the end-point.

4.1.4.3 Spectrophotometric methods

A wide range of spectrophotometric and chemiluminesence-based techniques have been developed for the determination of hydrogen peroxide. Numerous direct spectrophotometric methods are available that rely on the reaction of hydrogen peroxide with a substrate in the presence of peroxidase to yield a highly coloured species. Clapp and Evans [19] have reported on a method involving the reaction of hydrogen peroxide with Leuco Patent Blue Violet (Figure 4.3) in the presence of peroxidase leading to the regeneration of the dye Patent Blue Violet, the product can be then detected spectrophotometrically at 639 nm. Clapp has also discussed the employment of an ethyl acetate extraction step to eliminate interferences in this procedure. This extraction step eliminates substances that interfere in the direct method, such as poisons for peroxidase and materials that absorb at 639 nm.

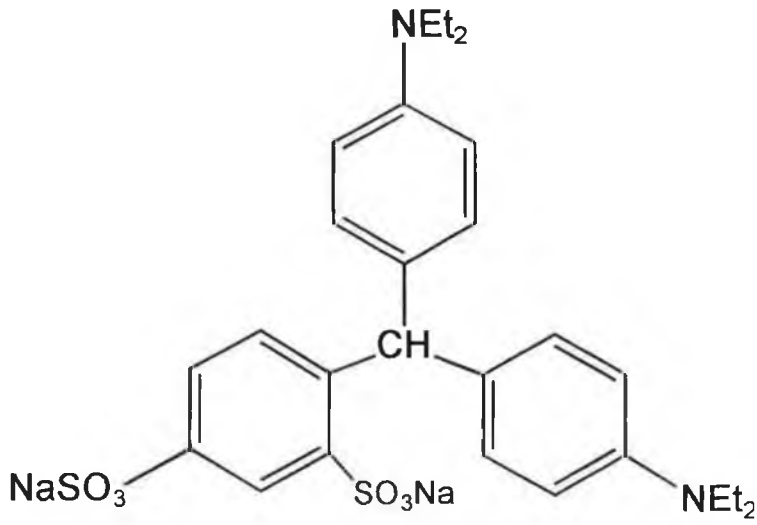


Figure 4.3 Structure of Leuco Patent Blue Violet.

The titanium sulphate method [20] is also a well known procedure for the determination of hydrogen peroxide. It consists of reacting acidic titanium sulphate with hydrogen peroxide to generate a compound with an intense yellow colour, which can be monitored spectrophotometrically. Flow injection analysis has been used by Madsen and Kromis [21] with limits of detection down to $6x10^{-6}$ M. It is based on the formation of a chromophore by the oxidative condensation of a hydrogen donor, in this case N-ethyl-(sulfopropyl)aniline sodium salt with 4-aminoantipyrene in the presence of peroxidase and hydrogen peroxide. The method has the advantage of high sample through-put, and is free from interference from manganese(II) and iron(III).

A water soluble titanium(IV) porphyrin complex has recently been found to enhance the spectrophotometric determination of hydrogen peroxide [22]. The quantity of hydrogen peroxide is determined by the decrease in absorbance of the parent complex at 432 nm as it forms a complex with hydrogen peroxide. This method of Matsubara et al.[22] has a limit of detection of $1x10^{-8}$ M and requires the presence of 1.6 M perchloric acid to promote the complexation. This method is based on the formation of a titanium(IV) complex with hydrogen peroxide and pyridylazo compounds; however, the molar extinction coefficient for hydrogen peroxide in this system was inadequate to allow determination of hydrogen peroxide at trace levels, such as in environmental samples. Therefore, the use of ligands with larger extinction coefficients compared with the pyridylazo pigments was investigated as a means of achieving greater sensitivity. Certain water soluble porphyrins have been used for highly sensitive spectrophotometric determination of various metals ions [23], as porphyrins have a strong absorption band in the 400-450 nm range. Subsequently, a highly sensitive method for hydrogen peroxide was developed using porphyrins as ligands for complex formation.

Traditionally, a lot of interest in the determination of hydrogen peroxide has been associated with clinical applications. The function of many oxidase enzymes is to oxidise their specific substrates in the presence of dissolved oxygen, which results in the production of a stoichiometric amount of hydrogen peroxide. The hydrogen

peroxide can then be measured, giving rise to an indirect method of analysis. Also the quantitative determination of hydrogen peroxide in atmospheric samples at very low levels is considered very important. It is believed that hydrogen peroxide may play a role in the oxidation of sulphur dioxide to sulphate in rain water and elevated levels of hydrogen peroxide have been implicated in deforestation.

Relative to spectrophotometric methods used in most clinical applications, greater sensitivities are generally required to determine hydrogen peroxide in environmental samples. Sensitive luminesence based methods abound for the determination of hydrogen peroxide. For instance, Ibusuki [24] discussed the influence of trace metal ion concentrations on the determination of hydrogen peroxide in rain water using a chemiluminesence technique. The technique is based on chemiluminesence oxidation of luminol (3-aminophthalhydrazide) by hydrogen peroxide which is catalysed by copper(II). In the presence of certain metals, peroxide reacts with luminol (Figure 4.4) in basic media to form an excited aminophthalate anion, which returns to the ground state by emission of a photon.

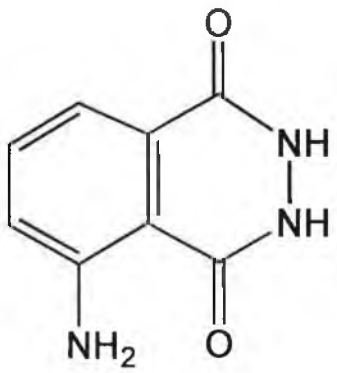


Figure 4.4 Structure of luminol

Generally, metal ions possessing oxidation states requiring a one-electron transfer are capable of promoting the chemiluminesence reaction between peroxide and luminol in water [25]. These include iron(III)-containing compounds, such as haem and $[Fe(CN)_6]^{3-}$; copper(II), cobalt(II) and $[SbCl_6]^-$ have also been cited as reagents capable of producing chemiluminesence in the presence of luminol and hydrogen peroxide. However, the authors found that the chemiluminesence intensity of this system is greatly diminished by the presence of manganese(II) and iron(III) at their environmental concentrations in rainwater. It was suggested that chromium could be employed as a catalyst, thereby allowing the use of EDTA to remove the interfering ions, without interfering with the analysis procedure.

The luminol reaction has been the most widely used chemiluminesence technique for the determination of hydrogen peroxide since its discovery by Albrecht in 1928. However it does have some drawbacks, most notably that it requires a very high pH (>10.0). This causes severe problems when dealing with clinical samples, as most enzymes will be denatured a such a high pH. Because of the problems with hydrogen peroxide detection based on luminol, the use of peroxyoxalate chemiluminescence has been investigated by Scott and Seitz [26]. This technique can be used for detection at neutral pH, but requires the employment of a t-butanol-water mixture, as the oxalates are insoluble in water. Triethylamine was employed as an 'activator' of the peroxyoxalate (2,4,5-trichloro-6-carbopentoxyphenyl oxalate) chemiluminescence and a detection limit of $2x10^{-8}$ M was achieved. This technique can also be employed in a similar procedure based on immobilising the peroxyoxalate (trichlorophenyloxalate) and 3-aminofluoranthene on glass beads in a flow cell, mounted close to a photomultiplier tube [27]. This system was designed for eventual use as a field monitor, with both manual and automated injections possible. Using methanol as a solvent, a detection limit of $1.5x10^{-8}$ M was obtained. The authors claimed a sample throughput of 100 h^{-1} is possible in the flow injection mode and 40 h^{-1} for manual injection.

Bostick and Hercules [25] used a prior absorption step to eliminate uric acid, a major cause of interference, from urine samples, thereby allowing the determination of urine glucose levels between $1x10^{-4}$ M and $1x10^{-8}$ M. The system was based on the enzymatic conversion of glucose to hydrogen peroxide, and subsequent reaction of the peroxide with a mixed luminol-ferricyanide reagent to produce chemiluminesence. The reaction was carried out at pH 10.5 and permitted the determination of hydrogen peroxide in the $1x10^{-5}$ M to $1x10^{-8}$ M range.

4.1.4.4 Electrochemical techniques

Titrimetry, spectrophotometry and chemiluminesence are the general methods used in industry for the determination of hydrogen peroxide, but electrochemical methods offer alternative approaches. Electrochemical techniques for the analysis of hydrogen peroxide can be carried out at bare electrode surfaces and modified electrode surfaces as well as using polarographic-based techniques. Methods involving the oxidation or reduction of hydrogen peroxide at bare electrode surfaces, have not been popular in recent times, however, because of the development of modified electrodes and the high overpotentials required to drive the reactions at the bare electrodes. For example, the direct oxidation of hydrogen peroxide at a glassy carbon electrode requires a potential of +1200 mV or greater vs saturated calomel electrode (SCE), and at such high potentials other electroactive species present in the sample will also be oxidised [28]. Hydrogen peroxide is oxidised electrochemically to oxygen and hydrogen ions according to:

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

Monitoring of the oxidation process is more often than not the favoured approach as against monitoring the reduction of hydrogen peroxide. Reduction is subject to interference from dissolved oxygen which itself can be reduced to hydrogen peroxide:

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$$

Thereby giving rise to inaccurate results. Also, development of an analytical method for the determination of hydrogen peroxide based on reduction is not advisable for samples that contain reducible metals.

Flow injection analysis has been used for detection of hydrogen peroxide based on oxidation at a glassy carbon electrode [29]. The electrode was held at +1500 mV with respect to a SCE with platinum serving as the counter electrode. While a detection limit of $1x10^{-6}$ M was obtained using 1 M sulphuric acid as the carrier electrolyte, a noisy signal was observed; however, at higher concentrations due to oxygen formation in solution, which adhered to the electrode surface. This was overcome by higher dilution factors.

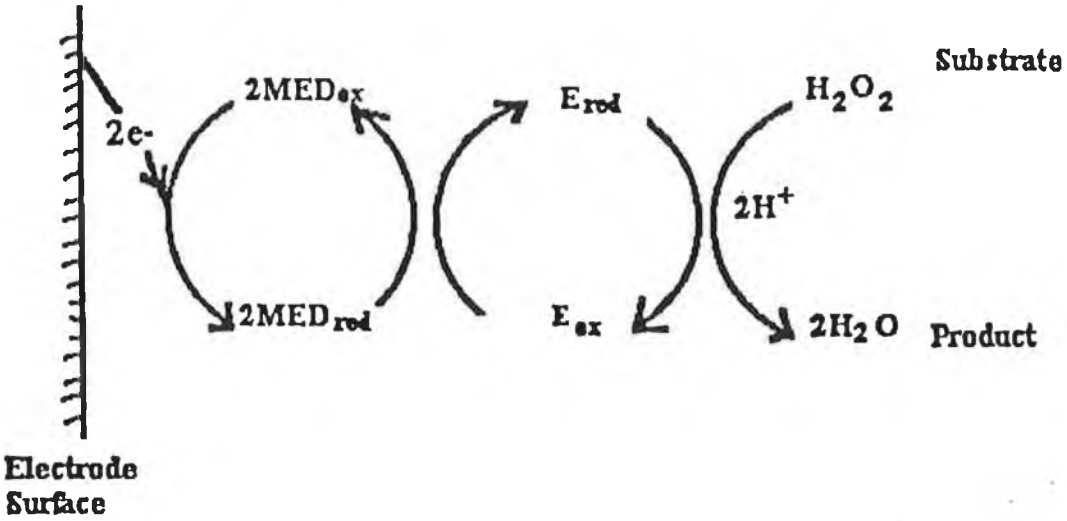
Hydrogen peroxide has also been determined using a rotating platinum disk electrode polarised at +400 mV vs SCE in 1.0 M potassium nitrate and phosphate buffer at pH 7.5 as electrolyte [30]. The neutral pH was selected as it is in the optimal range for the reaction of catalase enzyme which was used for the preparation of an analytical blank by 'in situ' selective decomposition of the hydrogen peroxide.

In recent years, modified electrodes have become popular for the determination of hydrogen peroxide. They have the distinct advantage over using bare electrode surface in that the lower overpotential required provides better selectivity, thereby avoiding interference from other electroactive materials present in samples. Many modified electrodes involve the use of biologically-derived recognition systems, such as enzymes and antibodies.

These are responsible for the very selective and sensitive recognition of the desired analyte. When these biological materials are immobilised on an electrode surface, the recognition of the analyte can be translated into an electrical signal.

In terms of the detection of hydrogen peroxide, use is invariably made of horseradish peroxidase, an enzyme that reacts specifically with peroxides. Immobilised enzymes are now widely used in analytical chemistry in conjunction with a variety of support materials such as acrylamide gel, nylon netting and Nafion polymer. For instance, Cosgrove et al. [28] described a method for the detection of hydrogen peroxide by immobilising horseradish peroxidase (HRP) on a nylon net using glutaraldehyde. In order to minimise the problem of interference from other electroactive substances, a soluble mediator, in this case hexacyanoferrate(II), was employed. The oxidation of the mediator by hydrogen peroxide is catalysed by HRP and the oxidised form of the mediator can be reduced at a lower potential (-100 mV vs Ag/AgCl) than would normally be required for direct hydrogen peroxide detection (Figure 4.5). This paper also described a second approach using catalase enzyme immobilised on nylon netting placed over the PTFE membrane of an oxygen electrode, which was used with a view to analysis in waste streams under alkaline conditions. Catalase converts hydrogen peroxide to oxygen, which can then be detected by the underlying oxygen electrode.

Tatsuma and Okawa [31] detailed the use of a modified electrode with HRP attached to a tin(IV) oxide glass plate electrode and when this was used in conjunction with a dissolved electron mediator, hydrogen peroxide could be detected down to $1x10^{-8}$ M levels. As an extension to the method, glucose oxidase could also be chemically attached to the HRP modified electrode using glutaraldehyde to yield a glucose electrode.



$$\text{A. } H_2O_2 + 2[Fe(CN)_6]^{4-} + 2H^+ \xrightarrow{\text{Peroxidase}} 2H_2O + 2[Fe(CN)_6]^{3-}$$

$$\text{B. } [Fe(CN)_6]^{3-} + e^- \longrightarrow [Fe(CN)_6]^{4-}$$

$$E_{cell} = -100 \text{ mV vs Ag/AgCl}$$

Figure 4.5 Schematic representation of the role of a mediator. E (ox/red) are the redox forms of the peroxidase enzyme and MED (ox/red) are the redox forms of the mediator, such as hexacyanoferrate(II).

In addition to the use of solid or modified electrodes, there are also polarographic-based techniques for the determination of hydrogen peroxide. Polarography refers to direct current voltammetry when a dropping mercury electrode is used as the working electrode, first developed by Heyrovsky [32] in 1922, for which he subsequently received a Nobel Prize in 1959. The dropping mercury electrode has many advantages over its solid counterparts; most notably, the constant renewal of the surface eliminates the problems caused by poisoning of the electrode surface which in the case of glassy carbon electrode necessitates the polishing of the surface between analyses. Secondly, the mercury electrode is useful over the range +0.30 V to -2.80 V in aqueous solutions. Perhaps the most unique characteristic of hydrogen peroxide is its position as the oxidation-reduction intermediate between oxygen and water. The reduction of oxygen at the dropping mercury electrode results in a polarogram with two steps, which has been known since the very early days of polarography [33]. The first wave of oxygen is due to its reduction to hydrogen peroxide,

In acidic solution, the reduction occurs by:

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$$

while in alkaline solution:

$$O_2 + H_2O + 2e^- \rightarrow HO_2^- + OH^-$$

The second wave, whose height is equal to that of the first wave, is a two electron transfer which results in the reduction of oxygen to water :

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$$

Although the reduction wave of hydrogen peroxide is drawn out, the diffusion current is well defined and can be used for quantitative analysis.

In hydrogen peroxide solutions above 1 mM, the oxygen wave is small in comparison to the hydrogen peroxide wave and hydrogen peroxide may be measured in fully aerated solutions. Giguere and Jaillet [34] have reported that at hydrogen peroxide concentrations above about 15 mM, a film of mercury peroxide forms on the cathode, and that at about 40 mM, catalytic decomposition of the hydrogen peroxide occurs. Therefore polarographic determination of hydrogen peroxide is limited to solutions having concentrations of less than 15 mM. Organic peroxides produce waves at more positive potentials (by about 0.20 V vs SCE) than does hydrogen peroxide [35].

Although polarography is well suited for the determination of hydrogen peroxide, the method does not appear to be widely used, probably because of the wide variety of titrimetric and spectrophotometric methods available.

Sandler and Chung [36] carried out a study of the determination of hydrogen peroxide, using polarography, in the presence of formaldehyde and acetaldehyde, products of the low temperature oxidation of hydrocarbons. It was found that buffered acidic media were most suitable for the determination of hydrogen peroxide, as it decomposed rather rapidly in neutral or alkaline media. The electrolyte employed consisted of 0.05 M lithium chloride and 0.05 M lithium hydroxide adjusted to pH 4.5; this yielded a half-wave reduction potential for hydrogen peroxide of -0.93 V. Preliminary experiments with unbuffered electrolytes gave inconsistent results and indicated the importance of a constant pH in this analysis. A lithium chloride electrolyte was also used by Kuta and Quackenbush [37] for the determination of a wide variety of organic peroxides. The electrolyte consisted of 0.30 M lithium chloride in a benzene/methanol mixture, and the hydrogen peroxide reduction wave was observed at -1.16 V compared to the reduction wave for benzoyl peroxide at 0.00 V. This highlights what was mentioned previously, that organic peroxides are reduced at considerably lower potentials than is hydrogen peroxide itself.

A polarographic method for the separation of hydrogen peroxide from organic hydroperoxides has been reported by MacNevin and Urone [38]. The method is based on elimination of the hydrogen peroxide polarographic wave by complexing with titanium(IV) ion and precipitating the complex in alkaline solution. The organic hydroperoxides in the study were unaffected by the complexing ion and its precipitation. Upon acidification the hydrogen peroxide is restored to the solution and a polarographic wave identical to the original is obtained. This separation is useful analytically, especially when the reduction potentials of hydrogen peroxide and the organic hydroperoxide differ only slightly.

4.1.4.5 Aims of the research project

The aims of the research project were two-fold. The first was to confirm unambiguously using various analytical techniques that it was in fact hydrogen peroxide which is generated in the autoxidation of DHP and not a hydroperoxide based on the pyridine ring. This is vital information in order to commercialise this technology, as it is the peroxide which is the vital component that leads to eventual polymerisation of the monomer. Its identification should lead to greater understanding of the DHP chemistry, and avoid complications of factors such as in the manufacture, packaging and prediction of shelf life of the adhesive formulations based on this chemistry. It has been proposed, based on work by Cilento and da Siva Araujo [3], that the autoxidation of DHP proceeds by hydrogen anion abstraction by molecular oxygen, with the formation of hydrogen peroxide and a pyridinium salt.

The second aim was to determine the exact role of transition metals in the cure chemistry. DHP is a strong reducing agent capable of reducing transition metals, and the inclusion of transition metals, particularly iron, and copper to a lesser extent, markedly improves the ambient stability of formulations. It would be anticipated that the higher oxidation states of these metals would be reduced to the

corresponding lower states by the action of the DHP. Hence the primary influence of the metal would arise from its lower oxidation state.

4.2. REACTIONS WITH TRANSITION METALS

4.2.1 Experimental

4.2.1.1 Reagents

All reagents were of Analar grade, unless otherwise stated. The DHP was commercial grade supplied by Loctite (Irl.) Ltd., which was further purified by fractional distillation. The electrolyte used consisted of 50% ethanol, 25% glacial acetic acid and 25% aqueous citrate-phosphate buffer, pH 7.0. A hydrogen peroxide solution (0.10 M) was prepared by dissolving 1.13 g of 30% (w/w) hydrogen peroxide in 100 cm^3 ethanol. For the polarographic analysis, a 0.10 M solution of DHP was prepared by dissolving 1.2 g of DHP in 50 cm^3 of ethanol. 0.10 M solutions of copper(I) and iron(II) were prepared from the chloride and acetate salts respectively. 0.05 M copper(II) solutions were prepared by dissolving 9.98 g of copper(II) acetate in 1 dm^3 of 50:50 ethanol/acetonitrile. 0.01 M solutions of iron(III) were prepared by dissolving 3.53 g of iron(III) acetylacetonate in 1 dm^3 of 50:50 ethanol/acetonitrile. Vanadium oxygen scrubbing solutions were made up by boiling 2.00 g of ammonium metavanadate in 25 cm^3 concentrated HCl, which was then made up to 200 cm^3 with distilled water. This brown coloured solution turned blue on addition of several grams of amalgamated zinc.

4.2.1.2 Apparatus

All polarographic experiments were carried out using an Princeton Applied Research (Princeton, N.J.) EG&G Model 303 dropping mercury electrode in conjunction with an EG&G model 384 polarographic analyser. The dropping mercury electrode was used with a small drop size, scan rate of 8 mV/second, drop time 0.5 second and a scan increment of 4 mV. The potentiometric titrations were carried out using a Metrohm Dosimatt model 655 auto-titrator linked to a Metrohm Model 670

titroprocessor. A double junction Ag/AgCl and Pt electrode were employed as reference and working electrodes respectively.

4.2.2 PROCEDURES

4.2.2.1 Reaction of Hydrogen Peroxide with Transition Metals

To 10 cm^3 of electrolyte 0.20 cm^3 of 0.10 M hydrogen peroxide was added. After analysing the solution using differential pulse polarography, 0.20 cm^3 of copper(I) was added and the concentration of hydrogen peroxide was monitored polarographically over the following 60 min. The same identical procedure was followed for the reaction of iron(II) with hydrogen peroxide. Also, as a means to confirm that in the reaction with the hydrogen peroxide that the iron(III) ions were being reduced to the iron(II) state, the reaction was monitored from the point of view of the metal in pyrophosphate buffer. For the reactions between DHP and the transition metals, 0.50 cm^3 of a 1000 ppm solution of the metal species were added to 0.60 cm^3 of 0.10 M DHP in 10 cm^3 of electrolyte and the concentration of DHP was monitored using differential pulse polarography. The samples were analysed in an oxygen free atmosphere by purging the electrolyte with argon prior to analysis and maintaining a "blanket" of argon over the samples during analysis.

4.2.2.2 Potentiometric Titrations

Titration solutions consisted of 30 cm^3 acetonitrile, 10 cm^3 glacial acetic acid and 100 µl DHP, while titration blanks had no DHP present. In the case of the iron(III) titrations, 20µl of DHP was used to adjust for only using 0.01 M iron(III) solution compared to 0.05 M copper(II) solution. Both titrant and titration solutions were purged with oxygen-free argon for five minutes prior to the start of the titration, and

an argon blanket was maintained over the solutions during the titration procedure. All titrations were carried out in triplicate to ensure reproducibility of the procedure.

4.2.3 RESULTS AND DISCUSSION

Polarography, although not as widely used as other instrumental techniques, can be used for the determination of hydrogen peroxide. On the assumption that hydrogen peroxide was the product resulting from the autoxidation of DHP, polarography was the technique chosen to assess the effect of copper(I) and iron(II) ions on the decomposition rate of hydrogen peroxide. Also, polarography was employed to determine if DHP was capable of reducing these transition metals from higher oxidation states to their lower states. It was hoped that if this 'dual role' of the transition metals could be proven, it might lead to a greater understanding of the role of the transition metals in the complex cure chemistry.

Potentiometry is a steady state electrochemical technique that entails measuring the potential difference at zero current. In a potentiometric type sensor, a membrane or sensing surface acts as a half-cell, generating a potential proportional to the log of the analyte concentration. This potential is measured relative to an inert reference electrode such as a silver-silver chloride, which is also in contact with the sample. Potentiometric measurements are made under conditions of essentially zero current flow, so as not to disturb the equilibrium at the sample membrane interface.

Potentiometric titrations were employed as a means of also confirming the ability of DHP to reduce transition metals, in addition to the polarographic results. It would also yield information on the stoichiometry of the reaction between the metal ions and DHP. In the presence of a weak acidic species such as glacial acetic acid, DHP reacts rapidly with oxygen; therefore vigorous purging of both titrant and titration mixture was necessary to eliminate this side reaction and concentrate solely on the reaction between the metals and DHP.

4.2.3.1 Polarography

Firstly, the reaction between iron(II) and hydrogen peroxide confirmed that iron(II) rapidly decomposes hydrogen peroxide (Figure 4.6), as all the hydrogen peroxide had been decomposed on initial analysis after addition of the iron(II) ions. It was also confirmed by monitoring the change in concentration of the two iron species in pyrophosphate medium, that the iron(II) ions on decomposing the hydrogen peroxide were oxidised to the iron(III) state, which can be monitored by means of the peak obtained at -0.91 V vs Ag/AgCl (Figure 4.7). In the case of copper(I) ions, however, no decrease in hydrogen peroxide response was observed even after 60 min. Therefore, it was concluded that this reaction does not proceed to any significant extent. In the experiments carried out involving DHP and iron(III) ions/copper(II) ions, in both cases a decrease of 30% in the DHP concentration was observed after addition of the metal to the DHP solution (Figure 4.8 and 4.9). In molar terms, this corresponds to a 2:1 reaction between the transition metal ions and DHP.

4.2.3.2 Potentiometric Titration

In the case of the iron(III) titrations, no end point was detectable even under an argon atmosphere. In the case of titrations of DHP with copper(II), however, the results shown in Table 4.1 for the copper(II) titrations clearly illustrate, firstly, the extremely high rate with at which DHP reacts with oxygen, and the necessity for carrying out such titrations under an argon atmosphere. More importantly, these results would also indicate that two moles of copper(II) react with one mole of DHP.

SAMPLE	END-POINT (cm^3)
Copper(II) under argon	14.81
Copper(II) in air	7.30
Copper(II) in air 24hrs	0.27

Table 4.1 Results of titrations of DHP with 0.05 M copper(II) solutions under different conditions. End point values is average of three titration values.

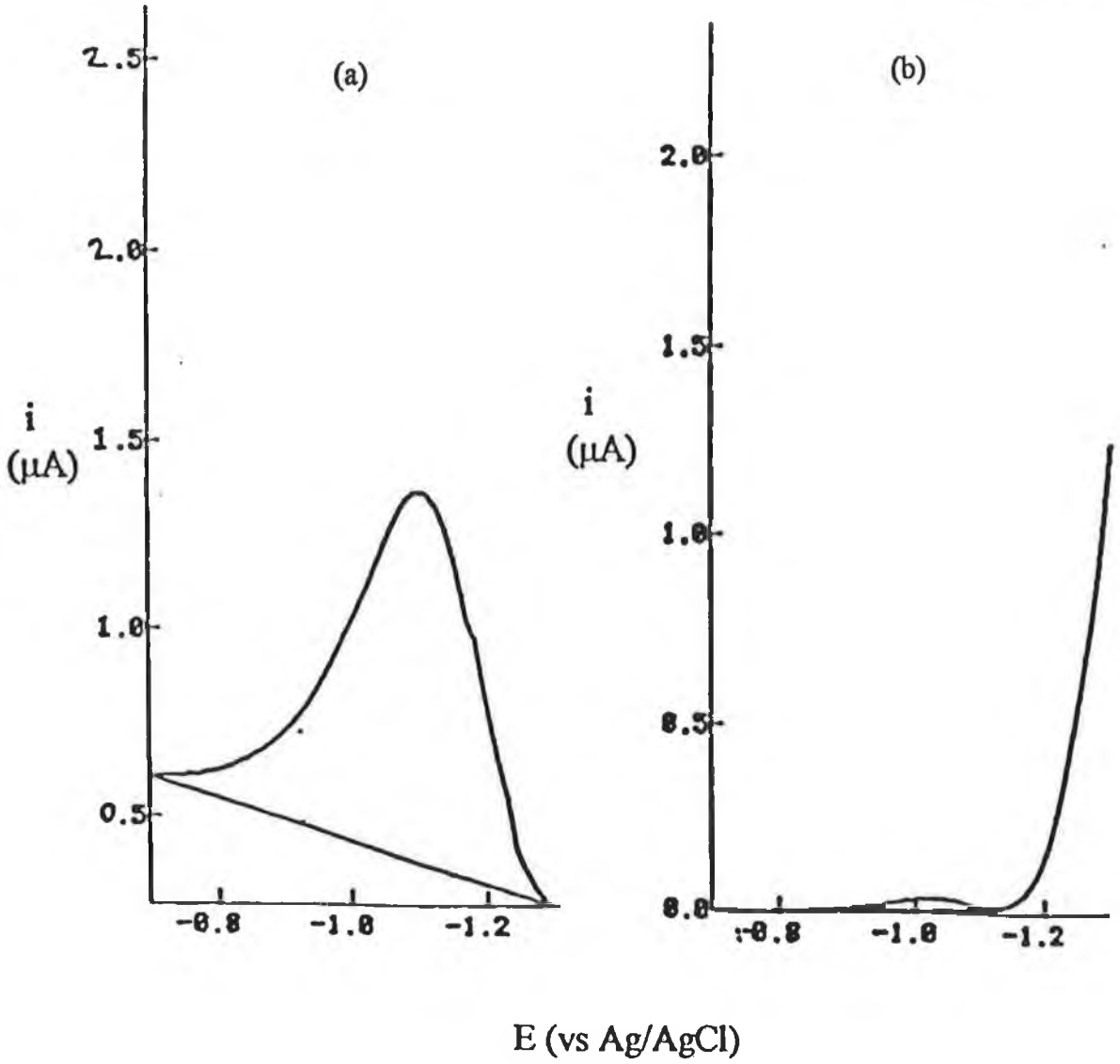


Figure 4.6. Differential pulse polarogram showing the rapid decomposition of hydrogen peroxide by iron(II) ions. (a) 0.20 cm^3 0.01 M H_2O_2 (b) on addition of 0.20 cm^3 0.01 M iron(II). Solution scanned after mixing with 30 seconds of argon purging.

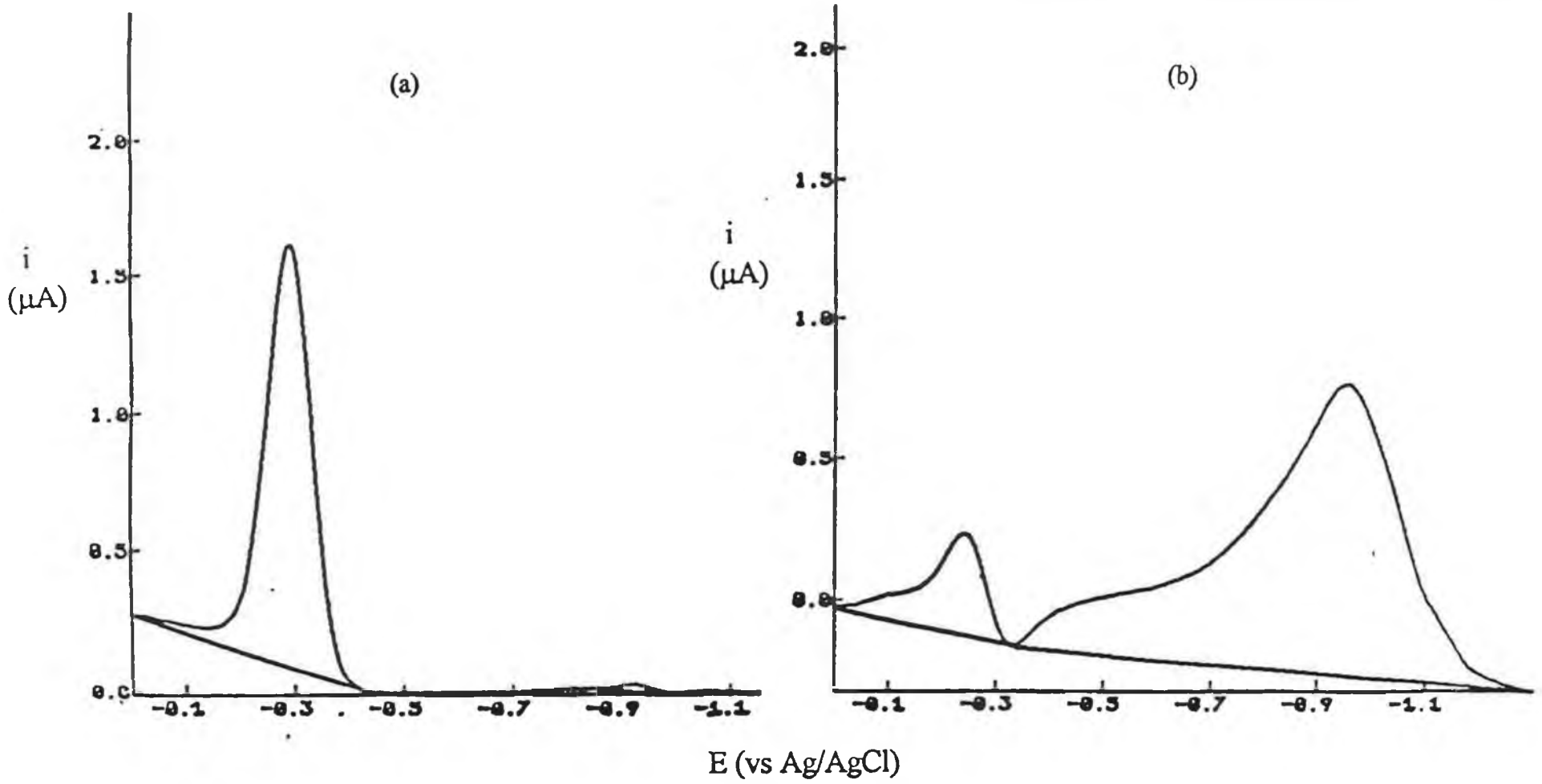


Figure 4.7 Differential pulse polarogram showing the oxidation of iron(II) to iron(III) by hydrogen peroxide in sodium pyrophosphate buffer, pH 8.0. (a) 0.10 cm^3 0.10 M iron(II). (b) on addition of 0.10 cm^3 0.10 M H_2O_2. Solution analysed after mixing and 30 seconds of argon purging.

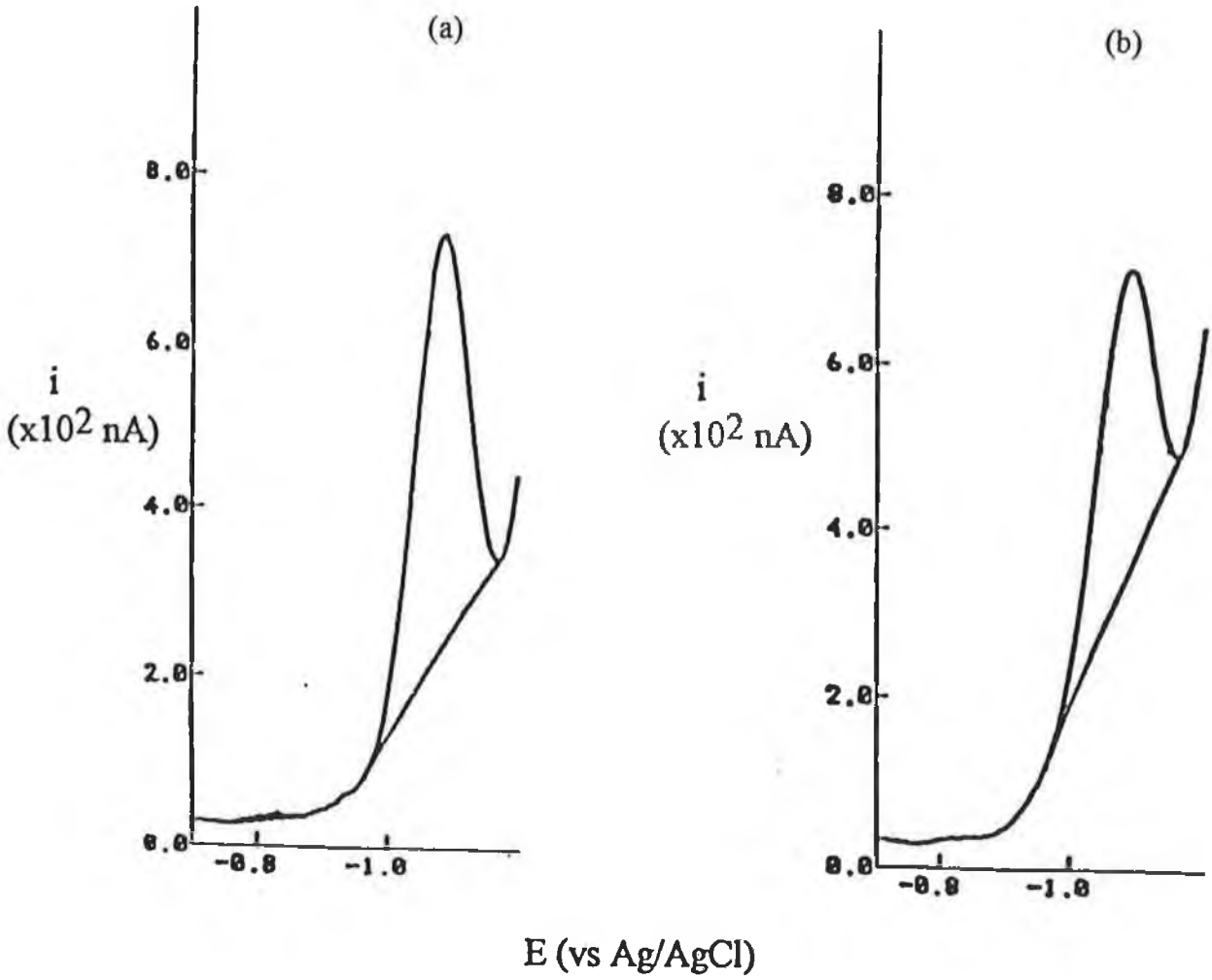


Figure 4.8 Reduction in DHP concentration following addition of Iron(III). (a) 0.60 cm^3 0.10 M DHP in glacial acetic acid/ethanol/citrate-phosphate buffer. (b) On addition of 0.50 cm^3 1000ppm iron(III); scan at t=0 min.

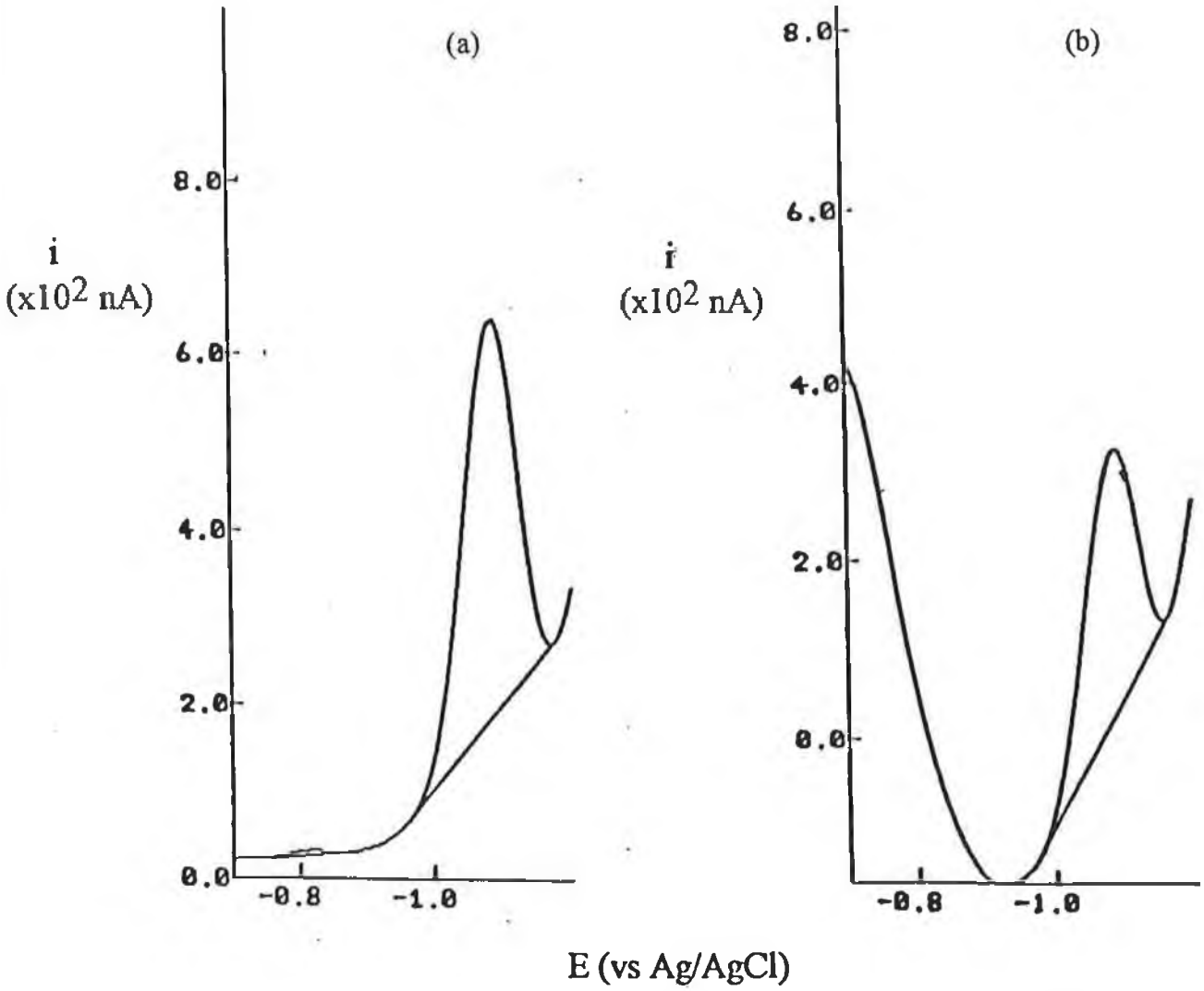


Figure 4.9 Reduction in DHP concentration following addition of copper(II). (a) 0.60 cm^3 0.10 M DHP in glacial acetic acid/ethanol/citrate-phosphate buffer. (b) On addition of 0.50 cm^3 1000 ppm copper(II), scan at t=0 mins.

4.2.3.3 Discussion

Polarography was selected as the analytical technique of choice for the monitoring of the reactions between hydrogen peroxide and certain metals primarily because of its ability to rapidly determine hydrogen peroxide concentrations with good sensitivity in the presence of a complex matrix and also, very importantly, the ease in setting up the instrumentation required. Polarography also had the ability to speciate between the different valence states of the metals of interest.

The results obtained using polarography to monitor the reactions between copper(I) ions and iron(II) ions with hydrogen peroxide agreed with previous observations made in Loctite's laboratories. The results generated for the iron(II) ions indicate that any hydrogen peroxide generated via autoxidation would be decomposed rapidly, generating free radicals capable of initiating polymerisation. The polarographic procedure for simultaneous determination of iron(II) and iron(III) was based on the work of Parry and Anderson [39]. This pyrophosphate media gave rise to two well resolved peaks with no significant reduction of iron(II) to iron(III) during the time scale of the polarographic experiments.

Experiments were carried out with both iron(II) ions and copper(I) ions, as it was believed that in the DHP-based cure chemistry, under the influence of the reducing ability of DHP, that the primary influence of the metals would be due to their lower oxidation states. Therefore the subsequent set of experiments were designed to confirm the ability of DHP to rapidly reduce certain transition metals to their lower oxidation states. In both cases, using iron(III) ions and copper(II) ions, on analysis of the reaction mixture just after addition of the metal, a decrease in 30% of the DHP concentration was observed. This would indicate a reaction ratio of 2:1 between the metal ions and DHP. It should be noted that the electrolyte used for this procedure contained glacial acetic acid, so in theory it is possible that the decrease in DHP concentration is due to its reaction with oxygen and not its reaction with the metal

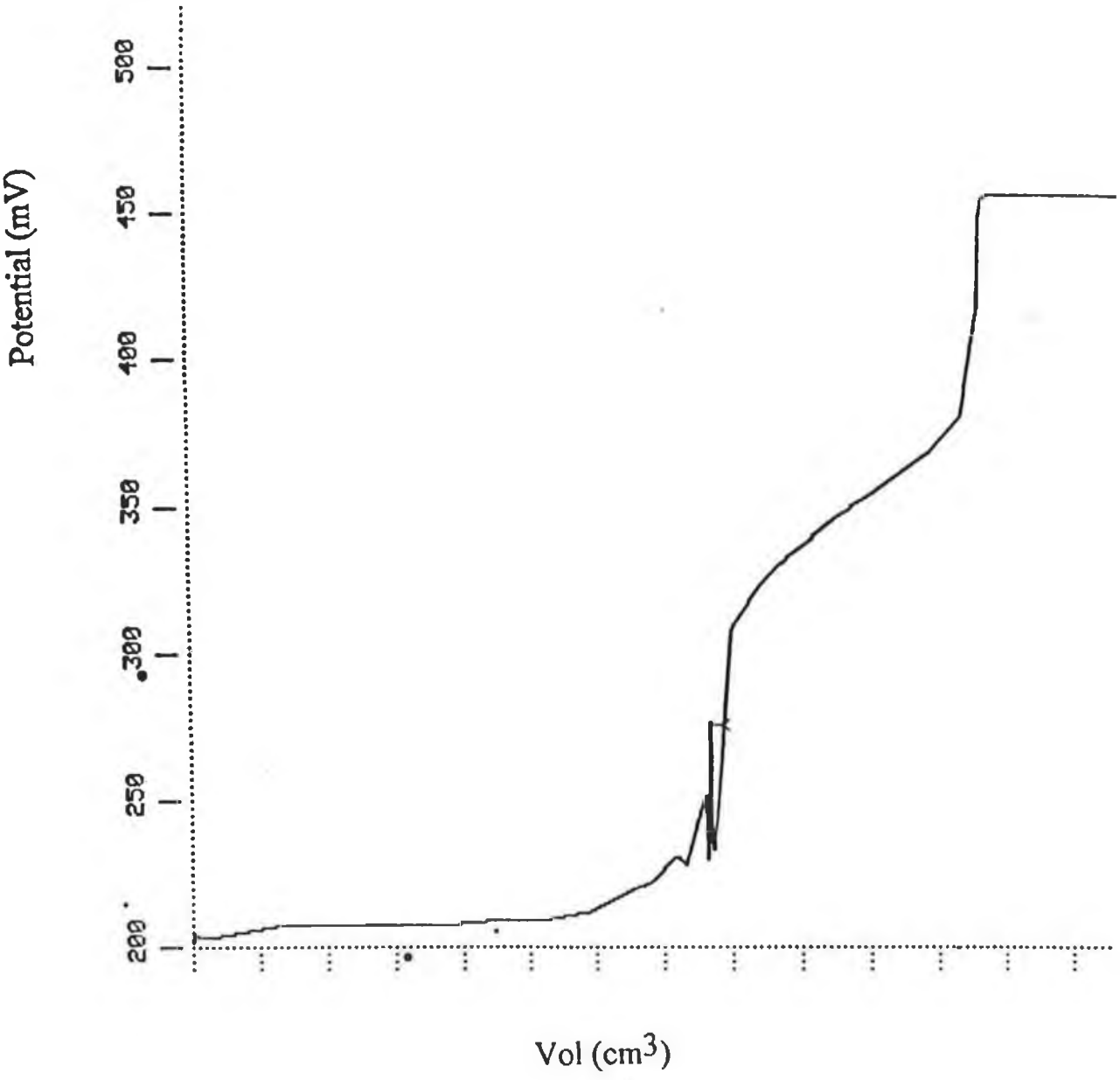


Figure 4.10 Potentiometric trace of titration of DHP with copper(II) under an argon atmosphere.

species. Although the electrolyte is not ideal, as it contained glacial acetic acid, it functioned effectively, most importantly in its ability to dissolve DHP. Great care was taken throughout to eliminate any interference from oxygen. In blank runs it was found that the concentration of DHP in this electrolyte did not decrease significantly over a period of 60 min and this was considered evidence in itself that oxygen did not interfere to any significant extent in this analysis procedure.

The potentiometric titrations were employed as an alternative analytical method to determine the ability of DHP to reduce transition metals. The copper(II) ion titrations yielded results that also indicated a stoichiometry of 2:1 between the metal and DHP. The titrations carried out in air also demonstrated the speed with which DHP undergoes autoxidation in the presence of glacial acetic acid. No matter how efficient the purging, there will always be some oxygen present which will lead to the generation of a peroxide product. The presence of some peroxide is the only plausible explanation for not detecting an end-point in the iron(III) titrations. Any peroxide present would react with the iron(II) ions produced by reduction of iron(III) ions by DHP, and these iron(II) ions would thus be oxidised by the peroxide and give no end-point detection. In the case of the copper-based titrations, however, this oxidation-reduction cycle cannot take place as hydrogen peroxide cannot oxidise copper(I), as demonstrated by the previous polarography-based experiments.

Subsequent research carried out by Loctite (Irl.) Ltd based on these results has confirmed that one mole DHP reacts with two moles of metal ions (Figure 4.11) and the products of the reaction have been isolated and identified [1]. The reactions were carried out in methanol under argon and the products identified GC-MS. DHP in the presence of glacial acetic acid was reacted with iron(III) acetylacetonate, and it was found that the hydrogen anion abstracted from DHP, reduced two metal ions with the formation of pyridinium acetate and iron(II) acetylacetonate.

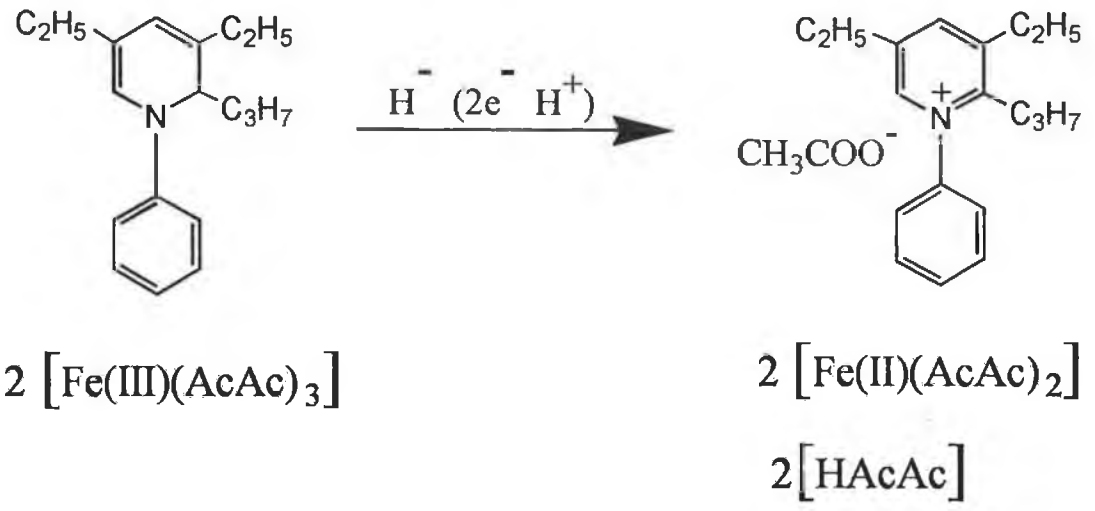


Figure 4.11 Stoichiometry of the reaction between DHP and iron(III) acetylacetonate, illustrating the 2:1 reaction ratio between the metal ions and DHP, as confirmed by GC-MS studies.

4.3. THE IDENTIFICATION OF HYDROGEN PEROXIDE AS THE AUTOXIDATION PRODUCT OF DHP

4.3.1 INTRODUCTION

It was the purpose of this work to confirm, using a variety of analytical techniques, that hydrogen peroxide is generated in the autoxidation of N-phenyl-2-propyl-3,5-diethyl-1,2-dihydropyridine (DHP). It has been proposed, based on work by Cilento and da Silva Araujo [3], that the autoxidation of DHP proceeds by hydrogen anion abstraction by molecular oxygen with the formation of hydrogen peroxide and a pyridinium salt. In isolation, the DHP reacts only slowly with air, and glacial acetic acid or some other weakly acidic species is required to bring about autoxidation.

4.3.2 EXPERIMENTAL

4.3.2.1 Reagents

All reagents were of Analar grade unless otherwise stated. The N-phenyl-2-propyl-3,5-diethyl-1,2-dihydropyridine used in all experiments was commercial grade supplied by Loctite (Irl.) Ltd., which was purified by fractional distillation. A saturated solution of sodium iodide was prepared by dissolving 160 g of sodium iodide in 100 cm^3 water. The sodium thiosulphate solution ($1x10^{-2}$ M) was prepared by dissolving 1.58 g of sodium thiosulphate in 1 dm^3. All reagent solutions were prepared in deionised water which was obtained by passing distilled water through a Milli-Q (Millipore, Milford, MA) water purification system.

The supporting electrolyte employed for polarographic studies consisted of 4.00 g of a solution of saturated lithium chloride in ethanol made up in 100 cm^3 acetonitrile. The saturated lithium chloride solution was obtained from Metrohm. A standard hydrogen peroxide solution (0.10 M) was prepared by diluting 1.13 g of 30% (w/w) hydrogen peroxide to 100 cm^3 with water.

The titanium porphyrin complex was synthesised according to the method of Inamo et al. [40] and was specially supplied by Tokyo Kasei Industries. The titanium porphyrin reagent ($5x10^{-5}$ M) was prepared by dissolving 34 mg of the complex in 1 dm^3 of 0.05 M hydrochloric acid. A perchloric acid solution (4.80 M) was prepared by diluting 68.60 cm^3 of 70% (w/v) perchloric acid solution to 100 cm^3 in water.

The tin(IV) oxide glass plates, 1/2 inch square and 0.38 inch thickness, were obtained from Nippon Sheet Glass (Japan). The tin(IV) oxide layer was 600-700 nm in thickness. The horseradish peroxidase enzyme (EC 1.11.1.7, type IV) was supplied by Sigma Chemical company. The electron mediator ferrocenemonocarboxylic acid, (3-aminopropyl)triethoxysilane and glutaraldehyde (25% w/v solution) were all obtained from the Aldrich Chemical company. Citrate-phosphate buffer (0.10 M, pH 5.9) was prepared by dissolving 21.09 g of citric acid (0.10 M) in 1 dm^3 of water and 17.80 g of disodium hydrogen phosphate dihydrate (0.10 M) in 1 dm^3 of water. The disodium hydrogen phosphate dihydrate was then titrated against the citric acid to pH 5.9.

4.3.2.2 Apparatus

The iodine titration experiments were carried out using a Metrohm Dosimatt 665 Auto-titrator linked to a Metrohm 686 titroprocessor. A Pt and Ag/AgCl double junction electrode were employed as indicator and reference electrodes respectively.

All polarographic determinations were carried out using a Princeton Applied Research (Princeton, N.J.) EG&G PARC Model 303 SMDE linked to an EG&G Model 384 Polarographic analyser and a Houston Omniscribe chart recorder. Standard electrochemical techniques were used throughout. Ag/AgCl in saturated lithium chloride in ethanol was employed as the reference electrode, with Pt and dropping mercury electrodes employed as counter and working electrodes

respectively. The supporting electrolyte consisted of saturated lithium chloride (4% w/v) in acetonitrile.

The spectrophotometric measurements were carried out using a Simadzu Model 160A spectrophotometer using quartz cells of 1 cm pathlength.

The biosensor signal was measured using an EG&G PARC Model 264A polarographic analyser with Pt and Ag/AgCl employed as counter and reference electrodes respectively. The applied potential was +150 mV vs Ag/AgCl. Electrical contact with the tin(IV) oxide layer on the glass was achieved by attaching wire to the surface using silver doped conducting epoxy resin which was then coated in epoxy resin to maintain electrical insulation in solution.

4.3.2.3 Procedures

4.3.2.3.1 Extraction procedure

The procedure consisted of dissolving 1.00 g DHP in 36 cm^3 chloroform and 4 cm^3 glacial acetic acid. After exposure of this solution to air for a fixed period of time an aqueous extraction into deionised water was carried out by mixing 9 cm^3 of the DHP containing solution with 1 cm^3 deionised water. It was expected that hydrogen peroxide, being ionic in nature, would be extracted into the aqueous layer with the DHP remaining in the chloroform layer. Furthermore, to ensure the removal of trace organics, the aqueous extract samples were passed through a Sep-Pak C_{18} cartridge.

4.3.2.3.2 Iodiometric titration

A 1 cm^3 volume of aqueous extract sample was first pipetted into 50 cm^3 of chloroform/glacial acetic acid (1:1 v/v) solution. To this was added 2 cm^3 saturated sodium iodide solution and three 'pea size' lumps of dry ice. The solution was then covered with parafilm and stored in the dark for 15 min. The dry-ice is required to exclude oxygen which interferes with the analysis procedure, giving rise to an increase in the apparent peroxide level. After 15 min, 25 cm^3 deionised water was added to the solution and it was then titrated with 0.01 M sodium thiosulphate, with the end point detected potentiometrically. A blank sample was prepared in an identical manner, using deionised water instead of the aqueous extract sample.

As a means to reinforce the hypothesis that the peroxide which was to be subsequently detected using an iodine titration was being generated via autoxidation, an aqueous extract sample was also prepared in an argon atmosphere. This involved the use of a glove box which was previously purged with argon for thirty minutes. It should be emphasised that this did not ensure the complete exclusion of oxygen. Also it should be noted that in this instance all DHP/glacial acetic acid samples were exposed to air (or argon where applicable) for three minutes. A blank prepared under the same conditions, but omitting the DHP, was also titrated and the result subtracted from the analysis samples.

4.3.2.3.3 Polarography

Both aqueous extract samples exposed to air/argon for three minutes were analysed using DP polarography. A sample volume of 0.10 cm^3 of the extract was added to 10 cm^3 of supporting electrolyte solution, which consisted of saturated lithium chloride (4% w/v) in acetonitrile. The solution was purged for three minutes with nitrogen prior to analysis to remove dissolved oxygen. The concentration of hydrogen peroxide was determined by DP polarography by scanning in the range -

0.70 V to -1.30 V vs Ag/AgCl. As a means of confirming the identification, the samples were subsequently spiked with 0.05 cm^3 0.10 M hydrogen peroxide and the polarographic scan repeated.

4.3.2.3.4 Spectrophotometric analysis based on formation of a titanium porphyrin complex

Utilising the aqueous extraction procedure detailed previously, the quantity of hydrogen peroxide generated in the autoxidation mixture over time was determined spectrophotometrically using a titanium porphyrin reagent. 250 µl of the aqueous extract sample, 250 µl of 4.8 M perchloric acid and 250 µl of titanium porphyrin reagent were mixed and allowed to stand for 5 min at room temperature. After diluting the sample to 2.50 cm^3 with water, the absorbance at 432 nm was measured (A_S). A blank sample was prepared in an identical manner, using deionised water instead of the sample and its absorbance (A_b) measured at 432 nm. The concentration of hydrogen peroxide was determined by the difference in absorbance between the blank and sample solutions:

$$\Delta A_{432} = A_b - A_s .$$

Further experiments were carried out following the procedure detailed above, but with quantities of transition metals present, namely iron(II) and copper(II). Also carried out were studies involving only hydrogen peroxide and transition metals.

4.3.2.3.5 Determination of hydrogen peroxide using a tin(IV) oxide biosensor

A tin(IV) oxide coated glass plate was treated successively with 10% aqueous solution of (3-aminopropyl)triethoxysilane (APTES) for 1 h at 50°C, then with a 2.5% aqueous glutaraldehyde solution for 30 min at room temperature and finally with a 0.10 M citrate-phosphate buffered solution (pH 5.9) of horseradish

peroxidase, containing 2 mg of HRP in 5 cm^3 of citrate buffer, for 30 min at room temperature, to obtain a HRP-modified electrode. The sensor signal was obtained as a cathodic current in 0.10 M citrate buffer at 30°C containing electron mediator (0.2 mM ferrocenemonocarboxylic acid).

4.3.3 RESULTS AND DISCUSSION

4.3.3.1 Aqueous Extraction

A first essential requirement for the investigation was the development of a method that eliminated the interference from the DHP itself, which is a strong reducing agent. Because of the organic nature of DHP, an aqueous extraction procedure was employed. This aqueous extraction procedure was carried out on all autoxidation samples prior to analysis, as it removed all traces of the DHP and placed the hydrogen peroxide in a less hostile matrix for analysis. The complete removal of the DHP in the aqueous extraction procedure was confirmed by thin layer chromatography. Also, a positive reaction for the presence of peroxide in the aqueous extract sample was obtained on TLC plates using N-N-dimethyl-p-phenylene-diamine-dihydrochloride, a specific peroxide spray reagent [41]. The hydrogen peroxide gave a typical purple response against a blue background. A liquid-liquid extraction procedure was employed because of the speed, ease and convenience of the technique requiring very simple equipment. Also it allows the separation of many sensitive compounds such as hydrogen peroxide with less danger of decomposition.

4.3.3.2 Iodometric titration

Quantitative determination of peroxides is the most commonly used analytical method for following the course of autoxidation reaction [42]. Many methods have

been developed for determining peroxides, but iodometric titration is the most popular [18,19]. Other more sensitive, less time consuming methods, such as gas chromatography and high performance liquid chromatography, are not employed to the same extent due to the sensitivity of peroxides to heat and surfaces. The iodiometric method is based on the assumption that sodium iodide (or any iodine salt) and glacial acetic acid when contacted with peroxide liberate iodine quantitatively, which can be then titrated against standardised sodium thiosulphate. Significant iodiometric titration results were observed for aqueous extract samples exposed to air, thereby indicating the presence of peroxide in the samples. A 70% decrease in titration values was observed for the samples which were exposed to the argon atmosphere. An average titre value of 11.00 cm^3 of 0.01 M sodium thiosulphate was required to react with the peroxide present in a 9:1 aqueous extract sample which was exposed to air for 3 min, while 3.20 cm^3 of 0.01 M sodium thiosulphate was required for a sample which was maintained in an argon atmosphere (n=3). All experiments were carried out in triplicate. Taking into account that the complete exclusion of oxygen was not possible, and the extremely high affinity of the DHP for oxygen, the complete elimination of the peroxide in the sample under argon was not expected. A blank sample, which consisted of the aqueous extraction procedure in the absence of the DHP, resulted in no detectable amounts of iodine being liberated.

4.3.3.3 Polarography

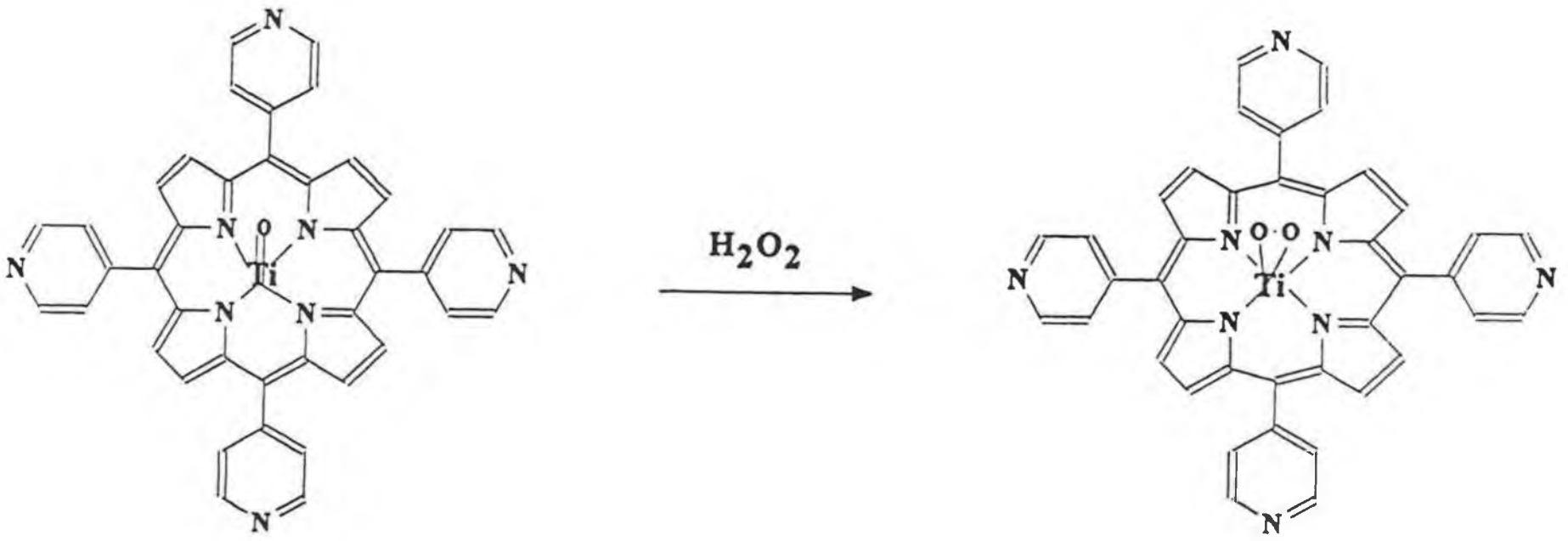
Polarography has been used frequently for the determination of hydrogen peroxide, and has lower limits of detection in comparison to iodine-based titration techniques. Polarography also has the advantage of greater selectivity, being able to distinguish between hydrogen peroxide and organic peroxides, which are reduced at more positive potentials compared to hydrogen peroxide [35]. On polarographic analysis of an aqueous extract sample which had been exposed to air for 3 min, one large broad peak, characteristic of peroxides, was observed with a reduction potential of -1.16 V vs Ag/AgCl, with a peak current of 4.74 μA, and on spiking that sample

with 0.05 cm^3 0.10 M hydrogen peroxide, the peak current increased to 7.28 μA. In the case of the aqueous extract sample, which was exposed to the argon atmosphere for comparison purposes, the observed peak had a peak current of 1.18 μA, which is equivalent to a 75% decrease in response compared to the samples exposed to air. The standard reduction potential for hydrogen peroxide as determined from standards was found to be -1.18 V vs Ag/AgCl which matches closely the reduction potential for the peak observed in the extract samples.

4.3.3.4 Spectrophotometric determination using titanium porphyrin reagent

A wide variety of spectrophotometric and chemiluminesence-based techniques are available for the determination of hydrogen peroxide. However, many of these techniques are affected considerably by interferences, especially from reducing agents. In this instance, the use of a specific reagent for the determination of hydrogen peroxide, which was not subject to interferences from reducing agents, would be an advantage. The method chosen involved using the oxo[5,10,15,20-tetra(4-pyridyl)porphyrinato]titanium(IV) complex [22]. This water soluble complex reacts specifically with hydrogen peroxide, with the quantity of hydrogen peroxide determined spectrophotometrically by the decrease in absorbance of the parent complex at 432 nm (Figure 4.12). This spectrophotometric reagent yields a very sensitive method, capable of detecting hydrogen peroxide in the range $1x10^{-6}$ M to $1x10^{-8}$ M. Perchloric acid was added to all samples as hydrogen ions assist the complexation reaction.

Figure 4.13 shows the results obtained using the specific titanium porphyrin reagent. These results not only confirm unambiguously the presence of hydrogen peroxide in the aqueous samples, but also demonstrate, as would be expected, that the concentration of hydrogen peroxide increases with the exposure time to air.



Max. abs@ 432nm

Max. abs@450nm

Figure 4.12 Titanium Porphyrin spectrophotometric reagent for the determination of hydrogen peroxide (Reproduced from M. Inamo, S. Funahashi and K. Tanaka, Analyst, 117 (1992) 1781).

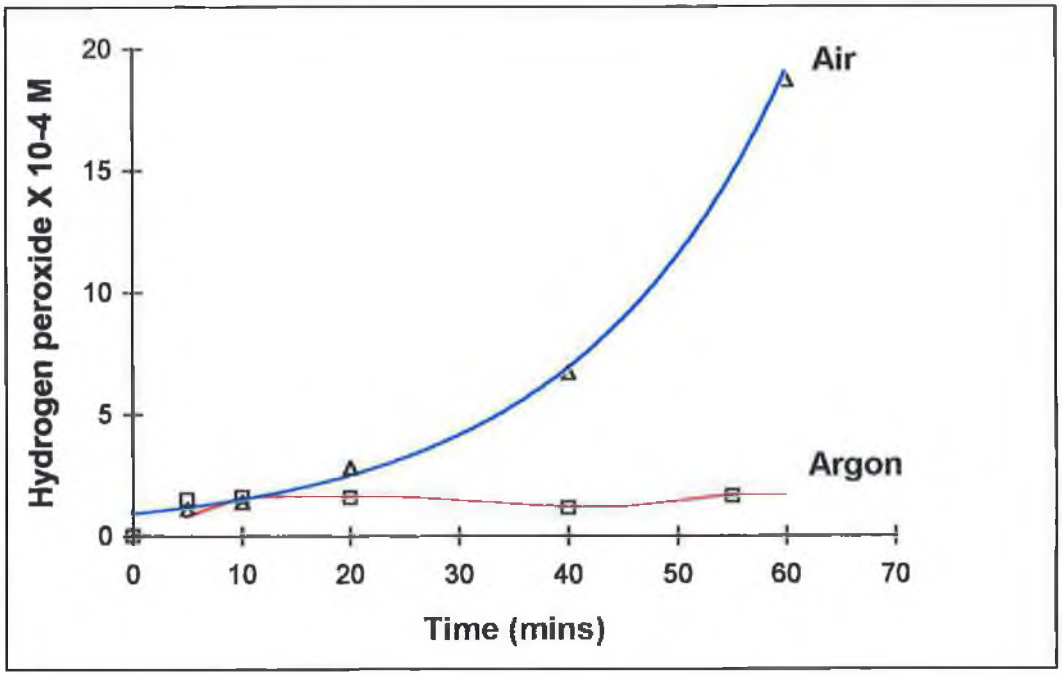


Figure 4.13 Quantitative analysis over time for hydrogen peroxide using the Titanium Porphyrin reagent, with a comparison of a sample exposed to air and a sample exposed to an argon atmosphere, thereby limiting the amount of hydrogen peroxide which can be produced via autoxidation.

In the absence of glacial acetic acid in the autoxidation mix, no hydrogen peroxide was detectable. Also shown in Figure 4.13 is the sharp decrease in the quantity of hydrogen peroxide generated when the autoxidation mixture was purged continuously with argon to reduce its exposure to oxygen.

4.3.3.5 Analysis using a tin(IV) oxide biosensor

A biosensor may be defined as a device that recognises an analyte in an complex sample and interprets its concentration as an electrical signal via a suitable combination of a biological recognition system and an electrical transducer [43]. The biological recognition system, typically an enzyme, multi-enzyme system, antibody, or whole slices of mammalian or plant tissue, is responsible for the selective and sensitive recognition of the analyte and subsequent response resulting in a change in one or more physiochemical parameters associated with the interaction. If generated in close proximity to a suitable transducer, it may be converted into an electrical signal.

An enzyme-based amperometric biosensor for hydrogen peroxide was constructed, based on work by Tatsuma, Okawa and Watanabe [31]. It employs the horseradish peroxidase (HRP) enzyme covalently attached to a tin(IV) oxide coated glass plate electrode. Tin(IV) oxide was chosen for its excellent chemical and electrochemical stability and the ease in chemically modifying the surface. The system is based on enzymatic reduction of hydrogen peroxide by horseradish peroxidase and subsequent electron transfer from the tin(IV) oxide electrode to the enzyme via an electron mediator. The electron mediator is required to enhance the electron transfer between the peroxidase and the electrode. Although hydrogen peroxide can be oxidised directly on a tin(IV) oxide electrode it requires a high overpotential of +900 mV vs Ag/AgCl. Therefore any electroactive substances present as impurities in the sample are liable to interfere in the analysis. The coupling of horseradish peroxidase with a mediator allows operation of the sensor at considerably lower potentials, reducing the risk of interference from contamination in the sample.

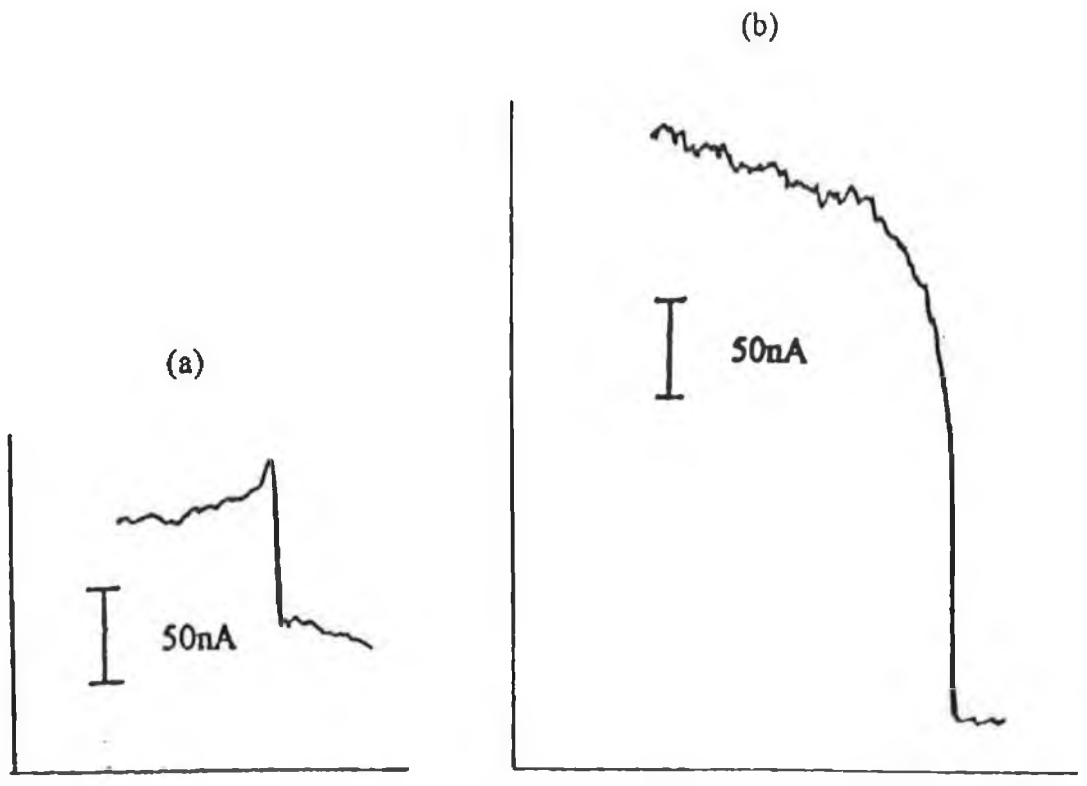


Figure 4.14 Results obtained using HRP modified tin(IV) oxide electrode. (a) Blank aqueous extract. (b) Aqueous extract of sample exposed to air for 120 min.

A small response of 83 nA was obtained for the blank sample, due to the presence of glacial acetic acid, as aqueous extract samples taken from solutions which consisted of only DHP in chloroform i.e. no glacial acetic acid present, did not affect the baseline current. With injection volumes of 0.10 cm^3, the response obtained for aqueous extract samples taken at 120 min and 160 min was 258 nA and 240 nA respectively (Figure 4.14). These correspond to hydrogen peroxide concentrations of 2.40×10^{-4} M and 2.10×10^{-4} M respectively, as determined by standard addition. These concentrations of hydrogen peroxide correlate well with the results obtained using the titanium porphyrin spectrophotometric reagent.

4.4. CONCLUSION

The main aim of this work was to confirm that hydrogen peroxide was being generated via autoxidation of N-phenyl-2-propyl-3,5-diethyl-1,2-dihydropyridine. This was further complicated by the presence of the hydrogen peroxide in an organic matrix with a strong reducing agent, namely the dihydropyridine. The employment of an aqueous extraction procedure removed the hydrogen peroxide from the organic environment, while also eliminating the possibility of any interference from the dihydropyridine.

Experiments carried out using the titanium porphyrin spectrophotometric reagent, carried out to determine the efficiency of the extraction procedure, indicated an extraction efficiency in excess of 95%. The possibility that a hydroperoxide based on the pyridine ring was generated in the autoxidation was discounted by the aqueous extraction procedure, as a hydroperoxide being quite organic in nature, would remain in the organic layer, and in contrast to the results obtained, no trace of peroxide would be detectable in the aqueous extract.

A multi-discplinary approach was taken to confirm the identity of the peroxide produced in the autoxidation of DHP, as that of hydrogen peroxide. The iodine-based titrations confirmed the presence of peroxide in the aqueous extraction samples. A 75% decrease in peroxide concentration was observed when the autoxidation mixture was exposed to an argon atmosphere, even though complete exclusion of oxygen was not possible. The iodine-based titration lacks the sensitivity of other instrumental techniques, with such factors as dissolved oxygen and moisture having marked affects on the results obtained. But in both instances, i.e. samples exposed to air and those maintained in an argon atmosphere, the results correlate very well with the results obtained using polarography in the lithium chloride/acetonitrile electrolyte. The reduction potential for the peak from the aqueous extract samples matches closely to the reduction potential for that of hydrogen peroxide standards. If in fact an organic peroxide was present, i.e. based on the pyridinium ring, it would be detected using polarography but would be

expected that it would be reduced at a consideraby more positive potential than that which was observed. The titanium porphyrin reagent offered a specific highly sensitive spectrophotometric based technique for the determination of hydrogen peroxide in an aqueous environment. Results obtained using this technique confirmed the presence of hydrogen peroxide in the aqueous extract samples and also demonstrated that the steady state concentration of hydrogen peroxide increased on prolonged exposure to air.

The tin(IV) oxide biosensor also yielded results consistent with those acquired using the other techniques and the biosensor had the benefit of being very selective for peroxides due to the presence of HRP covalently attached to the electrode surface.

All the various techniques employed yielded positive results to the presence of peroxide, while the high selectivity of the polarography and spectrophotometric methods provided further confirmation that it was in fact hydrogen peroxide.

The approach taken with this work was a qualitative one, to determine conclusively that hydrogen peroxide was generated in the autoxidation of the dihydropyridine. The process was made more difficult by the fact that the hydrogen peroxide was in fact only an intermediate, as it is continuously reduced by DHP; therefore the presence of hydrogen peroxide could only be determined if sufficient steady state concentrations were achievable. The kinetics of autoxidation reactions are very complex with a whole series of reactions occurring simultaneously and can be further complicated by the presence of even small quantities of transition metals. No attempt was made to investigate the kinetics of the reaction of this compound with oxygen or the factors that augment this reaction. Rather it was envisaged that the conclusive identification of the autoxidative peroxide product would lead to greater understanding of the chemistry in areas where this technology is in use.

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CHAPTER FIVE

THE DEVELOPMENT OF AN ANION EXCHANGE SEPARATION METHOD FOR THE DETERMINATION OF INORGANIC AND ORGANIC ACID ANIONS IN CYANOACRYLATE ADHESIVES

5.1. INTRODUCTION

5.1.1 General Chemistry

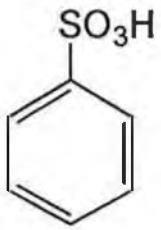
As has been previously outlined in chapter one, cyanoacrylate adhesives originally developed during World War II by Eastman Kodak are unique among the many classes of adhesives [1]. They are single component, instant bonding adhesives that cure or polymerize at ambient temperatures to form strong bonds between a wide variety of surfaces. Cyanoacrylates polymerize by an anionic mechanism, in the presence of a weak base, typically water, present as a thin layer of moisture on all surfaces.

Since the anionic cure of cyanoacrylates is a result of base catalysis, acids are predominantly used as stabilizers. Acids of either the Lewis type or protonic acids have been used, such as SO_2, BF_3, sulphuric acid, aliphatic and aromatic sulfonic acids (Figure 5.1). The acidic gases stabilize the monomer in both the liquid and gaseous phases, but the combination of non-volatile sulphonic acids with the gaseous stabilizers have been reported to offer synergistic effects [2]. The acid strength and levels are crucial variables in producing adhesives with the combination of desired performance in conjunction with a practical shelf-life. The protonic acid stabilizers are routinely determined using a non-aqueous potentiometric titration method [3].

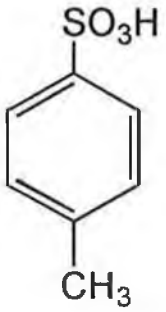
The synthesis of cyanoacrylates involves the reaction of an alkyl cyanoacetate with formaldehyde in the presence of a base to yield a poly(alkyl-2-cyanoacrylate) (Figure 5.2). This reaction is usually carried out in a non-aqueous organic solvent to facilitate the removal of water from the system. The polymer is then heated, forcing the de-polymerization of the polycyanoacrylate back to a cyanoacrylate ester. For this reaction to occur smoothly, without repolymerisation of the monomer, the base catalyst from the first reaction must have been neutralized by the addition of an acidic compound such as phosphoric acid or phosphoric anhydride.

Although water is known to be the most common initiator, it is widely accepted that cyanoacrylates can contain up to several thousand parts per million water, which in combination with strong acid stabilizers, can cause hydrolysis of the monomer forming carboxylic acids which can seriously affect adhesive performance [1]. The non-aqueous potentiometric titration method routinely used to determine the strong acid stabilizers lacks the sensitivity to detect these weaker carboxylic acid contaminants. Therefore, the need exists for the development of a technique for the determination of both the strong acid stabilizers and carboxylic acid impurities in cyanoacrylate adhesives. This pointed to the use of either high performance liquid chromatography (HPLC) or ion chromatography (IC) as techniques which can distinguish between the various acidic species present in the cyanoacrylate adhesive matrix. Both HPLC and IC have been used extensively in the determination of carboxylic acids in other matrices, which is reflected by the large number of publications available on the subject.

The following section will briefly review IC and HPLC based procedures for the separation and quantification of carboxylic acid anions and inorganic anions.



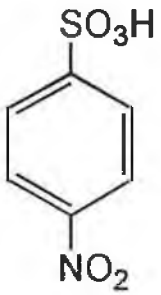
Benzene sulphonic acid



p-Toluene sulphonic acid (pTSA)

CH_3SO_3H

Methane sulphonic acid (MSA)



p-Nitrobenzene sulphonic acid

Figure 5.1 Examples of some non-volatile sulphonic acid stabilizers used in cyanoacrylate adhesives.

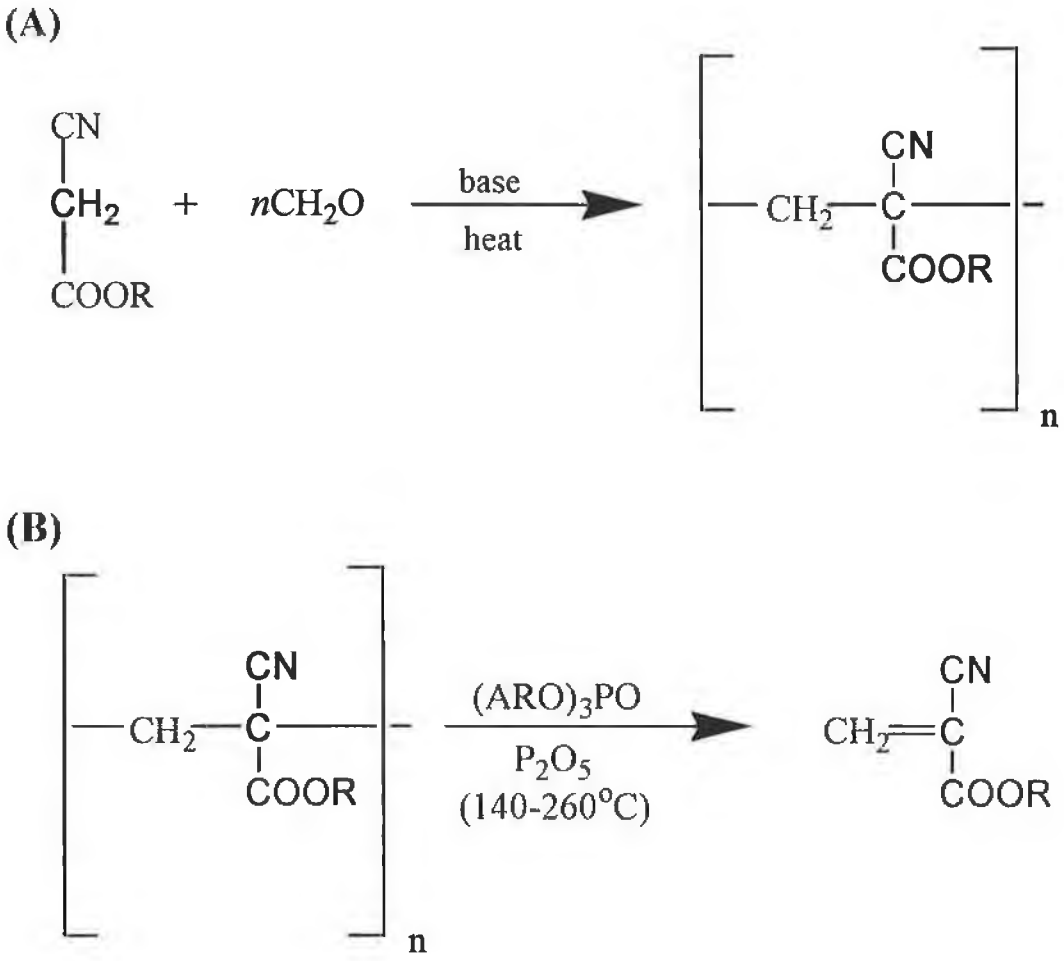


Figure 5.2 Synthesis of cyanoacrlayte adhesives by the Knoevenagel condensation method. (A) Reaction of an alkyl cyanoacetate with formaldehyde in the presence of a base to yield a poly(alkyl-2-cyanoacrylate). (B) The polymerised cyanoacrylate is then heated to a temperature of 140-260^0 C, forcing the depolymerisation of the polycyanoacrylate back to a cyanoacrylate ester.

5.2. LIQUID CHROMATOGRAPHY

Carboxylic acids can be analysed chromatographically by employing reversed-phase, ion exchange or size exclusion techniques. The main detection methods used in conjunction with these techniques can be classified as either electrochemical or optical. In terms of electrochemical detection, conductimetric, amperometric and potentiometric detection has been used, whereas for optical detection, UV-visible absorbance, indirect photometric, fluorescence and refractive index detectors have found some application.

5.2.1 Ion chromatography

5.2.1.1 Introduction

Ion chromatography is in essence a form of high performance liquid chromatography. Its true definition is a matter of debate, but it can be defined as simply the liquid chromatography of ions. According to literature, dating back approximately 3,000 years, Moses was one of the first to recognise the capabilities of ion exchange, when he threw a decomposed log (an ion-exchange resin) into a pool of brackish water at Marah. Apparently, the exchange of ions made the water potable (Exodus, Chapter 15, verses 24 and 25).

Ion chromatography encompasses both ion exchange and size exclusion chromatography, and is routinely used in the determination of organic and inorganic cations and anions. Modern ion chromatography was first described by Small and co-workers [4] of the Dow Corporation, when they reported a novel ion exchange chromatographic method using conductimetric detection. They used a low-capacity ion exchange column, which they called a "stripper", which served to reduce the background conductance of the eluent in order to enhance the signal from the eluted ions. The term 'ion chromatography' was then introduced by the Dionex Corporation who licensed the technology for commercial development.

5.2.1.2 Ion exchange chromatography

Ion exchange chromatography is based on the separation of ions on columns that have charge-bearing functional groups attached to a polymer matrix [5]. The functional groups are permanently bonded ionic groups associated with counter ions of the opposite charge. The most common retention mechanism is the simple exchange of sample ions and mobile-phase ions with the charged group of the stationary phase. Cation exchange resins bear negatively charged groups which can be either strong or weak acid cation exchangers. The most commonly used functional groups are the sulphonate type, which are strong acidic exchangers, whereas weak exchangers have carboxylic acid groups which permit the exchange of cationic species only when the pH is sufficiently high to permit dissociation of the carboxyl group.

The anion exchange resins are classified likewise as strong and weak basic exchangers. Quaternary amine functional groups form strong basic exchangers and less substituted amines form weak base exchangers. The strong exchange groups in the case of both the anion and cation resins are completely dissociated, and therefore, the exchange properties are independent of the pH of the mobile phase, unlike the weaker exchange groups [5].

There are four main types of stationary phases employed in ion exchange chromatography.

(1) Polymer-coated silica

A silica support is first coated with layer of polymer, such as polystyrene, silicone or fluorocarbon, and the layer is then derivatised to introduce functional groups. These particles have the advantage that diffusion within the thin layer of polymer is faster than that occurring with totally polymeric particles. This leads to favourable mass transfer characteristics and improved efficiency.

(2) Functionalised silica materials

The functional groups are chemically bonded directly to a silica particle. The silica particles for both polymer-coated and functionalised silica ion exchangers can be either pellicular or macro-particulate in nature. They provide similar chromatographic efficiency, but the pellicular materials are restricted to the separation of small amounts of sample because of their low surface area.

(3) Resin based ion exchangers

Organic materials, in the form of synthetic polymeric resins are the most widely used types of ion exchangers. They are manufactured by first synthesizing a polymer with the desired physical and chemical properties. The polymer is then further reacted to introduce the functional groups required. Most ion exchange resins are made from copolymers of styrene and divinylbenzene, with a small number consisting of copolymers of divinylbenzene and acrylic or methacrylic acid. These resins also have the advantage of being stable over a wide pH range, and in many instances are compatible with most organic solvents.

(4) Hydrous oxide ion exchangers

These act as ion exchangers because the skeleton or matrix material carries an excess charge which is compensated by the mobile phase counter ion. Typical examples of this class of ion exchanger are alumina, silica and zirconia. However, these exchangers show strong pH dependence effects.

The eluent or mobile phase in ion exchange chromatography generally consists of an aqueous solution of a suitable salt or mixture of salts, with a small percentage of an organic solvent sometimes being added. The salt mixture may itself be a buffer, or a separate buffer can be added to the eluent if required. The prime component of the eluent is the competing ion which has the function of eluting sample components through the column within a reasonable time. The role of the eluent is to compete with analyte ions for the functional group sites on the stationary phase and to separate the mixture of analyte ions into well defined bands. The main properties of

the eluent which affect the elution of analyte ions are the eluent pH, the nature of the eluent and its concentration. The eluent pH affects the form in which the functional group on the ion exchange matrix exists, and also the forms of both the analyte ions and in some cases the eluent. The eluting power of weak acid eluents increases with increasing pH until the acid is completely dissociated, while the opposite is true of weak base eluents. Solute ions derived from weak acids or bases will show pH effects because their charge is also governed by the eluent pH. Increased analyte charge results in increased retention; therefore, because the eluent pH effects retention behavior, it is desirable to use some buffering capacity. This enables the eluent to maintain a stable pH and to provide reproducible retention times even when acidic or basic samples are injected.

The selectivity coefficient, K, for an ion exchange reaction between an analyte ion and a competing eluent ion, describes the extent to which the analyte ion is able to displace the competing ion from the stationary phase. Retention increases as the value of K increases; so a suitable eluent is one which leads to an appropriate degree of retention for the analyte ion. Factors which affect the selectivity coefficients include size, charge, degree of hydration and polarizability of the eluent ion. Eluents of greater charge have stronger affinity for the fixed ions of the stationary phase and so are stronger eluents. The rate at which retention can be varied by changing the eluent ions concentration depends on the charges carried by both the analyte and eluent ions. For a particular analyte, retention changes more slowly with eluent concentration for a doubly charged eluent than for a singly charged ion. Complexation agents can be added to the eluent to alter the charge on the analyte by complexing it with a suitable organic agent to alter retention. The use of organic solvents can accelerate complex formation and it can also alter the selectivity for ions which exhibit hydrophobic interactions with the stationary phase. Most metals can be converted to a negatively charged complex ion through suitable masking systems, enabling them to be separated on an anion exchange column.

Carboxylic acids can be determined using anion exchange chromatography, although due to the large retention differences between ions of differing charge, gradient elution is required to elute mono-, di- and triprotic acids. Anion chromatography has the advantage that inorganic anions such as sulphate and chloride can be determined simultaneously with carboxylic acid anions. Ivey and Davies [6] used anion exchange chromatography with conductivity detection to investigate the occurrence of some organic acids in Antarctic ice, as well as measuring the concentrations of some inorganic anions. Boyles [7], using a Dionex Omni-Pac PAX 500 column, then determined 14 organic acid and inorganic anions in beer wort using gradient elution chromatography with a sodium hydroxide/methanol eluent (Figure 5.3).

5.2.1.3 Ion exclusion chromatography (ICE)

Ion exclusion chromatography was first introduced in 1953 by Wheaton and Bauman [5]. It involves the use of strong anion or cation exchange resins for the separation of ionic analytes from weakly ionised or neutral analytes. In ion exclusion chromatography, the charge sign on the resin used is the same as that of the weakly ionised analytes. So analytes with a partial charge, such as organic acids, are separated on a cation exchange resin having anionic sulphonate functional groups. This is the opposite situation to that occurring in ion exchange chromatography. The principle of ion exclusion is based on the consideration that the chromatographic system has three distinct phases. The first of these is the flowing eluent, which passes between the beads of the ion exchange resin. The second zone is the polymeric network of the resin material itself, together with its bonded functionalities, while the third zone is liquid occluded inside the pores of the resin bead. The functional groups on the polymeric resin can be considered to comprise a charged membrane separating the flowing mobile phase from occluded static mobile phase trapped in the pores of the resin.

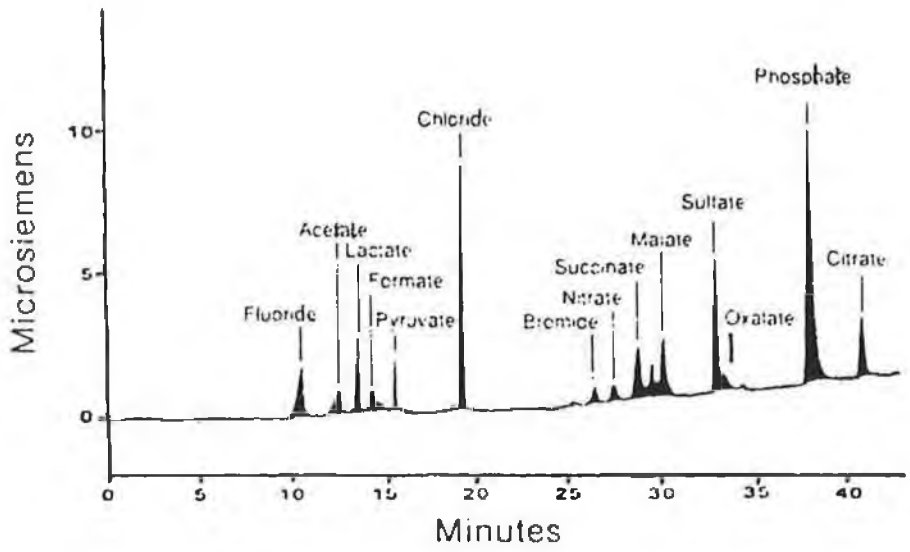


Figure 5.3 The separation of 14 organic acids and inorganic anions in beer wort using gradient elution anion exchange chromatography. (Reproduced from S Boyles, J. American Society of Brewing Chemists, 50 (1992) 61.

Retention in ion exclusion chromatography is dependent on a number of different mechanisms, including electrostatic interaction of the analyte with the charged functional groups on the resin surface. Ionic analytes are prevented or excluded from the resin because of their inability to penetrate the charged 'membrane' and so are eluted in the void volume of the column. The ionic analytes are repelled by the anionic functional groups on the resin referred to as the Donnan exclusion effect.

In contrast, non-ionic or weakly ionic species may partition between the occluded liquid phase and the flowing mobile phase. The degree of partition determines the extent of retention on the column.

Typical ion exclusion columns designed for the separation of carboxylic acids contain sulfonated polystyrene-divinylbenzene copolymers. There are, however, a range of stationary phase parameters which can influence the retention on the column. Typically 5-10 μm particles are used. The capacity of the ion exchange material should be high enough so that the number of functional groups on the resin is sufficient to exert an appropriate Donnan exclusion effect. Microporous or gel type resins are most commonly used, but some macroporous resin are also used. Highly crosslinked resins (8-12% divinylbenzene) show stronger Donnan exclusion effects than low cross linked resins (2% divinylbenzene). Ion exclusion columns usually have large dimensions because a considerable volume of resin is required to provide sufficient occluded mobile phase to permit a reasonable degree of retention of analytes. The eluent employed in ion exclusion chromatography for the determination of carboxylic acids can be water or a dilute solution of a mineral acid such as sulphuric or an aliphatic sulphonic acid. Weak acids such as phosphoric acid and benzoic acid have also been used. Earlier work was carried out in de-ionised water, but it was unsuitable for the analysis of stronger acids as they show too great a degree of ionisation to be retained. Also, analytes which are retained show poor peak shape with water as the mobile phase. The use of an acidic eluent gives improved peak shape and ensures that the retention time is independent of analyte concentration.

The choice between which eluent acid to use is usually governed by the method of detection being used. Sulphuric acid is usually used with UV spectroscopic detection, while sulphuric or hydrochloric acids, are generally used with conductivity detection following suppression. Sulphonic acids are used with conductivity detection in the absence of suppression. The pH of the eluent affects analyte retention, as changes in the eluent pH produce a change in the degree of ionisation of the analyte which induces a change in the retention of the analyte. Strong acids which are fully ionised are totally excluded and are eluted at the void volume. Very weak acids on the other hand ($pK_a > 6.4$) tend not to be excluded at all and permeate totally into the resin giving retention times which are independent of pK_a. Many acids with pK_a values intermediate between the two extremes shoe an elution order which can be predicted from pK_a values.

However, other acids, particularly aromatic and long chain aliphatic carboxylic acids, show retention times which are longer than that expected from their pK_a values alone. In those cases, hydrophobic adsorption effects are considered to contribute to the retention process i.e. a reversed-phase partition mechanism [8]. Finally, some acids, especially di-functional aliphatic carboxylic acids, show less retention than expected from their pK_a values. For these species it is believed that size exclusion effects may restrict access to the occluded liquid [8,9].

The degree of analyte ionisation is the most significant factor affecting analyte retention. As the analyte becomes more ionised the retention is decreased due to an increase in the Donnan exclusion effect. Fully ionised analytes are totally excluded from the resin and the retention volume of these fully ionised analytes is given by $V_r = V_o$, where V_o is the void volume. Neutral analytes, on the other hand, can move freely between the interstitial eluent and the occluded liquid without being influenced by the Donnan effect. For some analytes it has been observed that the degree of exclusion is greater than that expected by charge alone. Therefore, the retention volume is lower than expected. Waki and Tokunaga [10], as well as other authors, have suggested that size exclusion contributes to the retention process by restricting

the access of larger molecules to the occluded liquid in the pores of the stationary phase. Some of the effects resulting from size exclusion are that the retention volumes for large, partially ionised analytes are smaller than expected. Also, large, neutral molecules are eluted sooner than expected.

As mentioned previously, aromatic and long chain aliphatic carboxylic acids show retention volumes which are larger than might be expected and are eluted in order of increasing molecular weight (the opposite of what would be expected). It has been suggested that a mixed mode mechanism exists in which partitioning also exists due to hydrophobic attraction of the sample analytes for the polymeric resin matrix [11]. Hydrophobic adsorption effects can be expected to increase in magnitude as the alkyl chain length increases giving larger retention volumes.

Morris and Fritz [11] found that small chain alcohols added to an aqueous eluent has a dramatic effect on the chromatographic behavior of small polar compounds such as alkane carboxylic acids. They separated several acids on a lightly sulphonated polymeric resin using an aqueous eluent with varying amounts of various alcohols and other organic solvents as modifiers. Addition of a small amount of methanol to the eluent improved peak shape and reduced retention times. The separation was then carried out with ethanol and butanol as modifiers. It was found that the detection sensitivity increases as the chain length of the alcohol increased, and in the series of alcohols (methanol to butanol) the sensitivity increased as the percentage of alcohol decreased. Using an eluent of 5% butanol/ 95% water, the acids were baseline resolved and the separation was completed in 5 min. The function of the modifiers is to coat the resin surface and make it less hydrophobic. They concluded that the separation of carboxylic acids could be performed on columns containing either underivatised or lightly sulphonated macroporous resins. The separation mechanism is largely hydrophobic attraction between the analytes and the resin. If a suitable alcohol or other organic modifier is added to the aqueous eluent, no strong acid is necessary.

Temperature can affect retention in ion-exclusion chromatography by altering the chromatographic efficiency in the same manner as in most forms of chromatography. It is evident by the reduced retention times, improved peak shape and better separations at elevated temperatures due to faster mass transfer [5]. Blake et al. [8] found that the resolution of acids present in sugar cane process juice is improved by connecting two Aminex HPX-87H columns in series and equilibrating them at different temperatures. When both columns were equilibrated to the same temperature, not all of the acids were separated satisfactorily. Pecina et al. [12] found that 60^{O} C was the optimal temperature for the separation of eleven organic acids on a strong cation exchange resin (Aminex HPX-87H) with 0.01 N sulphuric acid as the eluent. Narrow and symmetrical peaks were observed for all acids under the optimal conditions.

The separation of carboxylic acids is the most common application of ion-exclusion chromatography. It has been used for instance to prove the authenticity of apple juice by showing the presence of D-maleic acid, which is not a naturally occurring isomer [13]. Ashoor and co-workers [14 ,15] used ion-exclusion to determine the amount of citric acid and lactic acids in certain foods, as these acids influence the flavour, stability and long term stability of the foods. Some other examples of foods tested for their organic acid content by ion-exclusion chromatography include: dairy products [16], fruit juices [17 ,18], sugar cane process juice [8], cocoa beans [19], potatoes [20], pepperoni [15] and pickles [15].

Ion-exclusion chromatography has also been used for the determination of organic acids in other matrices, such as precipitation samples [21]. A cation exchange column in the hydrogen form can be used not only to evaluate organic acids, but also for the simultaneous determination of sugars and alcohols; this is achieved by having two detectors in-line, one for the acids and one for the other compounds [19]. One area in particular where ion-exclusion chromatography has been important is in the wine industry. The malolactic fermentation process is important in controlling the acidity of wines. Several workers have described the use of ion-exclusion chromatography in this field. Ion-exclusion has several advantages over reversed-

phase methods, such as the possibility of determining carboxylic acids simultaneously with frutose, glucose, glycerol and ethanol. The sensitivity is not as good as reversed-phase chromatography, but is usually sufficient to determine the compounds in wine samples [22 ,23]. Poor resolution, however, between malic acid glucose and fructose peaks in wine samples, especially when the concentration of the two sugars is high, can make it impossible to determine malic acid. Therefore, many workers have applied it to dry wine samples only. Calull and co-workers, however, have used two ion exclusion methods for the separation of acids and other compounds in wine (Figure 5.4). The first involved optimisation of the process parameters using a computer program, while the second method involved the separation of the acids from the neutral compounds before determination, thereby making it possible to determine acids in wine or grape must independently of the sugar content. A quaternary amine bonded silica support with strong anion exchangers was used to separate the neutral and acidic compounds.

5.2.1.4 Ion-pair chromatography

The term 'ion-pair' describes species formed between two ions of opposite electrical charge; ion-pairs therefore, have a low net electrical charge and a low polarity [5]. Adding hydrophobic counter ions to a mobile phase to enhance retention and resolution has been widely used in the liquid chromatographic separation of charged organic species on alkyl modified silica. Studies focusing on identifying the interactions that occur in the presence of the counter ions have been carried out, and several retention mechanisms have been proposed [24]. The ion-pair mechanism is one where it has been suggested that ion pairs form between analyte ions and the hydrophobic counter ions prior to sorption on a hydrophobic alkyl-modified silica stationary phase. Experimental evidence supporting this are isotherm measurements and correlation of retention with counter ion concentration. Other workers suggested an ion exchange mechanism, where the counter ions are first sorbed and these charged sites then serve as exchange sites for the analyte ions.

Retention of the counter ion on the stationary phase and correlation of this to analyte retention has been the major supporting evidence for an ion exchange mechanism, called "dynamic ion exchange". The third mechanism, ion interaction chromatography, can be seen as intermediate between the two previous models, in that it incorporates both the electrostatic effects which are the basis of ion pair chromatography and the absorption effects which form the basis of the dynamic ion exchange model.

In the ion pair model, an ion pair is seen to form between the analyte ion and the counter ion. The resultant neutral ion-pair can then be absorbed onto the hydrophobic stationary phase in the same way that any neutral molecule with hydrophobic characteristics is retained in reversed-phase chromatography. Retention therefore results solely as a consequence of reactions taking place in the eluent.

The degree of retention of the ion-pair is dependent on its hydrophobicity, which in turn depends on the hydrophobicity of the counter ion. An increase in percentage of organic solvent in the eluent decreases the interaction of the ion pairs with the stationary phase and therefore reduces their retention.

In the dynamic exchange model, the counter ions of the ion-pair reagent are adsorbed onto the stationary phase and can be seen as another means of preparing an ion exchange column. The adsorbed counter ion imparts a charge onto the stationary phase, causing it to behave as an ion exchanger. The total concentration of the counter ion adsorbed is dependent on the percentage organic solvent in the eluent; the higher the percentage the less of the counter ion on the stationary phase. In addition, the more hydrophobic the counterion, or the higher its concentration, the greater is its adsorption onto the stationary phase. Constant interchange of counter ions occurs between the eluent and the stationary phase, so it is called a dynamic ion exchanger. When a analyte ion of opposite charge is introduced, retention results by a conventional ion exchange mechanism.

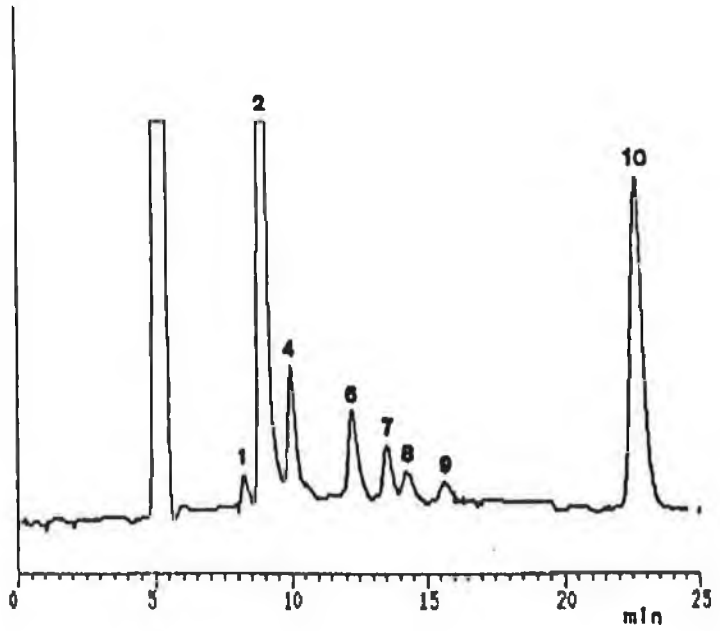


Figure 5.4 Chromatogram of acid fraction of wine sample as determined by anion exchange in conjunction with refractive index detection. Peaks: 1 = Citric acid; 2 = tartaric acid; 4 = malic acid; 6 = succinic acid; 7 = lactic acid; 8 = glycerol; 9 = acetic acid; 10 = ethanol. (Reproduced from Callul et al., J. Chromatogr., 590 (1992) 215.)

Analytes of the same charge as the counterion are repelled and show decreased retention, while neutral analytes are unaffected by the counter ion. The hydrophobic counterions of the ion pair reagent are considered to form a dynamic equilibrium between the eluent and the stationary phase in the ion interaction model. This results in the formation of an electrical double layer at the stationary phase surface. The adsorbed counterions are expected to be spaced evenly over the stationary phase. This adsorbed layer forms a primary layer of charge to which is attached a secondary layer of opposite charged ions.

The amount of charge in both the primary and the secondary charged layers is dependent on the amount of counter ion adsorbed, which in turn depends on the hydrophobicity of the counter ion, the counter ion concentration and the percentage of organic solvent in the eluent. Transfer of analytes through the double layer to the stationary phase surface is due to electrostatic effects and hydrophobic interactions. An analyte ion of opposite charge to the counter ion then competes for a position in the secondary charged layer, from which it tends to move into the primary layer as a result of electrostatic attraction and hydrophobic effects. The presence of such an analyte in the primary layer causes a decrease in the total charge of the layer, so to maintain charge balance, a further counter ion must enter the primary layer. The overall result is that analyte retention involves a pair of ions as distinct from an ion pair. This process leads to increased retention of the analyte. This method, and that of the dynamic exchange mechanism, are the most common mechanisms used in the literature to explain this type of chromatography.

Ion-pair chromatography has been performed successfully on a wide range of stationary phases, including neutral polystyrene divinylbenzene (PS-DVB) polymers and bonded silica materials with C_{18}, C_8, phenyl and cyano groups as the chemically bound functionality. Each of the stationary phases gives satisfactory retention of ionic analytes, provided the eluent composition is such that an appropriate amount of the ion pair reagent is adsorbed. A major advantage of the PS-DVB stationary phase over the alkyl modified silica is its stability throughout the entire pH range.

Therefore, a strongly basic mobile phase can be used to ensure that even very weak organic analytes are completely ionised in the presence of the hydrophobic cations. Examples of reagents used as counter ions are tetraalkylammonium, iron(II) 1,10-phenanthroline and ruthenium(II)-1,10-phenanthroline salts for anion separations and alkylsulphonates and diethyldithiocarbamates for cation separations.

The separation and determination of organic acids, such as carboxylic, phosphoric and sulphonic acids in complex matrices is a difficult problem encountered in health, food and environmental sciences. Often the procedures used must be applicable to the separation of complex mixtures of simple organic acids including short alkyl chain mono- and diprotic acids. In the ion-pair chromatographic separation of organic acids, a hydrophobic ion, such as tetraalkylammonium ion, is used as a mobile phase additive in combination with a reversed stationary phase in conjunction with conductivity or spectrophotometric detection.

An enhanced retention occurs because of the interactions between the hydrophobic cations, its counter ion, the organic acid analyte anion, any other mobile phase solvent and the pH are the major parameters that can be altered to affect retention and resolution of analyte anion mixtures. Iskandarani and Pietrzyk [25], Smith and Pieyrzyk [26] and Rigas and Pietrzyk [27 ,28] have all carried out much work on the ion-pair chromatography of both inorganic and organic ions. Rigas and Pieyrzyk [27] used iron(II)-1,10-phenanthroline [$Fe(phen)_3^{2+}$] salts as mobile phase additives for the separation of organic acids as anionic analytes on a reversed-phase column. The problem encountered in the liquid chromatographic separation and determination of simple non-chromophoric organic acids, is their detection at favourable detection limits. In this method it was found that the [$Fe(phen)_3^{2+}$] salt served as both the counterion and a chromophoric marker for the presence of the organic analyte anion with spectrophotometric detection. The pH range of [$Fe(phen)_3^{2+}$] salt stability is broad and extends into the basic side; therefore, the conditions are favorable so that even weak organic acids are dissociated and can be separated as anions. Rigas and Pietrzyk [28] have used ruthenium(II)-1,10-phenanthroline and 2,2-bipyridine complexes as ion pair reagents for the

determination of organic and inorganic anions on a reversed-phase column. The Ru(II) complex acts as an ion pair reagent and because the complexes fluorescent the anions can be detected by fluorescence detection, although photometric detection is also possible.

5.2.2 Reversed-phase chromatography

This is a form of chromatography where the stationary phase is relatively non-polar and the mobile phase relatively polar. Retention is governed by the interaction of the non-polar components of the analytes and the non-polar stationary phase [5]. The most commonly used stationary phase is octadecyl alkyl hydrocarbon chain (C_{18}) which is chemically bonded to a silica substrate. For reversed-phase chromatography, the mobile phase is made by choosing one solvent in which the sample is soluble and another solvent in which the sample is less soluble. The mobile phase is then prepared by adjusting the amount of the strong and weak solvents to a ratio where the attraction of the analytes to the stationary phase is in competitive equilibrium with the attraction of the analytes to the mobile phase. The equilibrium of the analytes in the mobile phase relative to the stationary phase determines the retention time and effects the separation.

The separation of ionisable analytes by reversed-phase can be optimised by suppressing the ionisation of the analytes by adding a buffer of appropriate pH to the eluent. The separation is really only suitable for those weak acids (and bases) for which ionisation can be suppressed using buffers having pH values in the range 3-8, because C_{18} columns are unstable outside this pH range [5]. A wider range of analytes can be separated using ion-suppression technique on a non-polar polymeric stationary phase because the eluent pH of the eluent is not restricted to between 3-8. Slingsby [29] showed that a polymeric reversed-phase column using ion suppression coupled with gradient elution and suppressed conductivity detection enables the separation of butyric through to stearic acid.

The reversed-phase chromatographic method is slightly limited in that it is difficult to separate low molecular weight acids. The more hydrophilic carboxylic acids are not retained on the column and are eluted with the void volume. Lee [30] showed that polar carboxylic acids could be retained longer by using a hydrophilic end-capped C_{18} column. Other methods have also been used to improve the results obtainable from reversed-phase chromatography using a buffered mobile phase, such as pre-column derivatisation, to improve detection limits. One of the most widely applied derivatisation procedures is alkylation using reagents containing bromo- or iodoalkyl groups, e.g. 4-bromomethoxycoumarin and 7-(diethylamino-3-{-4-[(iodoacetyl)amino]-phenyl}-4-methylcoumarin. These reagents react with organic acids to form esters in polar aprotic media. Derivatisation is usually performed at 50-60^{o} C in acetone or acetonitrile with crown ethers and carbonate as a base catalyst [31]. Many other pre-column derivatisation methods have also been used [31,32], such as the NPH method, and pre-column derivatisation with 2-nitrophenylhydrazine with UV absorbance detection.

Coenan et al. [32] carried out a comparison of four organic acid determination methods; two pre-column derivatisation methods, ion suppression reversed phase HPLC with UV absorbance detection and an ion exclusion method with conductivity detection. They found that the reversed-phase methods were more suited to less polar carboxylic acids, with the NPH method being the most successful of the three, and recommended ion exclusion for more polar acids.

Some of the areas in which reversed-phase chromatographic analysis of organic acid has found itself useful is in the food and drinks industry. Both direct and indirect methods of organic acid analysis have been used for products such as oranges, and other citrus fruits [33], green beans [34], sweet peppers, tomatoes, wines [35 ,36], wine must, apple and cranberry juice. Direct methods have been used for the determination of ascorbic, dihydroascorbic and other organic acids in Calamondin (a citrus fruit) using a Spheri-5 (RP-C_{18}) column at a pH of 2.3 with diode array detection [37].

A simple method for improving results has been exploited by a number of workers, which involves the use of two columns in tandem to achieve better separation [33]. Indirect methods of analysis involve the derivatisation of the acids with either fluorescence or chemiluminescence detection. Nakabayashi et al. [38] used 9-anthryldiazomethane to esterify gluconic acid. The reaction mixture was directly chromatographed by LC using a C_{18} reversed-phase column with fluorescence detection. They showed that the method was suitable for the determination organic acids in sea water. Elbert et al. [39] used 4-methyl-7-methoxycoumarin as a fluorescent label for the determination of dicarboxylic acids.

Chromatographic methods are considered to be valid alternatives to enzymatic methods for the determination of organic acids in wine, with shorter analysis times and greater accuracy [23]. The determination of carboxylic acids in wine has considerable importance in enology as they are used to control the vinification process. They also have a great influence on the biological stability and the organoleptic (taste) properties of wines. Derivatisation method are preferred over the more conventional direct methods as they offer greater sensitivity. Mentasti et al. [35] described a method for the derivatisation, identification and separation of carboxylic acids in wines and beverages by reversed-phase HPLC. Derivatisation with phenacyl bromide was optimised for application to acids in wines and separation was accomplished on a standard C_{18} column. Tusseau and Benoit [40] used a C_8 column with UV detection and an eluent adjusted to pH 2.1, in order to have maximum protonation of the acids.

5.2.3 Detection methods

The main detection systems employed in chromatography can be classified as either electrochemical or optical.

5.2.3.1 Electrochemical detectors

Conductimetric detection is based on the application of an alternating voltage, E, to the cell electrodes and the measurement of the cell current, i, which is directly proportional to the conductance G, of the solution between the electrodes through Ohm's law. Conductivity detection can be used in the non-suppressed mode, which was first reported in 1951 by James et al. [41] However, in non-suppressed ion chromatography, sensitivity is directly proportional to the difference in equivalent conductivity between the analyte and the eluent ions. If the eluent contains low-conductivity ions such as benzoate and/or phthalate, then sensitivity will increase as the analyte ions equivalent conductivity is increased. If the eluent on the other hand contains a high conductivity ion, then sensitivity will be greatest for ions of the lowest conductivity. In this case, the detector response is a decrease in measured conductivity, producing a dip instead of a peak [42].

However, conductimetric detection did not gain wide spread popularity until the introduction of suppressor columns for post-column signal enhancement. Small et al. [4] described how the analysis of certain organic species was frustrated by the presence of the background electrolyte, because available detectors were not able to detect the species of interest against the background. They described how conductimetric detection was desirable as a means of monitoring ionic species in a column effluent, since conductivity is a universal property of ionic species in solution and conductance shows a simple dependence on species concentration. However, as mentioned before, the conductivity from the species of interest is 'swamped out' by the background signal.

But by using a combination of resins which strip out or neutralize the ions of the background electrolyte, only the species of interest was left as the major conducting species in the effluent. All ions in solution contribute to the total conductivity regardless of whether they are analyte ions or part of the eluent. Therefore, maximum sensitivity is produced by maximising the difference in equivalent conductivity between the analyte and eluent ions. In chemically suppressed ion chromatography, this is accomplished by reducing the eluent conductivity to a very low value, either by removing it completely or by neutralizing it (Figure 5.5). All analytes then produce conductivites greater than the eluent ions displaced; so sensitivity is directly proportional to the conductivity of the ion.

Recent advances in suppressor technology have seen the introduction of electrodes into suppressor columns to increase the efficiency and suppression capabilities, as illustrated for an anion suppressor column in Figure 5.5. The water regenerant undergoes electrolysis to form hydrogen gas and hydroxide ions at the cathode, while oxygen gas and hydronium ions are formed in the anode chamber. Cation exchange membranes allow hydronium ions to move from the anode into the eluent to neutralizse hydroxide. While sodium ions in the eluent, attracted by the electrical potential applied to the cathode, move across the membrane to the cathode to maintain electrical neutrality with the hyroxide ions at the electrode. This system has the advantage that it can employ either water as a regenerant or it can recycle the detector effluent, thereby eliminating the requirement to make up separate solutions of sulphuric acid and reducing the quantities of water used.

Conductivity detection has also been found to be the optimum detection method for many small inorganic and organic ions, especially those which do not absorb UV light and are difficult to detect by optical means. This includes all strong acid anions such as chloride, nitrate, sulphate and organic sulphates and phosphates.

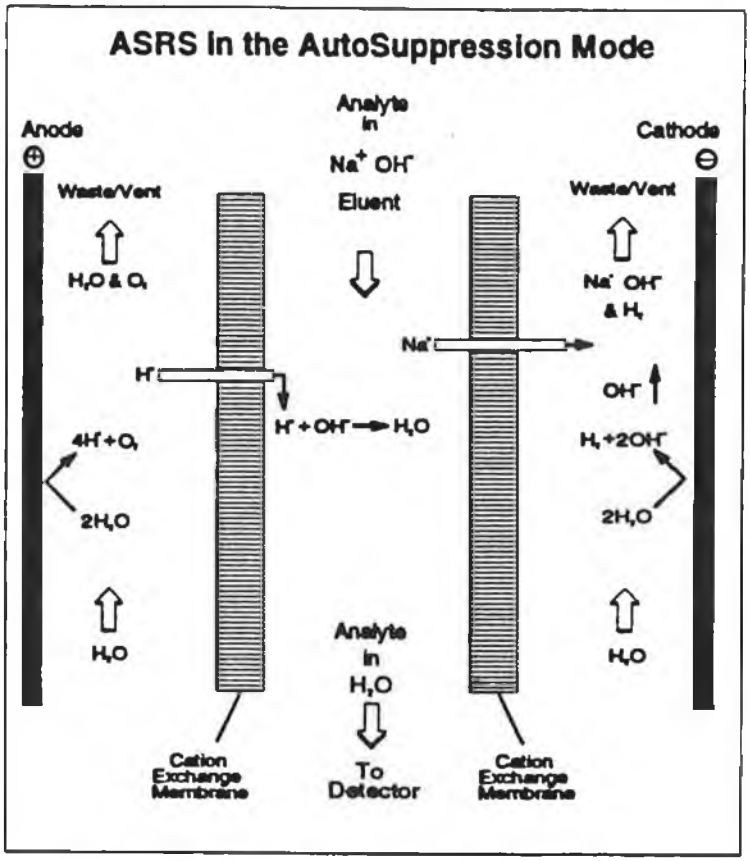


Figure 5.5 Diagram illustrating the operation of an anion micro membrane suppressor, which can be operated in either the chemical suppression mode, with sulphuric acid used as regenerant or as an Anion Self-Regenerating Suppressor (ASRS), with water used as the regenerant. (Reproduced from Dionex, ASRS Manual, 1994.)

Amperometric detection is based on the oxidation or reduction of analyte molecules at surface of the working electrode in a flow through cell. A single constant potential is applied to the electrode and the current is measured directly and reported to a data recording device. Under the right conditions, any molecule can be oxidised or reduced. However, to be detected by D.C.. amperometry, it must be electrolyzed at a lower potential than the components of the mobile phase. Also, if the electrolysis is an oxidation process, it must also occur at a lower potential than the oxidation of the working electrode itself. There are several major classes of analytes that fit into this category. The most important are molecules containing aromatic rings substituted with amine or hydroxyl functional groups [42]. Aromatic amines, phenols, and catechols have in common the presence of a pair of non-bonding electrons on the nitrogen and oxygen atoms. These are able to shift towards the aromatic ring and stabilize the positive charge resulting from oxidation, making the reaction favorable at a relatively low potential. Aromatic nitro compounds can be detected by reduction as the electron-withdrawing group stabilizes the negative charge on the aromatic ring. Amperometric detection is generally not applied to the detection of organic acids as they are electrochemically inactive. They can, however, be detected after derivatisation.

Potentiometric detection is a process in which potential changes at an indicator electrode are measured with respect to a reference electrode under constant current flow. The potential of the indicator electrode varies with the concentration of a particular ion in solution. Although there has been research published on potentiometric detection in ion chromatography, it has generally not been used as a routine analytical method. Potentiometric detection has the main disadvantage of having somewhat lower sensitivity than conductivity detection, a slower response time and a less stable baseline. Perhaps the major reasons why potentiometric detection is not used more commonly is that it possesses no major advantages over other forms of detection, and that there are also no commercial detectors available.

Detection using post column reaction (PCR) involves the chemical reaction of analytes as they elute from the column, before they go to the detector. The main aim

of this method is to increase the specificity and sensitivity of the detection method. For instance, Mongle et al. [43] described a method in which post-column derivatisation was used to enable the detection of electrochemically inactive substances with amperometric detection. The carboxylic acids were separated by HPLC and converted on line by immobilized enzymes which were covalently bound to a synthetic carrier. Hydrogen peroxide, which was produced in the reaction with oxidases, made amperometric detection possible. The procedure combined the separation efficiency of HPLC, the high substrate selectivity of enzymes and the high sensitivity of electrochemical detection.

5.2.3.2 Optical Detection methods

Spectrophotometric detection is based on the direct measurement of the visible or UV light absorbance by the analytes. Although all ions absorb light at some wavelength, spectrophotometric detection is only useful for those ions with appreciable absorbance above the solvent cutoff wavelength, approximately 200 nm. Although "appreciable absorbance" is a relative term, ions with an extinction coefficient above 1000 mol^{-1} dm^3 cm^{-1} can be detected with good sensitivity [42]. Unfortunately, not all ions fit into this category. In fact, many of the important ions such as chloride, sulphate and the alkali metals cannot be detected by direct absorbance. This was, of course, the driving force for the development of conductivity detection. The major category of UV-light absorbing ions are aromatic and heterocyclic acids and amines. Most of these compounds have one or more wavelengths of maximum absorbance in the ultraviolet with extinction coefficients between 1000 mol^{-1} dm^3 cm^{-1} and 10,000 mol^{-1} dm^3 cm^{-1}.

For analytes with strong UV absorbance, direct photometric detection provides several advantages compared to conductivity detection. High concentrations of salts from either the sample matrix or the eluent do not interfere with detection. Highly conductive eluents can be used, expanding the list of permissible eluents and thereby

increasing the control the user has over the chromatography. Also, a suppressor column is not required.

An example of the use of UV-absorbance detection is the determination of nitrite and nitrate in potassium chloride soil extracts. The diluted extracts contain 1% potassium chloride, which greatly overloads both the column and the detector when standard carbonate-bicarbonate eluent is used with conductivity detection. However, using UV-absorbance detection allows potassium chloride to be used as the eluent. This matching of eluent to the sample matrix greatly increases the amount of potassium chloride which may be loaded onto the column, since the form of the resin (chloride) does not need to be converted back to carbonate from the eluent. The same technique can also be used to determine UV-light absorbing anions in sea water, using sodium chloride as the eluent. Another example is the detection of precious metal cyanides, usually contained in high ionic strength sample matrices such as mining leachates.

As in electrochemical detection, post column reaction (PCR) methods can be used to increase the sensitivity of spectrophotometric detection. A post-column system was studied by Wada and co-workers [44]. For the post-column reaction, dicyclohexylcarbodiimide, bromocresol purple (BCP) and promophenol blue have been used.. The principle of detection using a pH indicator such as BCP, is that when an organic acid is added to a buffer solution, which contains the pH indicator, the pH of the buffer solution decreases causing the indicator to change colour. Therefore, the organic acid can be detected photometrically by monitoring the wavelength of maximum absorption of the pH indicator in its acidic form.

The most common application of photometric detection following post-column derivatisation is the detection of metals ions derivatised with 4-(-2-pyridylazo)resorcinol (PAR). The post-column reagent usually contains less than 1 mM PAR dissolved in a high pH buffer. The PAR forms a visible light absorbing complex with most transition metals with a λ_{max} at 520 nm. The major advantage of post-column derivatisation is that ions maybe detected which cannot be detected by

other means. This far out weighs the disadvantages of requiring the addition of a post-column pump [42].

Fluorescence detection is rarely used in ion chromatography as a direct detection method since very few ions fluoresce, and fluorescence detection is only used following post-column derivatisation.

Refractive index detection is a general, non-selective method, but because of only moderate sensitivity, poor selectivity and sensitivity to baseline fluctuations, it is rarely used in ion chromatography.

Of the many forms of detection used in ion chromatography, conductimetric detection is still the most useful. New detection methods originally developed for HPLC are now being used in ion chromatography; for example ion chromatography-mass spectroscopy. Ion chromatographic separation may also be coupled with atomic spectroscopy providing exceptional selectivity for certain metals ions.

5.3. EXPERIMENTAL

5.3.1 Anion Exchange Chromatography

The samples were separated on a Dionex system which incorporated a gradient pump, anion trap pre-column and an anion micro membrane suppressor (Dionex, Sunnyvale, CA). The sample loop size was 50 µl and the flow rate was 2.0 ml/min. The separation of organic acid anions was accomplished in a 22 min analysis run, with a 7 min re-equilibration step using gradient elution with a Dionex IonPac AS-11 (4 mm x 250 mm) column (Dionex, Sunnyvale, CA) and suppressed conductivity detection, with 25 mM sulphuric acid as regenerant. The regenerant flow rate was 12-15 ml/min. The column, consisting of 55% divinylbenzene microporous beads, allows for separation of a large number of inorganic and organic anions, and has the advantage of being 100% compatible with HPLC solvents.

5.3.2 Reagents

All eluants, standards and the regenerant were prepared using de-ionised water, which was purified by passing distilled water through a Waters Associates Milli-Q Water purification system. All salts, organic acids and cyanoacrylate adhesive samples were supplied by Loctite (Irl.) Ltd. The sodium hydroxide was of semiconductor grade (99.99%) and was obtained from Aldrich. The eluant solutions were carefully prepared, minimising contact with air and stored under helium. Prevention of carbon dioxide absorption in the eluant is essential, as it leads to the production of carbonate contamination, leading to high background conductivity and erratic results. The citric acid extraction solution was prepared by dissolving 0.32 g of Analar grade citric acid in one litre of water.

5.3.3 Sample Preparation

1.5 g of ethyl cyanoacrylate monomer was dissolved in 20 ml chloroform, and to this was added 5 ml of citric acid solution, pH 3.0. After thorough mixing, the solution was left standing to allow complete separation of the organic and aqueous layers, before removal of the aqueous layer and filtering through a 0.45 μm filter prior to analysis.

5.3.4 Standardisation and Quantification

A linear regression analysis of peak areas versus the concentration of each component was used to calculate the slope, intercept and correlation coefficient of each component. The equation for the calibration curve of each component was then used to calculate its concentration from peak areas of the organic acid anions in each cyanoacrylate sample. In all samples, phthalic acid (30 ppm) was used as an internal standard.

5.4. RESULTS AND DISCUSSION

5.4.1 Sample preparation

Cyanoacrylate adhesives (CA) are inherently difficult to work with, mainly due to the limited solubility of these compounds in a small number of organic solvents and the extreme reactivity to any aqueous environment. The difficulties encountered in the analysis of cyanoacrylate adhesives in general, revolves around the development of a practical sample preparation procedure. For instance, ethyl cyanoacrylate monomer is soluble in tetrahydrofuran (THF) and chloroform; while the polymer is soluble in these solvents it is more difficult to get into solution. The initial approach taken in this project involved dissolving the CA monomer in THF followed by direct injection onto the AS-11 column. The Dionex AS-11 column was chosen for its compatibility with HPLC solvents, including THF, up to a maximum concentration of 40%. The approach using THF had the obvious advantage of minimal sample preparation, which up to this was the major obstacle encountered with CA samples. In order to ensure the CA remained in solution when injected onto the column, the THF concentration in the eluent had to be maintained at a level of 40%. In practice, using a sodium hydroxide/THF (40%) eluent resulted in non-retention of the anions on the column as they eluted with the solvent front, presumably due to the high THF concentration.

Therefore, a fresh approach in terms of sample preparation had to be taken to develop an analytical technique which was reproducible and effective at removing the anions from the cyanoacrylate matrix, yet with as few steps as was practically possible. The use of an aqueous extraction procedure offered the practical means of removing anions from the cyanoacrylate matrix into an aqueous environment which was ideal for analysis using anion chromatography. Cyanoacrylate-based adhesives polymerize rapidly on contact with water adding to the practical difficulties, as the process of dissolving the polymer is slow and difficult.

But more importantly, the polymer is also susceptible to alkaline hydrolysis, in even mildly alkaline conditions, which leads to unzipping of the polymer chain, and this further complicates the sample preparation and subsequent analysis. Therefore, carrying out the extraction into the NaOH-based eluent or even deionised water was not practical. However, by carrying out the extraction into an acidic environment (pH 3-4) the problems of polymerization and alkaline hydrolysis are minimised. In order to accomplish this a number of acids were tested including oxalic, tartaric and acetic; however, citric acid proved to be the most suitable, as the citrate peak elutes subsequent to all of the analyte peaks of interest and was not present in any cyanoacrylate samples. The success of the aqueous extraction procedure was dependent on obtaining the correct balance between the amount of cyanoacrylate dissolved in the chloroform and the ratio of organic phase to aqueous phase, in terms of volume. Initially working with 20 cm^3 of chloroform, the concentration of cyanoacrylate and the volume of aqueous solution used in the extraction with which to obtain the best results was investigated. It was found that if in excess of 1.5 g of cyanoacrylate was dissolved in 20 cm^3 of chloroform, on carrying out an aqueous extraction, the separation of the aqueous and organic layers was affected by the accumulation of precipitate at the interface, which interfered with the removal of the aqueous layer. Also, in order to minimise the dilution of the anions in the cyanoacrylate samples and yet not interfere in the extraction procedure, 5 cm^3 was the minimal volume of aqueous phase required. Therefore, the extraction procedure consisted of dissolving 1.5 g CA in 20 cm^3 chloroform, with the anions being extracted into 5 cm^3 of aqueous solution; although some precipitate was present in the aqueous layer, it did not interfere in the successful operation of the procedure.

It should also be noted that a third approach was also investigated, which involved initially dissolving the CA in THF, polymerizing the CA in solution by addition of a suitable initiating agent, then adding water to precipitate the polymer out of solution. Although a precipitate was formed in the solution, attempts to filter this solution prior to analysis by anion chromatography proved impossible due to the density of the precipitate.

This yet again highlights the difficulties in terms of sample preparation when dealing with cyanoacrylate adhesives.

5.4.2 Separation

The biggest obstacle in the separation of the anions was to develop the optimum gradient conditions to achieve the best resolution, in particular to obtain baseline resolution of the early eluting anions. Sodium hydroxide was chosen as the counter ion for gradient elution to minimise the background shift, while gradient elution had the advantages of greater resolution with shorter analysis time compared to isocratic elution and more importantly, it is possible in a single analysis run to determine mono-protic, di-protic and tri-protic acids. The chromatogram shown in Figure 5.6 demonstrates the resolution eventually obtained in one analysis run, using gradient elution ion chromatography with suppressed conductivity detection, for the twelve anions examined. The optimum gradient conditions are given in Table 5.1. The resolution obtained for the first three eluting anions is reasonable and problems are only encountered in HPSA-stabilised adhesives samples where considerable levels of formate are present. In some instances, p-toluene sulphonic acid (pTSA) is also employed as an in-process stabiliser, and therefore it is possible that some of this acid could be present in purified monomer samples. As can be seen from Figure 5.6, the malonic acid and p-toluene sulphonic acid peaks are poorly resolved; however the levels of pTSA detected in purified monomer samples is so low that there is minimal interference in the determination of malonic acid.

The main difficulty encountered with this method was obtaining separation of the first three eluting peaks; namely formate, hydroxypropane sulphonate and methane sulphonate. The addition of 15% methanol as an organic modifier aided in this regard, by decreasing the retention time of the more hydrophobic species and thereby increasing the separation.

Standard curves were linear within the ranges given in Table 5.2 and all have linear regression values greater than 0.995. The standard curves were generated by addition of anion standards to a cyanoacrylate adhesive sample which was dissolved in chloroform and the standards were separated from the cyanoacrylate matrix using the aqueous extraction procedure discussed previously. A blank, consisting of just cyanoacrylate adhesive, was then analysed for residual levels of anions and these quantities were subtracted from the standard solutions.

The narrower range of linearity for formate and hydroxypropane sulphonate was attributable to the limited resolution obtained for these compounds using this column. The limit of detection was determined as a signal to noise ratio of 3, and the results of the study of reproducibility of the method for a cyanoacrylate sample and the relative standard deviation are given in Table 5.3.

The criteria for an effective internal standard were fulfilled by phthalic acid, which was used as an internal standard at a concentration of 30 ppm, in all samples. Although a number of carboxylic acids were tested for their suitability, such as oxalic and tartaric acids, phthalic acid was determined to be the most suitable as it does not interfere in the analysis of the anions as the citrate peak elutes after all the analyte peaks, is practically insoluble in chloroform and is not inherently present in cyanoacrylate adhesives.

Species determined by ion chromatography are invariably nearly all ionic, so that conductivity detection is the detection method of choice for the vast majority of applications. The introduction of suppressor technology to remove most of the background conductance of the eluent revolutionised ion chromatography. Modern suppressors are capable of suppressing eluants with sodium ion concentrations as high as 150 mM and the detection of anions using this method would not be possible without this suppressor technology. The background conductivity for 100% of eluent 1 and eluent 2 was 1-2 and 3-7 μS, respectively.

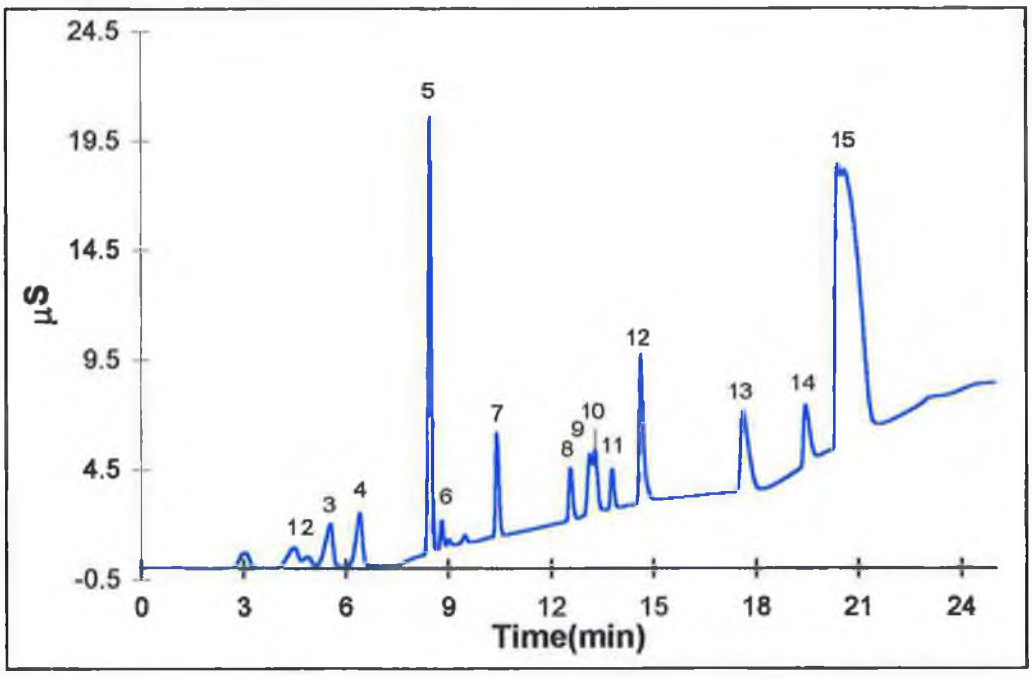


Figure 5.6 Chromatogram of inorganic and organic acid standards. Peaks: [1] formate (3 ppm), [2] HPSA (12 ppm), [3] MSA (12 ppm), [4] cyanoacetate (12 ppm), [5] chloride (10 ppm) [6] nitrate (1 ppm), [7] unknown contaminant, [8] succinate (12 ppm), [9] p-TSA (20 ppm), [10] malonate (6 ppm), [11] maleate (6 ppm), [12] sulphate (30 ppm), [13] phthalate (30 ppm), [14] ortho phosphate (18 ppm), [15] citrate (from aqueous extraction).

Time (mins)	%E1	%E2	%E3	%E4
0	80	5	0	15
2	80	5	0	15
4	75	10	0	15
5	75	10	0	15
12	0	70	15	15
16	0	65	20	15
22	0	5	80	15
Eluent 1: De-ionised water Eluent 2: 5mM NaOH			Eluent 3: 50mM NaOH Eluent 4: Methanol	

Table 5.1 Optimum gradient conditions.

Anion	Retention time (min)	Working range (ppm)
Formate	5.94	2-30
HPSA	6.27	2-120
MSA	6.89	2-160
Cyanoacetate	7.69	2-160
Chloride	8.44	0.5-100
Nitrate	8.80	0.5-100
Succinate	13.13	2-160
Malonate	13.91	1-80
Maleate	14.43	1-80
Sulphate	15.21	1-400
Phthalate	18.79	Internal Standard
Phosphate	20.19	1-240
Citrate	21.65	N/A*

N/A* : not applicable

Table 5.2 Retention times and working ranges for inorganic and organic acid anions in cyanoacrylate adhesives.

Anion	Reproducibility (n=6)		L.O.D**(ppm)
	Mean (ppm)	R.S.D.	
Formate	51.0	1.14	1.0
HPSA	8.1	3.65	1.0
MSA	450.0	0.76	0.4
Cyanoacetate	10.7	2.92	0.4
Chloride	6.6	1.25	0.1
Nitrate	8.4	1.40	0.1
Succinate	N/D*	N/D*	0.5
Malonate	130.6	2.66	0.2
Maleate	3.4	4.87	0.2
Sulphate	10.8	3.89	0.1
Phosphate	170.8	6.52	0.5

N/D* : not determined, as succinic acid was present in any cyanoacrylate samples.
LOD** : Limit of detection was based on a signal to noise ratio of 3

Table 5.3 Study of the reproducibility and L.O.D. for organic acid and inorganic anions in cyanoacrylate adhesive samples.

5.4.3 Analysis of cyanoacrylate samples

The chromatograms of the aqueous extract of two in-process cyanoacrylate monomer samples are shown in Figure 5.7 and 5.8. As can be seen from these figures, the in-process sample contains a large variety of organic acid and inorganic anions and although these samples were taken on different days, they are very similar in terms of the anions detected and their levels. MSA, pTSA, sulphuric acid and ortho phosphoric acid are added as an in-process stabiliser while the other anions are present as contaminants. It should be noted that in all instances peak identities were confirmed by spiking with standards.

However, the anion profile of an in-process sample contrasts sharply with that of a pure monomer, as shown in Figure 5.9. The cyanoacrylate sample shown in Figure 5.9 was stabilised using BF_3, hence the presence of fluoride in the sample. It is interesting that all of the major impurities present in the in-process samples are absent in the purified monomers, with the notable exception of low levels of formate and chloride. This trend is continued in two other pure monomer samples shown in Figure 5.10 and 5.11, which are stabilised by hydroxypropane sulphonic acid and methane sulphonic acid, respectively. The pure monomer samples which differ only in the acidic stabiliser present, are very similar in terms of the contaminating anions present and their respective levels. From these results there is some evidence that acid hydrolysis is occurring in the process of cyanoacrylate production, but the vast majority of these acidic impurities, with the exception of formic acid are removed during the distillation process to produce the pure monomer. It was not possible to identify the large early eluting peak in the in-process sample, but more importantly this impurity is not carried over into the final product in the form of the purified monomer samples.

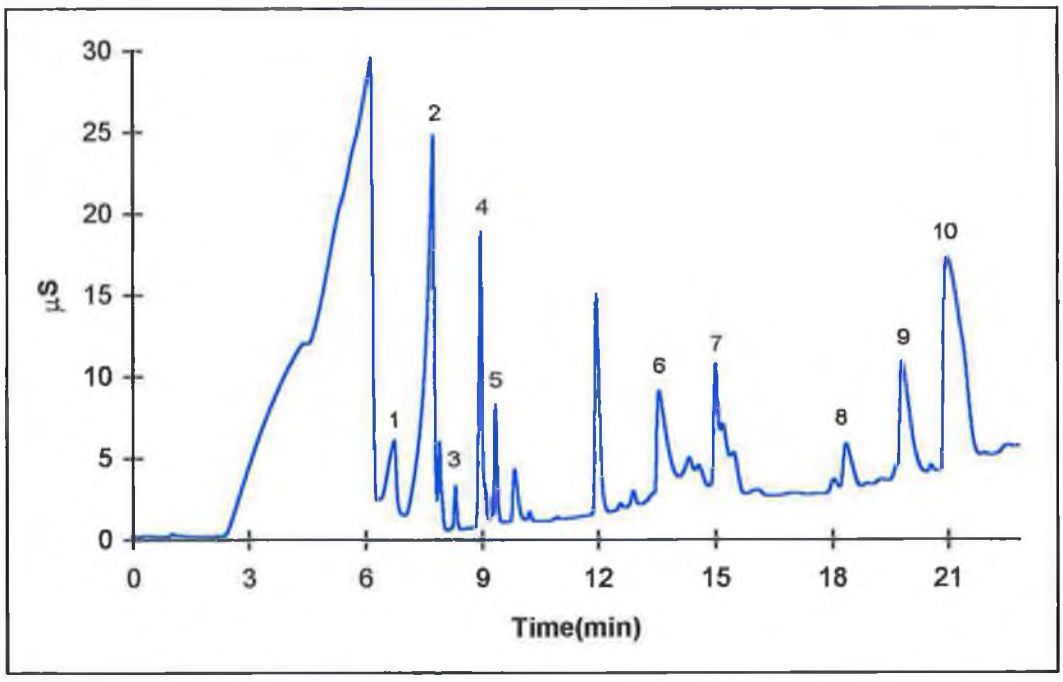


Figure 5.7 Chromatogram of the anions present in an 'in-process' cyanoacrylate sample (sample A). Peaks: [1] formate (50.0 ppm), [2] MSA (450.0 ppm), [3] cyanoacetate (10.7 ppm), [4] chloride (31.2 ppm), [5] nitrate (28.6 ppm), [6] malonate (130.6 ppm), [7] sulphate (10.8 ppm), [8] phthalate (30 ppm), [9] phosphate (170.8 ppm), [10] citrate (from aqueous extract solution).

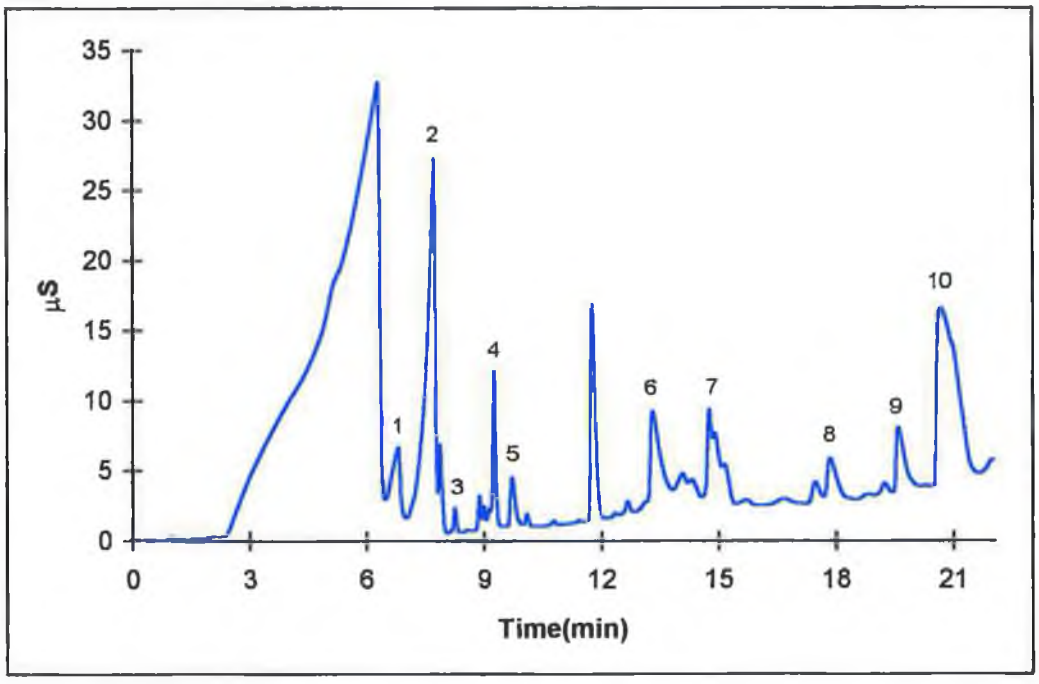


Figure 5.8 Chromatogram of the anions present in an 'in-process' cyanoacrylate sample (sample B). Peaks: [1] formate (40.3 ppm), [2] MSA (425.6 ppm), [3] cyanoacetate (4.3 ppm), [4] chloride (21.4 ppm), [5] nitrate (16.9 ppm), [6] malonate (124.6 ppm), [7] sulphate (9.3 ppm), [8] phthalate (30 ppm), [9] phosphate (78.0 ppm), [10] citrate (from aqueous extract solution).

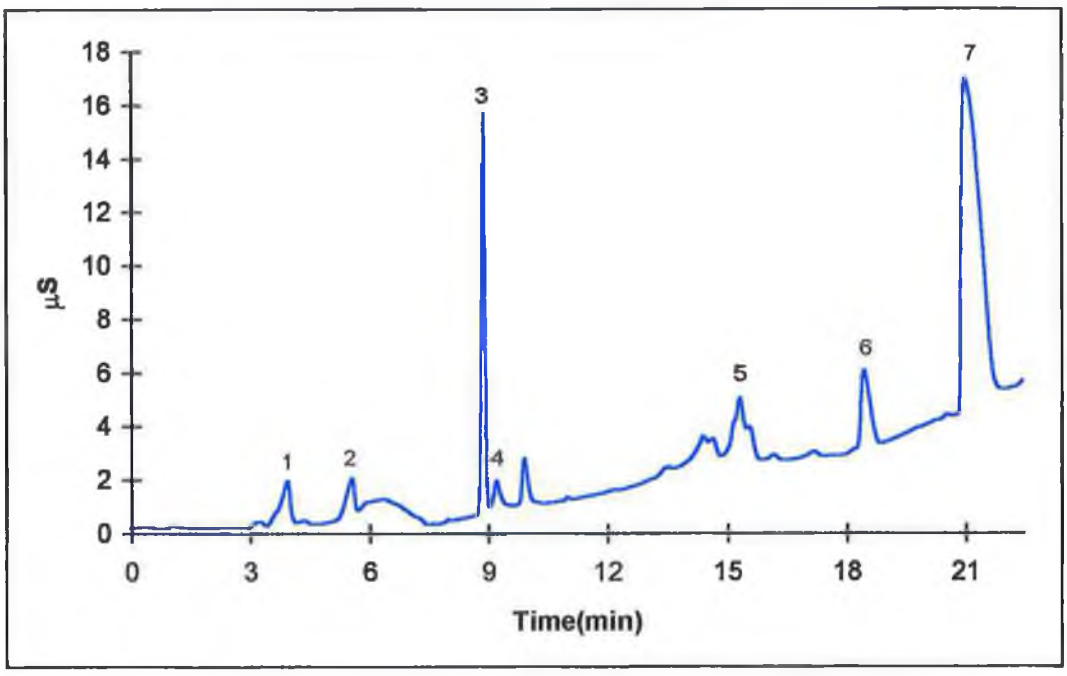


Figure 5.9 Chromatogram of a pure BF_3 stabilized cyanoacrylate sample. Peaks: [1] fluoride (N/D), [2] formate (13.8 ppm), [3] chloride (27.8 ppm), [4] nitrate (10.6 ppm), [5] sulphate (3.3 ppm), [6] phthalate (30 ppm), [7] citrate.

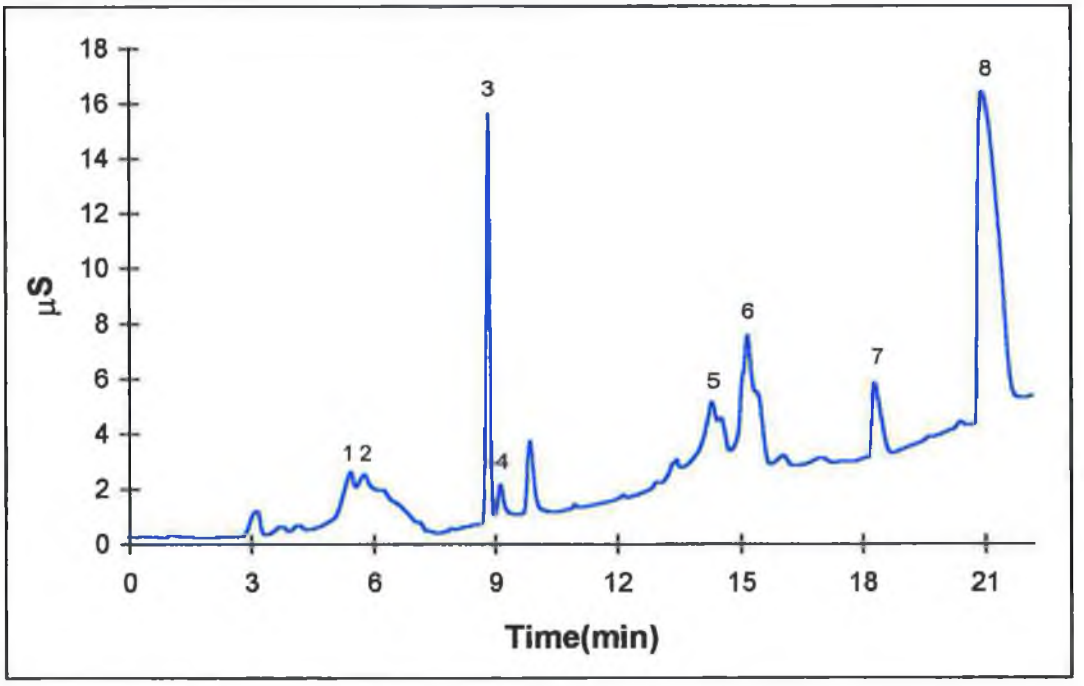


Figure 5.10 Chromatogram of a pure HPSA-stabilized cyanoacrylate adhesive sample. Peaks: [1] formate (5.6 ppm), [2] HPSA (18.1 ppm), [3] chloride (27.1 ppm), [4] nitrate (11.2 ppm), [5] malonate (3.4 ppm), [6] sulphate (7.2 ppm), [7] phthalate (30 ppm), [8] citrate.

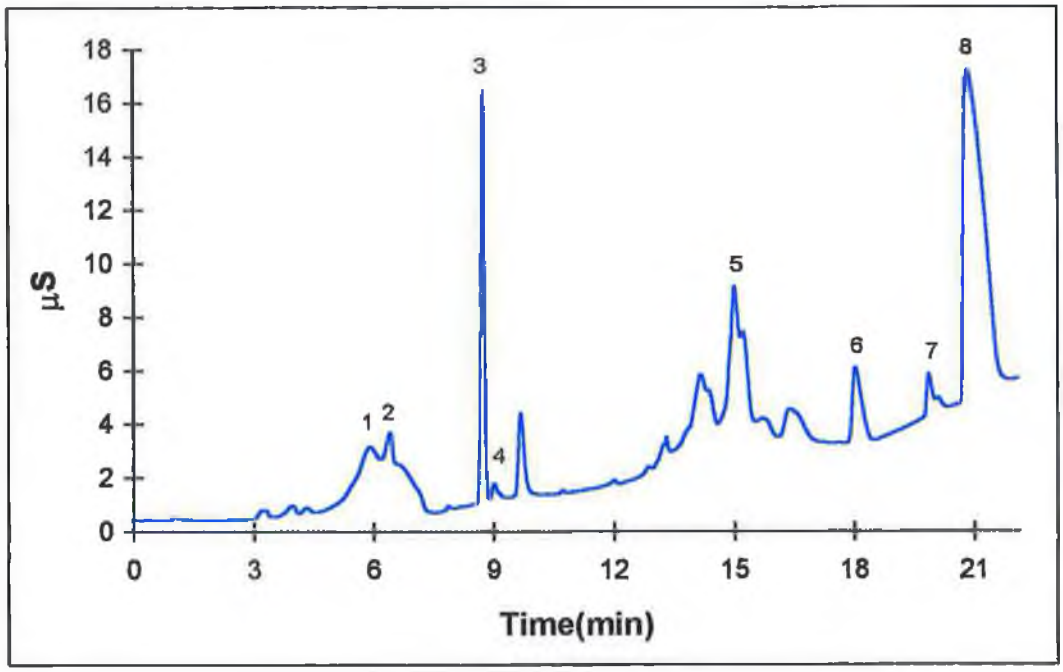


Figure 5.11 Chromatogram of a pure MSA-stabilized cyanoacrylate adhesive sample. Peaks: [1] formate (11.2 ppm), [2] MSA (8.5 ppm), [3] chloride (29.3 ppm), [4] nitrate (5.3 ppm), [5] sulphate (13.2 ppm), [6] phthalate (30 ppm), [7] phosphate (11.0 ppm), [8] citrate.

5.5. CONCLUSION

A method has been developed for the analysis of organic acid and inorganic anions in cyanoacrylate adhesives. Up to this, chloride and nitrate were determined by using a difficult time consuming process involving bomb combustion of the adhesive sample prior to analysis by ion chromatography. While the strong acid stabilizers were determined by means of a non-aqueous titration with which it was not possible to determine weak acidic contaminanting species. The sample preparation difficulties were overcome by using an acidic aqueous extraction procedure and this was followed by analysis using anion exchange chromatography. Eleven anions are separated and quantified within a 22 min run. This is an effective method not only for determining organic acid anions but also inorganic ion such as chloride and nitrate in particular, the levels of which are crucial for certain speciality applications of cyanoacrylates.

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CHAPTER SIX

OVERALL CONCLUSIONS AND FUTURE WORK

6.1. GENERAL CONCLUSION

This thesis comprises a study of three contrasting adhesive technologies using a variety of analytical techniques to gain further insight into the cure chemistries. The adhesives cure chemistry varies from transition metal based decomposition of hydroperoxides with anaerobic adhesives to autoxidative generation of hydrogen peroxide with air activated adhesives, and finally anionic polymerisation with cyanoacrylates.

6.1.1 Anaerobic Adhesives

Anaerobic adhesives were originally developed in the 1950's and research at that time concentrated on acquiring sufficient knowledge to commercialise the technology. Hence, over 40 years later, research is still being carried out to try and understand the complex cure chemistry. The complexity of the cure chemistry is illustrated by the diverse roles of the accelerators used in anaerobic adhesives. They firstly act as reducing agents, reducing transition metal ions to their lower oxidation state. And secondly, they act as complexing agents for copper(I) to catalyse the decomposition of cumene hydroperoxide.

In a similar manner saccharin, which is a weak acid, provides soluble metal ions from the substrate surface, yet does not protonate the accelerators to the extent that would be expected from its pK_a value and thereby interfere in the reduction of iron(III) by the accelerators. Also, saccharin is required for the reduction of copper(II) by APH and THQ. This apparent unique role of saccharin is not due to its acidic character as proven by replacement of saccharin with other organic acids.

Contrary to the general approach taken in the literature on the decomposition of cumene hydroperoxide by transition metal ions, the rate of decomposition of CHP is not only dependent on the oxidation state of the metal but also dependent on the metal itself. In particular, copper(I) is incapable of decomposing CHP unless complexed with two molecules of either APH or DMPT. Only in the case of APH did the presence of maleic acid actually increase the decomposition rate; in all other instances maleic acid, and saccharin to a lesser extent, inhibited the decomposition. Through all this research it has been highlighted time and time again that the cure chemistry of anaerobic adhesives is complex due to the variety of compounds and the numerous reactions and interactions that occur in adhesive formulations. And also, the chemistry of accelerators, of maleic acid and saccharin and that of the transition metals must be dealt with individually and not in a general fashion.

Future research will have to look in more detail at saccharin and the interaction of saccharin and transition metal ions, in particular copper(II). Tert-butylperbenzoate and tert-butylhydroperoxide are also employed as initiators in anaerobic adhesives; future research should investigate the interactions of these compounds with transition metal ions and the influence of accelerators on these reactions.

6.1.2 Air Activated Adhesives

Aerobic adhesive chemistry is very similar to that of anaerobic adhesives, with the notably exception that the substrate plays no role, as such, in the initiation chemistry and as in anaerobic adhesives free radicals are derived from the decomposition of a peroxide. However, in this instance the peroxide is generated via autoxidation of DHP. Therefore aerobic adhesives may find applications in instances that are not suitable to anaerobics due to lack of catalytic activity on the substrate surface where otherwise a primer would be required.

It was conformed using a variety of analytical techniques that hydrogen peroxide is generated from the autoxidation of DHP in the presence of glacial acetic acid. Also the ability of DHP to reduce copper(II) to copper(I) was demonstrated using potentiometric titrations. However, these titrations also indicated the high reactivity of the DHP/glacial acetic acid system to air.

DHP based adhesive formulations are stabilised by trace levels of transition metal ions but this stabilisation chemistry is not fully understood. The key to the commercialisation of this technology depends on gaining sufficient understanding of this stabilisation chemistry so that adhesives can be formulated that possess the right balance between reactivity and stability.

6.1.3 Cyanoacrylate Adhesives

In comparison to the two previous examples, cyanoacrylate adhesives are very simple and straight forward in terms of cure chemistry, consisting basically of monomer. But the nature of the monomer makes cyanoacrylates highly reactive compounds and as a consequence when it comes to analysis, sample preparation is very difficult. The method developed for the analysis for organic acid anions and inorganic anions was based on an acidic aqueous extraction to remove the anions from the cyanoacrylate matrix followed by analysis by anion exchange chromatography This method allows for the simultaneous determination of strong acid stabilizers and inorganic anions such as chloride and nitrate, in a single analysis run. But, more importantly it also allows for the determination of weaker contaminating acidic species such as formic acid and o-phosphoric acid, which cannot be detected using the conventional non-aqueous potentiometric titration method.

In the future this method could be included in the quality control testing schedule for cyanoacrylate adhesives as a sensitive and reliable method for the determination of contaminating acidic species which can have a deleterious effect on adhesive performance as well as for the assessment of strong acid stabilisers levels. Both p-toluene sulphonic acid and hydroquinone elute very close to peaks of interest, notably malonate and sulphate, therefore slight modification in the gradient elution profile would be required to obtain better resolution of these peaks. Hydroquinone and p-TSA are only present in appreciable quantities in 'in-process' samples, therefore no resolution problems were encountered for analysis of pure monomer samples. It would be interesting to identify the large early eluting peak detected in the 'in-process' cyanoacrylate samples. Although this peak is not carried over into the pure monomer samples, the size of the peak indicates that there are considerable quantities of a compound present which may have been generated from side reactions in the production process.

Appendix A: PUBLICATIONS

1. An Electroanalytical Investigation of Reactions undergone by Elemental Iron and Copper in the Presence of Anaerobic Adhesive based Cure Components. D.P. Raftery, M.R. Smyth, R.G. Leonard and M.C. Brennan, Intl. J. Adhesion and Adhesives, in press.

2. Reactions undergone by 1-Acetyl-2-Phenylhydrazine in the presence of active metal. D.P. Raftery, M.R. Smyth, R.G. Leonard and D. Heatley, Intl. J. of Adhesion and Adhesives, in press.

3. Identification of Hydrogen Peroxide as the Autoxidation Product of N-phenyl-2-propyl-3,5-diethyl-1,2-dihydropyridine. D.P. Raftery, M.R. Smyth, R.G. Leonard, B.J. Kneafsey and M.C. Brennan, Anal. Comm., in press.