



*The Application of Capillary
Electrophoresis and Liquid
Chromatography/Mass Spectrometry
to the Analysis of Anaerobic and
Cyanoacrylate Adhesives*

By

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A Thesis Submitted for the Degree of
Master of Science

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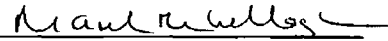
September 2002

Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Master of Science (MSc) 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

September 2002



Niamh M McCullagh

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For Mum and Dad

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Abbreviations

ACN	acetonitrile
APH	1 - acetyl – 2 phenylhydrazine
API-ES	atmospheric pressure ionisation - electrospray
APCI	atmospheric pressure chemical ionisation
CHP	cumene hydroperoxide
CE	capillary electrophoresis
DHP	1,2-dihydropyridine
DMPT	N,N – dimethyl – para-toluidine
DPM	dipiperidinomethane
DPP	differential pulse polarography
DPV	differential pulse voltammetry
EOF	electroosmotic flow
EPC	ethylpiperidine carboxylate
ESA	ethane sulphonic acid
FTIR	fourier transform infrared spectroscopy
GC	gas chromatography
GPC	gel permeation chromatography
HPLC	high performance liquid chromatography
LC/MS	liquid chromatography/mass spectrometry
LOD	limit of detection
MEKC	micellar electrokinetic chromatography
MeOH	methanol
MS	mass spectrometry
MSA	methyl sulphonic acid

NACE	non – aqueous capillary electrophoresis
PVSA	poly-vinyl sulphonic acid (sodium salt)
SDS	sodium dodecyl sulphate
TEA	triethylamine
THQ	1,2,3,4 tetrahydroquinoline
TLC	thin layer chromatography

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Abstract

This thesis details the application of separation techniques to the analysis of anaerobic and cyanoacrylate adhesives. Chapter 1 is an introduction to adhesives, where the chemistry, composition and analysis of both types of adhesives are discussed.

Chapter 2 represents the analytical study into the determination of the products of decomposition of two reducing agents in anaerobic adhesives. The decomposition of the reducing agents, 1,2,3,4 tetrahydroquinoline and dihydropyridine, in the presence of saccharin, maleic acid, cumene hydroperoxide and the metals, iron and copper, was investigated utilising Liquid Chromatography/Mass Spectrometry. A system was developed which was applied to the LC/MS determination of the products. This chapter also includes a brief discussion on LC/MS and the cure chemistry of the cyanoacrylate adhesives.

In Chapter 3 the application of Capillary Electrophoresis to the separation of basic components found in cyanoacrylate adhesives is detailed. This chapter includes a literature survey on the analysis of basic compounds with Capillary Electrophoresis. An investigation into the optimum system for the separation of piperidine, dipiperidinomethane and ethylpiperidine carboxylate was carried out. The study includes an assessment of the suitability of a variety of electrolytes for the separation.

In Chapter 4 suggestions for future research are made and conclusions are drawn from the work carried out in the thesis.

Chapter 1

The Chemistry and Analysis of Cyanoacrylate
and Anaerobic Adhesives

1.1 Introduction

Adhesives, also known as glues or pastes, are defined as ‘substances capable of holding materials together by surface attachment’ [1]

Lime, bitumen and animal or vegetable resins were used by ancient civilisations as adhesives [1] With the exception of the introduction of rubber and pyroxylin cements in the 1900s, there was little advance in adhesive technology until the advent of synthetic resins in the 1930s

Nowadays, adhesives have a myriad of industrial and domestic applications Modern industrial processes in areas diverse as footwear manufacture, production of DVDs and printed circuit boards, the building and construction industry, and vehicle manufacturing, require adhesives

Recently, medical adhesives have been developed These adhesives seal wounds cancelling the requirement for sutures after surgery and reducing the risk of infection to the patient [1]

1.2 Anaerobic Adhesives

1.2.1 Introduction

As their name suggests, anaerobic adhesives polymerise in a relatively oxygen free environment to form a strong heat and solvent impermeable material At room temperature, these adhesives remain stable in liquid form for prolonged periods of time However, upon confinement between closely fitting metal surfaces that exclude air, the adhesive rapidly cures

Burnett and Nordlander [2], researchers at the General Electric Company (GE), identified the unique properties of these adhesives in the 1950s. They bubbled oxygen at 60 – 80°C through triethylene glycol dimethacrylate and found that the monomer remained in liquid form when cooled, provided that the aeration was continued. Discontinuation of the air bubbling or pressing the monomer between two glass plates caused it to polymerise with crosslinking, to form a strong, hard material. GE marketed the adhesive as 'Permafil'. However, it was inherently unstable and could not be stored for long periods of time.

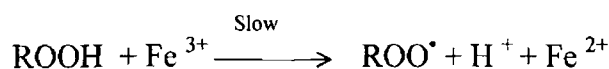
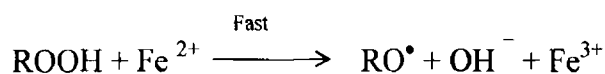
Kriebel [3], of the Loctite Corporation, solved the instability problem of the adhesive by adding controlled amounts of cumene hydroperoxide to the methacrylate monomer. This made the oxygenation process redundant and greatly reduced the amount of oxygen required to prevent premature polymerisation of the adhesive. Air permeating through a polyethylene bottle was sufficient to maintain the adhesive in its liquid form for up to a year [4].

The speed and efficiency of the adhesive cure has been improved over the years through the addition of many other components. However, since their inception in the 1950s, the combination of a methacrylate monomer and an organic hydroperoxide has remained the basis for most anaerobic adhesive preparations.

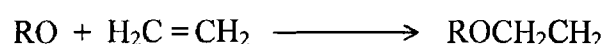
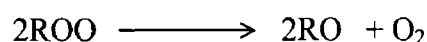
1.2.2 Cure Chemistry

The chemistry of the anaerobic adhesives is complex. However, it is known that curing occurs via a metal catalysed free radical polymerisation mechanism. This is a three-step process comprising initiation, propagation and termination (Fig. 1.1).

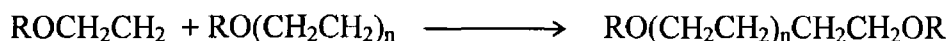
Initiation



Propagation



Termination



(R = alkyl group)

Fig 1.1 The reaction scheme of the redox –based cure chemistry of the anaerobic adhesives. The key catalytic role played by the active metal surface is evident in the initiation step where the metal catalyses the decomposition of the peroxide [5]

The unique feature of this polymerisation is the initiation step. On confinement of the adhesive between metal surfaces, an active free radical is formed due to the metal-catalysed decomposition of the hydroperoxide. This active radical has the ability to initiate polymerisation. The oxidation state of the metal governs its rate of reaction, the lower oxidation state allowing the reaction to proceed at the faster rate. In the presence of oxygen, the peroxy radicals react with molecular oxygen to produce inactive radicals that cannot initiate polymerisation. Suffice it to say that the amount of initiator in the formulation, and its balance with the accelerator and inhibitors, is crucial to the fast cure speed of the adhesive.

The type of metal substrate is also very important to the cure speed of the adhesive. Copper and iron have been proven to be active metals [6], while zinc and cadmium require a primer composition that contains active components capable of initiating polymerisation [7].

1.2.3 Development of an Efficient Cure System for Anaerobic Adhesives

An anaerobic adhesive preparation containing only the hydroperoxide initiator and the methacrylate monomer would have an extremely inefficient cure speed because the oxygen produced would retard the curing process. Therefore, a modern adhesive is a complex formulation also containing accelerators, stabilisers and modifiers.

Krieble [8] found that a combination of an amine with a formamide or succinamide increased the cure speed of the anaerobic adhesives. Concurrent with this increase in cure speed was a decrease in the overall stability of the adhesive. The combination of a sulphimide with small amounts of a di-, or tri-alkylarylamine increased the cure speed of the adhesives without compromising their stability during storage [9].

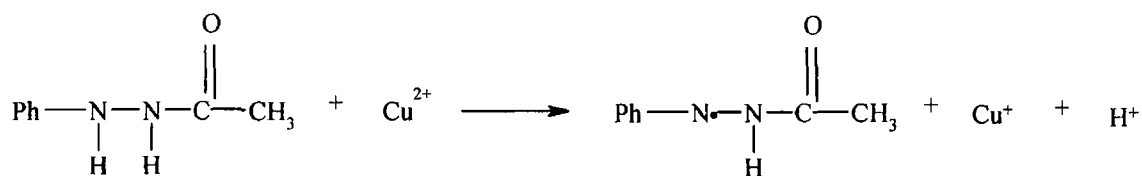
Krieble generally concentrated on cure systems consisting of N, N - dimethyl - para toluidine (DMPT) or 1,2,3,4 tetrahydroquinoline (THQ) combined with saccharin. While each of these compounds is an accelerator in their own right, the amine interacts with the saccharin to produce a significant increase in the overall cure speed of the adhesive.

In 1986, Rossi et al [10] patented the incorporation of a polymerisable accelerator that was also claimed to increase cure speed. Studies carried out by Rich [11] in the 1980s found that hydrazine accelerators further increased the cure speed of the anaerobic adhesives.

A key example of this complex interaction is the polymerisation of methylmethacrylate in the presence of copper using the combination of 1-acetyl-2-phenylhydrazine (APH) with cumene hydroperoxide (CHP). This mechanism has been extensively studied by Okamoto [12] and Raftery et al [13]. From the studies a mechanism for the reaction was proposed, which is detailed in figure 1.2. Initially, the APH acts as a reducing agent reducing Cu (II) to Cu (I) in Equation 1 (Fig. 1.2). A cumyloxyl radical is also produced in this step. The Cu (I) then decomposes the CHP in Equation 2 to generate the free radical. Equation 3 shows the reaction between the cumyloxyl radical from Equation 1 and the CHP to produce further free radicals. Finally, the cumyloxyl radical can react with the Cu (II) resulting in the regeneration of Cu (I) and the formation of a side product, 1-acetyl-2-phenyldiazene. The free radicals generated from Equations 1- 4 initiate the polymerisation of methylacrylate monomer (Equation 5) resulting in the methylmethacrylate polymer as shown in Equation 6.

The advent of more active cure systems increased the instances of premature polymerisation in the adhesives. Previous attempts to combat this problem by adding greater amounts of inhibitor to the adhesive preparations, e.g. hydroquinone [14], failed because the excess of inhibitor only served to reduce the overall performance of the adhesives. Chelating agents, added to the adhesives to scavenge any trace metals in the formulations, prevent the free radical generation process. These stabilisers greatly increased the inherent stability of the final adhesive preparations.

Equation 1

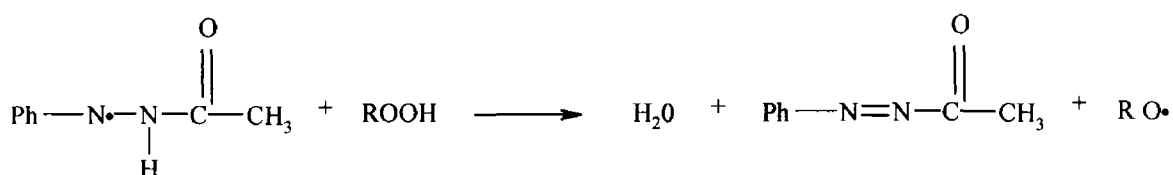


1-Acetyl-2-phenylhydrazine

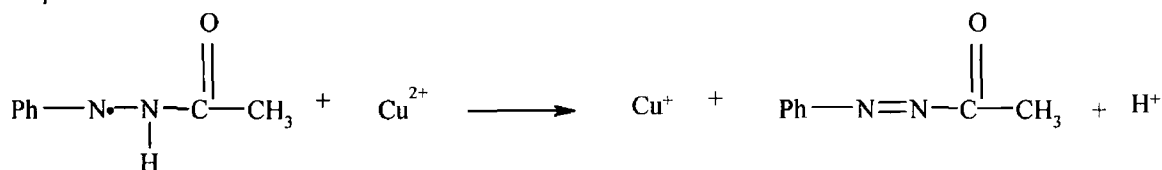
Equation 2



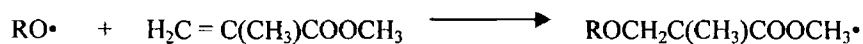
Equation 3



Equation 4

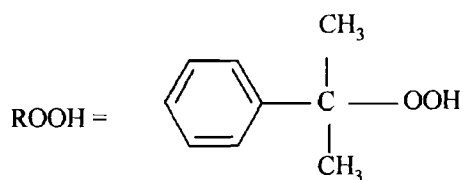
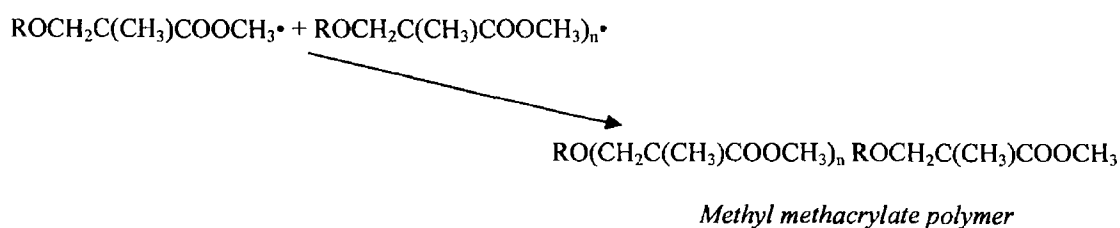


Equation 5



Methyl methacrylate monomer

Equation 6



Cumene hydroperoxide

Fig 1 2 Proposed mechanism for the curing of methylmethacrylate monomer [11, 12]

1.3 Analysis of Anaerobic Adhesives

1.3.1 Introduction

The wide variety of components required to produce an anaerobic adhesive are detailed in Table 1.1

Component	Function	Examples	
Monomer	Fundamental component of the anaerobic adhesive on which the curing efficiency is dependant	<ul style="list-style-type: none"> • Polyethylene glycol dimethacrylate • Trimethylpropane trimethacrylate • Methyl methacrylate 	
Accelerators	Interact with each other to promote the polymerisation of the monomer	Organic Peroxides	<ul style="list-style-type: none"> • Tert butylhydroperoxide • Cumene hydroperoxide
		Reducing Agents	<ul style="list-style-type: none"> • 1-Acetyl-2-phenylhydrazine • 1,2,3,4-Tetrahydroquinoline • N,N-dimethyl-<i>p</i>-toluidine
		Organic Acids	<ul style="list-style-type: none"> • Saccharin • Maleic Acid
Chelating Agents	Scavenge any metal ions present in the adhesive, thereby increasing its shelf – life	<ul style="list-style-type: none"> • Ethylene diamine tetracetic acid (EDTA) • 8-hydroxyquinoline 	
Stabilisers and Inhibitors	Prevent the premature polymerisation of anaerobic adhesives	<ul style="list-style-type: none"> • <i>p</i>-Methoxyphenol • Hydroquinone 	
Modifiers	Incorporated into the adhesive to achieve the desired physical form and appearance	<ul style="list-style-type: none"> • Thickeners e.g. polyester resins • Plasticisers e.g. polyethylene glycol octanoates 	

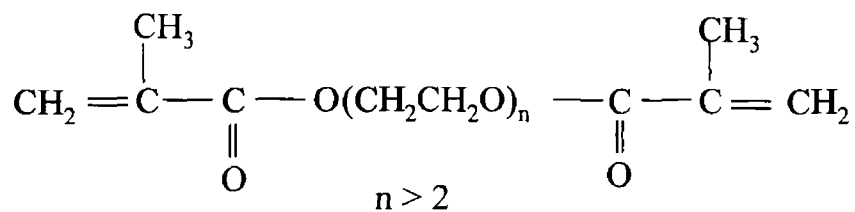
Table 1.1 The main components in an anaerobic adhesive preparation [6]

1.3.2 Components of Anaerobic Adhesives

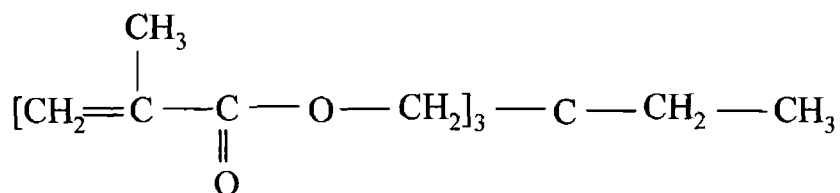
1.3.2.1 Monomers

The dominant component in any anaerobic adhesive preparation is the monomer. The cure efficiency of the adhesive is, therefore, dependent on the type of monomer used. Adhesives based on methacrylate are preferred because it is easy to synthesise, cures efficiently and has a lower sensitivity to oxygen than acrylates.

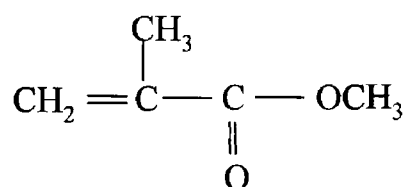
Initially, the efficiency of the anaerobic adhesives was limited by the brittleness of their cure [4]. These adhesives were based on polyethylene glycol dimethacrylate (Fig. 1.3 (I)). Introduction of flexible hydrocarbons and urethane functionality into the monomer have increased the quality of the adhesive cure. The most widely produced anaerobic adhesives remain those based on the polyethylene glycol dimethacrylate monomer [6]. Figure 1.3 illustrates some commonly used monomers.



Polyethylene glycol dimethacrylate (I)



Trimethylpropane trimethacrylate (II)



Methyl methacrylate (III)

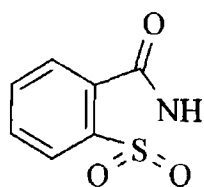
Fig 1 3 Examples of monomers used in anaerobic adhesives

1 3 2 2 Accelerators

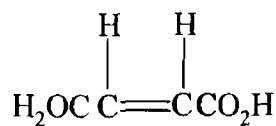
The cure system used governs the optimum performance of an anaerobic adhesive, particularly with respect to cure speed and stability of the packaged product. Typically, a cure system contains an organic acid, organic peroxide and an organic reducing agent. It is the balance of these promoters in the cure system that determines the rate of curing and the degree to which the monomer polymerises.

Commonly employed organic acids include para-toluene sulphonic acid, maleic acid (Fig 1 4 (V)) and saccharin (Fig 1 4 (IV)). Organic peroxides such as CHP (Fig 1 4 (XI)) and tert - butyl hydroperoxide (Fig 1 4 (XII)) are preferred because they can be readily reduced.

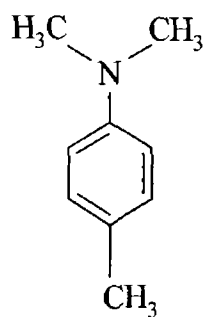
The most frequently used reducing agents are aromatic amines e.g. n,n-dimethyl-p-toluidine (DMPT) (Fig 1 4 (VI)) and 1- acetyl, 2- phenylhydrazine (APH) (Fig 1 4 (IX)). Other aromatic amines employed include 1,2,3,4-tetrahydroquinoline (THQ) (Fig 1 4 (VII)) and n-phenyl-2-propyl-3,5-diethyl-1,2-dihydropyridine (DHP) (Fig 1 4 (X)).



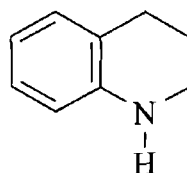
Saccharin (IV)



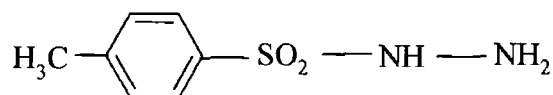
Maleic acid (V)



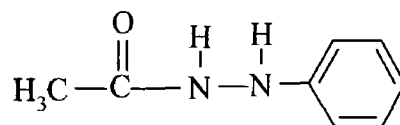
N,N-dimethyl-*p*-toluidine (VI)



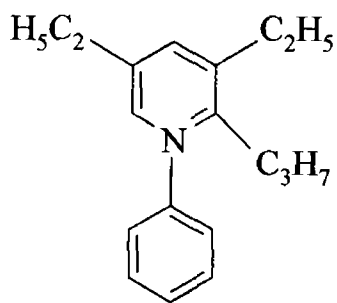
1,2,3,4-Tetrahydroquinoline (VII)



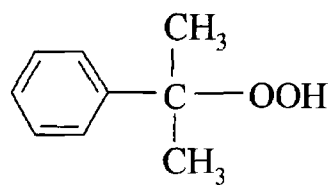
p - Toluenesulphonylhydrazine (VIII)



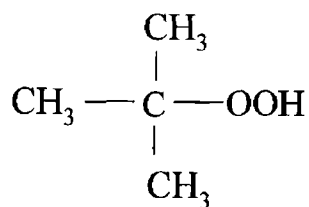
1-Acetyl-2-phenylhydrazine (IX)



N-phenyl-2-propyl-3,5-diethyl-
1,2-dihydropyridine (X)



Cumene hydroperoxide (XI)



Tert butylhydroperoxide (XII)

Fig 1 4 Examples of Accelerators

1 3 2 3 Chelators

The stability of anaerobic adhesives can be affected by the presence of trace levels of transition metals in the final adhesive formulation, which causes premature polymerisation of the adhesive

Chelating agents can be added to scavenge any metal ions present in an adhesive, thereby increasing its shelf life. The preferred chelating agents are sodium salts of ethylene diamine tetracetic acid (EDTA) which possess both oxygen and nitrogen ligands [9]. The type and concentration of the free metal ion chelator used is critical to the overall stability of the adhesive.

1 3 2 4 Stabilisers and Inhibitors

As oxygen is a natural inhibitor of the anaerobic curing process, anaerobic adhesives are packaged in low-density polyethylene containers as this allows the adhesive access to oxygen during storage. However, oxygen alone is insufficient to prevent premature polymerisation of the adhesives. Stabilisers and inhibitors are added to increase their shelf life. A stabiliser prevents premature polymerisation by reacting with the chemical species that can cause initiation, while inhibitors stop polymerisation once it has begun.

Chelating agents are regarded as stabilisers because they sequester transition metals preventing premature polymerisation. The most commonly employed inhibitors are phenols and quinones (Fig 1 5, (XIV – XV)). The phenolic inhibitors function by forming a phenoxy radical, which cannot initiate further polymerisation [7]. The mechanism of action of the quinone inhibitors remains unclear.

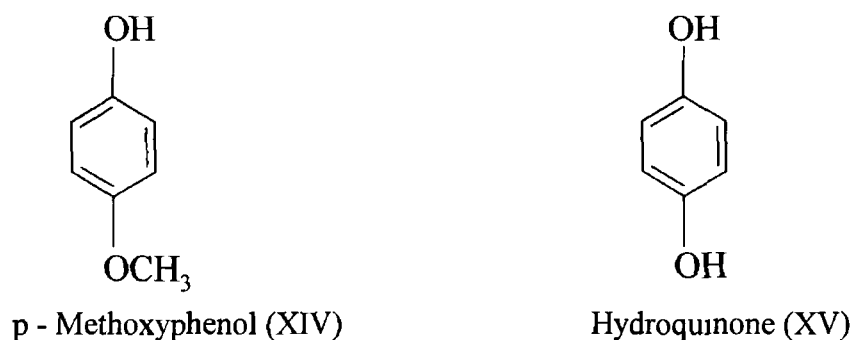


Fig 1 5 Examples of inhibitors used in anaerobic adhesives

1 3 2 5 Modifiers

A modifier is a material that is incorporated into the adhesive in order to obtain a desired physical form and appearance. However, it should not interfere with the cure chemistry in any way. The range of modifiers includes tougheners, viscosity modifiers, inorganic fillers, thixotropic agents, dyestuffs and pigments.

Examples of viscosity modifiers include thickeners such as polyester resins and polybisphenol A maleate. Non-reactive plasticisers, which lower the bond strength of the fully cured adhesive, include polyethylene glycol octanoates.

Thixotropic modifiers are generally silica – based, while calcium carbonate is frequently used as filler in anaerobic adhesives.

1.3.3 Analysis of Anaerobic Adhesives

1.3.3.1 Introduction

Analysis of anaerobic adhesives focuses on two areas: qualitative analysis of an adhesive preparation in the manufacturing environment and research into the mode of action of anaerobic adhesives.

The analysis of adhesives for quality control purposes encompasses a wide spectrum of analytical methodologies because the chemistries of these adhesive components are quite different.

1.3.3.2 Analysis of Monomers

A combination of techniques is used to analyse the monomers in adhesive preparations. Both nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy are used to determine the presence and identities of the monomer.

The quantity of monomer in an adhesive formulation is determined with either gas chromatography (GC) or high performance liquid chromatography (HPLC), depending on the volatilities of the monomers.

Wide bore or capillary columns with methylsiloxane - based stationary phases are used in the GC analysis of the methacrylate esters, while a C18 column is utilised in the reverse phase HPLC analysis of urethane-methacrylate resins. Typically, the HPLC analysis utilises a tetrahydrofuran (THF) in water mixture as the mobile phase with UV detection. Flame ionisation detection (FID) is commonly used for the gas chromatographic analysis of monomers, however, unambiguous identification of all the plasticising and monomeric components is accomplished with mass spectroscopy (MS) [15].

1.3.3.3 Analysis of Accelerators

Organic Acids

Thin layer chromatography (TLC) is used regularly in the analysis of anaerobic adhesives because it is a fast, inexpensive technique capable of determining the presence of accelerators in the cure systems

Following separation of the cure components on a silica plate with an appropriate solvent system, specific spray reagents can be used to visualise the components of interest (An example of a solvent system is diethyl ether adjusted with volumes of petroleum ether 40 – 60°C) TLC analysis of organic acids involves spraying with a solution of 2,6 - dichlorophenolindophenol sodium whereupon the acids can be visualised [16]

The total organic acid content is quantified by titration with either sodium hydroxide or tetrabutylammonium hydroxide in aqueous or non-aqueous solution, respectively The potentiometric endpoint is determined with a glass-reference electrode combination [6]

McManus [17] developed a HPLC method for the detection of a mixed ‘acid’ solution APH, saccharin, maleic acid and CHP This solution was separated using a mobile phase consisting of acetonitrile buffer, 45:55 (v/v), at a flow rate of 0.9 ml min⁻¹ The buffer consisted of a 3% (v/v) solution of triethylamine adjusted to pH 3.0 with ortho-phosphoric acid A 300 mm x 3.4 mm μ - Bondapak column was used and all components were detected at 254 nm

The method developed by MacManus [17] was a progression from the system used by Loctite [6] for the identification of all components in an anaerobic adhesive, with the benefit that the acidic components elute early. In the original method, the mobile phase was THF/water in varying ratios with a C18 column. UV detection at 254 nm was employed.

Organic Peroxides

TLC is frequently used for analysing the organic peroxides present in anaerobic adhesive preparations. The peroxy initiators are visualised by spraying the TLC plate with a methanolic solution of N, N - dimethylparaphenylenediamine dichloride followed by gentle heating. The peroxides give a purple response against a blue background [18].

Iodimetric titration determines the active oxygen content of peroxides in the adhesive. In an acidic environment the peroxy groups are reduced by the iodide ions and the liberated iodine is titrated with sodium thiosulphate. Potentiometry is used to detect the endpoint with a platinum-reference electrode combination [19].

Iodimetric titrations are cumbersome and time-consuming in comparison to chromatographic methods for quantifying peroxides. GC and HPLC allow for the identification of individual peroxides, which may be present in anaerobic adhesives.

Normally in GC analysis, peroxides decompose in the injector port. They must, therefore, be reduced to alcohols to allow for their detection by FID. Loctite have overcome this pre-analysis step by using a technique known as on-column injection. Typically, a dimethylsiloxane (OV1) column, in either narrow- or wide-bore column types is used for the separation [17].

Since the peroxides and hydroperoxides in anaerobic adhesives have a tendency to decompose at elevated temperatures, investigations were carried out by Heatley [20] into the feasibility of their analysis by HPLC. The samples do not have to be thermally stable to undergo HPLC analysis because this technique is carried out at ambient temperatures. This makes HPLC ideal for the analysis of organic peroxides. Ultra-violet detection is sufficient for analysis because the peroxides contain chromophores allowing for detection at 254nm.

The studies carried out by Heatley [20] determined that GC or HPLC could be substituted for iodimetric titration for the analysis of benzoyl peroxide and cumene hydroperoxide. The GC analysis of tert – butylperoxide suffered a loss in accuracy compared with the iodimetric titration. However, it was established that GC was the better of the two techniques because of its higher precision. HPLC was found to be a reasonable substitute for iodimetric titration in the analysis of tert-butylperbenzoate.

Studies by Moane et al [21] with differential pulse polarography (DPP) have also been carried out on peroxides. Voltammetry is based on the principle that, if at a particular potential a component of the solution is oxidised or reduced, then a current will flow at the working electrode known. The potential at which this occurs identifies the component and the amount of the current produced is proportional to the concentration of that component in the solution. In DPP, the working electrode is a small, polarizable electrode, which is produced by forcing a stream of mercury through a fine-bore glass capillary under the pressure of an elevated reservoir of mercury. A steady flow of mercury is produced. This electrode is known as the dropping mercury electrode and polarography refers specifically to the methods which use this type of electrode.

Knockers can be used to selectively detach the drop, thereby producing a more reproducible rate. The dropping mercury electrode is useful over the range 0.3V to -2.8V.

In polarography the dropping mercury electrode is the working electrode in a three-electrode potentiostat, of which the other electrodes are a reference e.g. saturated Calomel (Ag/AgCl) and counter electrode e.g. inert metal (platinum). In DPP a series of potential pulses are superimposed on a DC voltage ramp which is applied across the dropping mercury electrode and the counter electrode. The current is measured twice during the lifetime of the mercury drop, immediately after applying the pulse and directly before the drop is detached. A plot of the difference between the first and second current samples versus applied potential produces a stepped peak-shaped polarogram, from which the current can be calculated.

Moane et al [21] used DPP to determine the decomposition of hydroperoxides and perbenzoates during anaerobic adhesive cure. A number of difference cure systems were studied and the extent to which the peroxides decomposed was determined. Raftery et al [22] also applied DPP to the investigation of CHP-based cure chemistry of the anaerobic adhesives. However, DPP is not routinely used for the determination of peroxides for quality control purposes.

Reducing Agents

A wide range of nitrogen-based reducing agents are found in anaerobic adhesives, varying from hydrazines to toluidines, pyridines and quinolines. A good general technique for their identification in an adhesive preparation is TLC. After spraying with a solution of iron (III) chloride and potassium hexacyanoferrate, the aromatic adhesives are visualised as blue/green spots on the plate.

The total aromatic amine content is determined by titration with perchloric acid in an acetic acid solution [18]. The endpoint is again established using potentiometric detection with a glass-reference electrode combination.

Hai-Lin et al [5] carried out a study using Differential Pulse Voltammetry (DPV) in which the anaerobic adhesive formulation was coated on a glassy carbon electrode. The principle of DPV is similar to that of DPP except that the working electrode in voltammetry can be of any type except the dropping mercury electrode. In addition to quantifying Cu (II) and Fe (III), the investigators were able to detect N, N-dimethyl-*p*-toluidine (DMPT) and 1-acetyl-2-phenylhydrazine (APH) in the formulations tested.

1.3.3.4 Analysis of Chelating Agents

A commonly used technique for quantifying the amount of EDTA in an adhesive formulation is DPV. Metal – EDTA complexes are reduced at more negative potentials than the free metal ion. If an excess of a metal ion, such as copper (II), is added to a supporting electrolyte (e.g. 0.1M acetate buffer at pH 5.0), followed by a known amount of the adhesive sample dissolved in methanol, then two separate peaks appear on the voltammogram. One peak corresponds to the free metal ion while the other represents its complexed form. Using the method of standard additions, the chelator can be quantified by the addition of aliquots of standard chelator of known concentration to the electrolytic cell [6].

To minimise the risk of premature polymerisation, both raw materials and finished formulations must be screened for trace metal content. Atomic absorption spectroscopy (AAS) is routinely used to quantify the total trace metal content, however, the technique suffers from the inability to distinguish between free and chelated metal ions [6].

This has led to an exploration into the feasibility of using ion chromatography (IC) to distinguish between both free and complexed forms of a metal ion. Also investigated was the possibility of using IC to speciate between different metals and valencies of metals [23 – 27]

Mooney et al [23] accomplished the separation of copper (II) and iron (III) using HPLC by complexation of the metals with 8-hydroxyquinoline followed by detection at 400 nm with a spectrophotometric detector

O'Dea et al [24] used a cation-exchange column to effect the separation of copper (II), iron (III) and a number of other transition metal ions, which commonly cause the premature curing of the anaerobic adhesive. O'Dea [25] investigated the use of a mobile phase containing ethylenediammonium (EDA) and one of a selection of organic acids, including citric and maleic acids. The EDA competed with the metal ions for sites on the cation exchange column while the organic acid interacted with the metal ions to form weak anionic complexes in the eluent. These interactions determined the retention time of the metal ions and the selectivity of their separation. The ions were detected at 520nm following post-column derivatisation with 4-(2-pyridylazol-2)-resorcinol (PAR) reagent. This technique was an improvement on the method developed by Mooney et al [23] because it allowed for the separation of a wider range of metals. However, the technique suffered from an incomplete resolution of all metal ions investigated.

The use of dynamically coated reverse phase HPLC combined with post column derivitisation using PAR reagent was investigated by Deacon et al [26]. With detection at 500 nm, a successful separation of seven divalent metal cations in an anaerobic adhesive preparation was achieved. The limit of detection for all of the ions ranges from 40-70 ppb.

The method was an improvement on the previous IC method developed by Mooney [23], which had limits of detection of 100 ppb for copper (II) and 250 ppb for iron (III). The resolution between metal ions was also an improvement on the method developed by O'Dea et al [24].

Multivalent ions can be analysed with DPP. This technique allows for speciation between iron (II) and iron (III). This was demonstrated in a study carried out by Raftery et al [28], which used the ability of DPP to speciate between the valencies of iron (II) and iron (III) and copper (I) and copper (II) to determine the curing process of anaerobic adhesives. The presence of chromium, a contaminant, which appears as chromium (III) and chromium (VI), can also be determined with DPP [6].

1.3.3.5 Analysis of Stabilisers and Inhibitors

Occasionally it is necessary to quantify the amount of dissolved oxygen in an adhesive formulation. Using GC analysis with a thermal conductivity detector (TCD), a column packed with molecular sieve 5Å and a pre-column of 10% OV17 on Chromosorb W, a linear calibration plot of oxygen volumes at standard temperature and pressure (STP) vs peak area can be constructed. Using this calibration curve the amount of oxygen dissolved in an anaerobic adhesive can be determined [29].

TLC can be used to analyse these inhibitors [30]. Visualisation of the phenols and quinones is accomplished by reaction between rhodamine, ammonia and the inhibitor, which results in a coloured complex.

1.3.3.6 Analysis of Modifiers

Modifiers cover a wide range of compounds and, as a result, many techniques are used in their analysis. Infra-red (IR) spectroscopy can be used to determine the presence of a thickener in a sealant formulation. The thickener is precipitated from the adhesive formulation matrix into methanol. Following evaporation of the methanol the sample can be analysed with IR spectroscopy. Further analysis can be carried out using pyrolysis gas chromatography. In this technique the thickener, which is involatile, is decomposed into smaller volatile components at high temperature, yielding a “fingerprint” chromatogram characteristic of that thickener. A mass spectrometer may also be coupled to the GC to identify the products of the pyrolysis [5]

Gel permeation chromatography (GPC) can be used to determine the molecular weight range of the polymeric thickener in an anaerobic sealant. A column is packed with a highly cross-linked spherical polystyrene/divinylbenzene matrix, the pore diameter of which is strictly controlled. The largest molecules will elute first, being unable to penetrate the gel particles.

The degree to which the smaller molecules are retained on the column depends on their size. The smallest molecules are able to fully penetrate the gel particles, and therefore, take the longest time to progress through the column. The molecular weight distribution (MWD) of an unknown can be determined from a calibration curve constructed using standards of known weight and weight distribution [31]

1.3.4 Investigations into the Cure Chemistry of Anaerobic Adhesives

A number of investigations into the complex cure chemistry of the anaerobic adhesives have been undertaken. Some of the earliest work involved kinetic studies of polymerisation. Okamoto [32] studied the effect of the addition of peroxide to a cure system of methyl methacrylate and N, N, dimethyl – p – toluidine (DMPT) with saccharin. He found that polymerisation was 20%-50% greater in the presence of cumene hydroperoxide (CHP) and that the rate of polymerisation was independent of the CHP concentration. He also discovered that the cure speed decreased upon addition of an electron - donating group to saccharin and by any substituents on the nitrogen of the saccharin. The speed of polymerisation was also found to decrease by introduction of an open chain analogue of saccharin. Okamoto found that the addition of electron donating groups to DMPT in the ortho - position actually increased the speed of polymerisation. From this he proposed that the polymerisation was proceeded by a redox radical mechanism and that the reducing agent of the redox system was a charge transfer complex of DMPT and saccharin (Fig. 1.6).

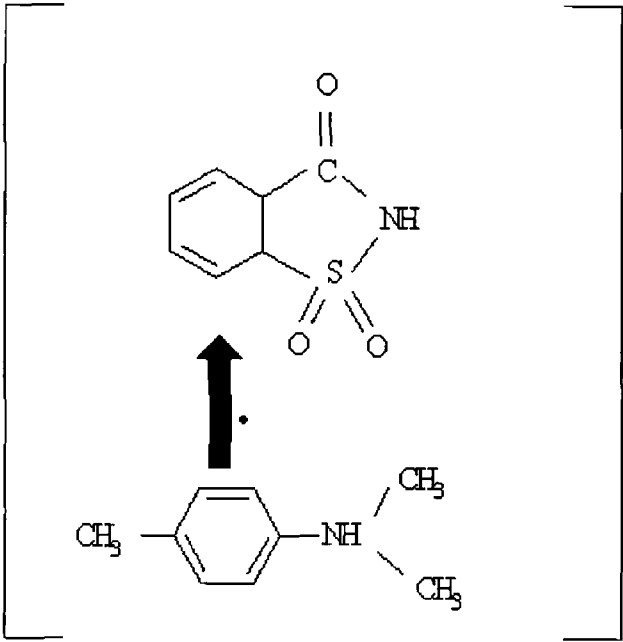


Fig 1 6 The charge transfer complex for DMPT and saccharin proposed by Okamoto [32]

Okamoto also investigated the APH/ CHP/ Copper redox system [12] from which he postulated that polymerisation was initiated by a cumyloxy radical and that APH behaved as a reducing agent, while CHP acted as an oxidising agent. Copper was the catalyst for the system (Fig 1.7)

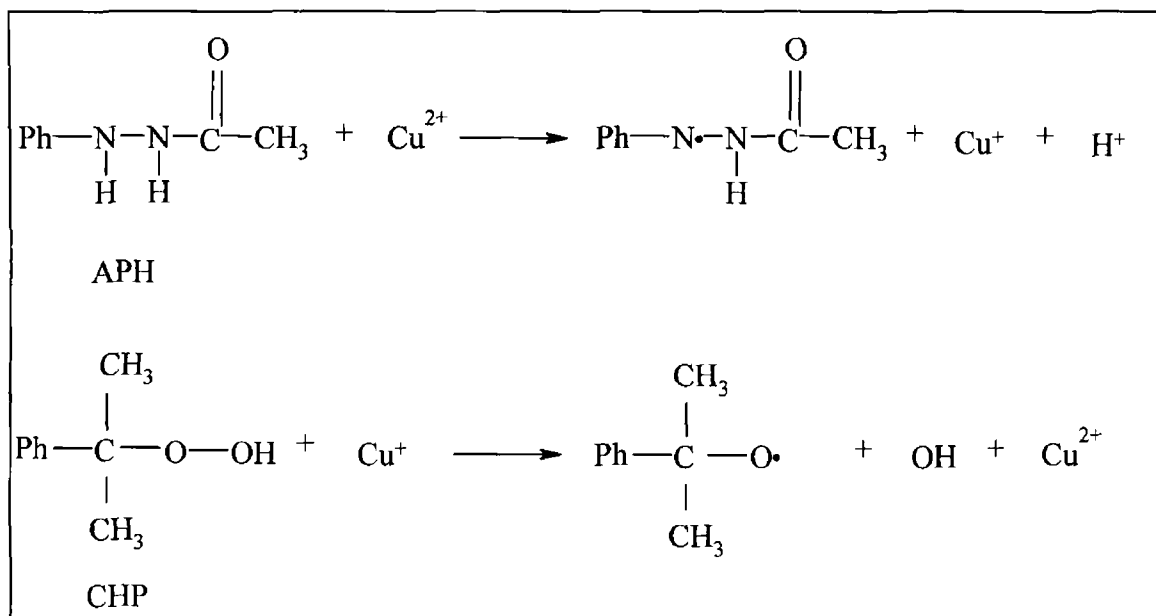
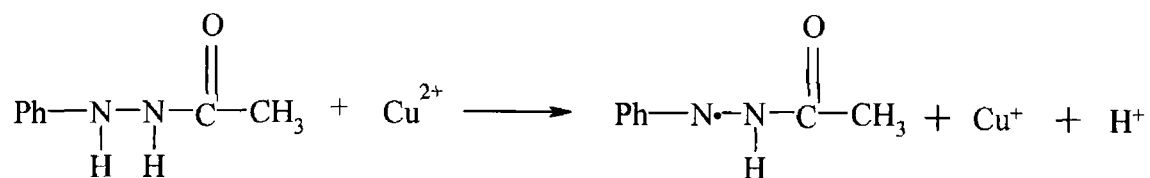


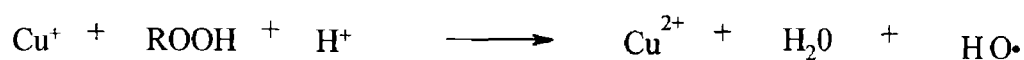
Fig 1.7 Postulated APH/CHP/Copper polymerisation mechanism [12]

A study carried out by Leonard [33] led to the following reaction scheme between APH, CHP and copper being proposed. Initially, the APH behaves as a reducing agent, reducing Cu (II) to Cu (I), as shown in equation (3) in Fig 1.8. In equation (4) the Cu (I) decomposes the hydroperoxide to generate free radicals. The hydrazine radical generated in equation (3) also reacts with the hydroperoxide to generate further radicals, as shown in equation (5). Finally, the hydrazine radical reacts with Cu (II) to regenerate Cu (I) and form 1-acetyl-2,2-phenyldiazene, which is represented in equation (6).

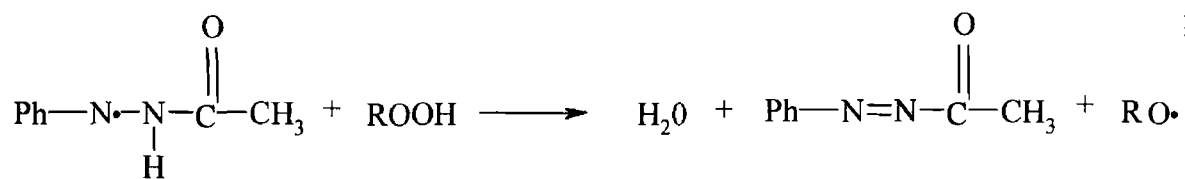
Equation 3



Equation 4



Equation 5



Equation 6

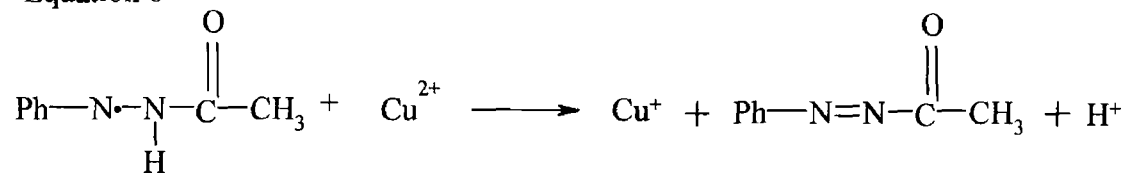


Fig 1 8 Proposed cure mechanism for APH/copper based cure systems [33]

The authors also discovered that the reaction took place at different rates depending on whether iron or copper was present. When Fe (III) was substituted for Cu (II) the only reaction that took place was the decomposition of CHP to form cumyl alcohol and methyl cumyl ether. The hydrazide based cure system was, therefore, much more sensitive to copper than iron.

Raftery and co-workers [34] also investigated the role of the co-accelerators, saccharin and maleic acid, in the APH cure system. They were not able to elucidate their exact mode of action, however, it was discovered that in the presence of saccharin the major product of the reaction was 1, acetyl-2, - diphenylhydrazine (66%) while in the presence of maleic acid 1, acetyl -2 - methyl - 2 - phenylhydrazine (33%) was the major product.

The complex nature of the anaerobic adhesive cure mechanism was demonstrated in two kinetic studies into the role of DMPT and saccharin in the radical polymerisation of methylmethacrylate [35, 36]. The first study focused on the redox initiation by CHP and copper saccharinate [36]. It was proposed that the CHP rapidly decomposed Cu (II) to Cu (I), whereupon some of the Cu (I) ions were complexed with DMPT. The complexed ions were strong reductants with respect to CHP whereas the uncomplexed copper ions were inactive. Saccharin, owing to its acidic character, was thought to protonate the oxygen atom linked to the hydrogen of the peroxide, thereby lowering the energy required to cleave the peroxide bond and increasing the rate of CHP decomposition to form free radicals.

Raftery et al [22] disagreed with this postulated role of saccharin in the curing process. Using polarographic studies it was claimed that the only evidence that the acid actually catalysed the decomposition of CHP by protonation was found in the case of APH with a 2:1 excess of maleic acid to CHP. It was also discovered that the addition of saccharin to the DMPT cure system had no effect in a 1:1 ratio but in a 2:1 ratio of saccharin to CHP the reaction was inhibited.

The second study by Beaunez and his colleagues [35] focused on the DMPT redox polymerisation initiated by CHP and iron saccharinate. Again, it was claimed that the addition of saccharin led to an increase in the decomposition of CHP. It was also proposed that DMPT reduces iron (III) to iron (II) and that uncomplexed iron was much more reactive than ions complexed with DMPT. The authors stated that uncomplexed iron (II) was unable to decompose CHP regardless of the concentration of saccharin.

It was demonstrated by Raftery and coworkers [22] that both iron (II) and iron (III) were quite efficient at decomposing CHP. The authors proposed that, while the acids protonated CHP to some extent, they also affected the reducing ability of the accelerators and, moreover, the ability of the accelerators to form complexes with the Cu (II) and Cu (I) ions.

An investigation into the decomposition of other peroxy initiators with differential pulse polarography (DPP) was carried out by Moane et al [21]. It was discovered that maleic acid reduced the rate of peroxide decomposition. The exception to this was the decomposition of *t*-butyl perbenzoate by Cu (II) with THQ, where protonation by the acid activated the decomposition of the CHP.

The study also demonstrated that the saccharin curtailed decomposition rates of all the peroxides except tert – butyl perbenzoate, where the THQ - Cu (II) and DMPT – Cu (II) complexes actually accelerated its decomposition. This was also the case with the decomposition of t – butyl hydroperoxide by DMPT-Cu (II) and benzoyl peroxide by APH – Fe (III). In all the studies it was shown that the metal-catalysed decomposition of CHP to produce a free radical is the critical step in the cure mechanism of anaerobic adhesives.

The studies carried out by Moane [21] confirmed the findings of Raftery [22] the presence of saccharin increased the decomposition rate of the peroxide by Cu (II) ion in comparison to the metal ion/reducing agent combination alone. The substitution of saccharin with maleic acid significantly reduced the rate of peroxide decomposition. In contrast, in the case of Cu (II) decomposition of the peroxide in the presence of the reducing agent (APH, THQ etc), the presence of saccharin or maleic acid reduced the peroxide decomposition rate.

An electroanalytical study by Raftery et al [28] into the reactions of elemental iron and copper in the presence of anaerobic adhesive based cure components noted that saccharin and maleic acid played key roles in the liberation of metal ions from a substrate surface. Solutions containing reducing agent and metal only generated trace levels of metal ions. However, upon addition of saccharin and maleic acid, higher levels of metal ions in the lower oxidation state were detected. Solutions containing saccharin were observed to generate greater levels of metal ions at the important lower valence state, in comparison to solutions with maleic acid.

The authors postulated that the maleic acid, being a stronger acid than saccharin, had the stronger tendency to protonate the reducing agents, thereby interfering with their reducing ability. APH, being a hydrazine rather than an amine, has less basic character and therefore is less influenced by the acid.

George et al [37] carried out a study into the reactivity of the anaerobic adhesive with a viscometer and determined that, because saccharin is a weak acid, it interacted with copper to make metallic cations available. The study demonstrated that the concentration of metallic ions (free or complexed) increased if copper was left in contact with saccharin. They also determined that copper saccharinate was formed in solutions after a prolonged period of time. George and his colleagues hypothesised that the Cu saccharinate was essential to the initiation of anaerobic adhesives cure.

Kincaid [38] carried out a number of studies into the decomposition of key reducing agents in anaerobic adhesives. He investigated whether the decomposition of the reducing agents occurred along different pathways in the absence and presence of metals. He also carried out time-based reactions to determine if there was a loss in performance of the adhesive with time. The results from his study of APH decomposition agreed with the findings of Raftery et al [34] that the main product of decomposition in the presence of CHP and saccharin was 1 - acetyl - 2, 2 - diphenylhydrazine. He determined that the main product of the decomposition of DMPT was N - methyl - N - phenyl formamide, a product of the oxidation of the amine. Also, produced in this reaction was a trace amount of methyl - p - toluidine. Kincaid found that *p*-toluene sulphonyl hydrazine (p-TSH), which is also used as a reducing agent in anaerobic adhesives, decomposed to produce a toluene sulphonic salt (Fig 1.9).

Kincaid concluded that in order to maintain optimum stability of the adhesive upon storage, the concentration of the reducing agent would have to be carefully controlled

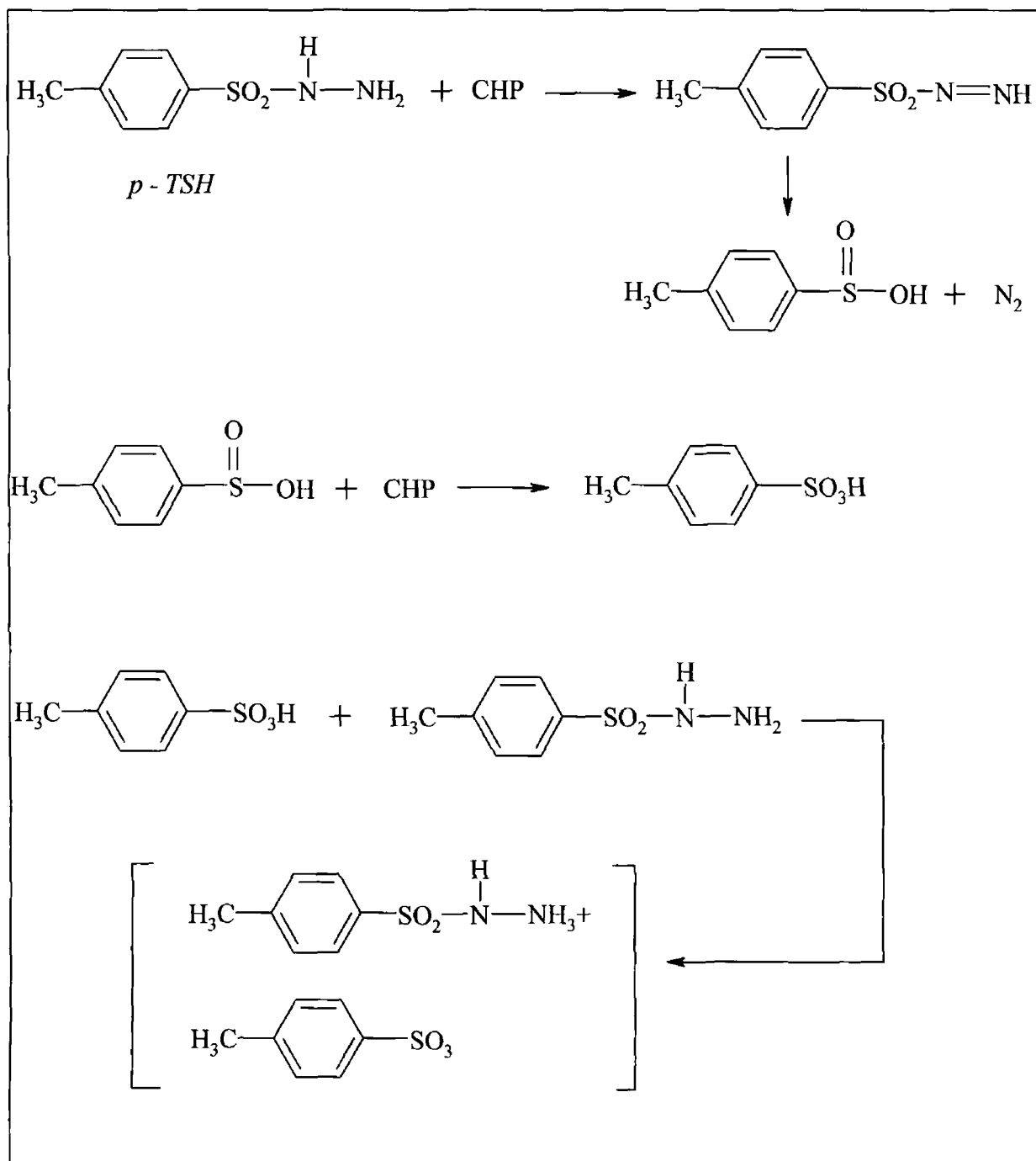


Fig 1 9 The product formed by the decomposition of p-TSH in the presence of CHP

1.4 Cyanoacrylate Adhesives

1.4.1 Introduction

Alkyl cyanoacrylate adhesives are single component, instant bonding adhesives that cure at ambient temperatures in the absence of any external energy source. In 1947 Alan Ardis of B F Goodrich reported that he had synthesised compounds that formed “hard, glass-like resins” when heat cured [39, 40]. However, the adhesive properties of these compounds, the alkylcyanoacrylates, were not discovered until the 1950s. A research team led by H W Coover at Eastman Kodak was involved in the development of new adhesives. One of the compounds under investigation was ethyl cyanoacrylate. While characterising the monomer, the prisms of an Abbe refractometer were inadvertently bonded together alerting Coover to its adhesive property [41].

In 1958, Eastman Kodak released its first cyanoacrylate adhesive made under the name Eastman 910 [42]. The adhesive had limited sales for a number of years, primarily because of two factors, firstly, their instability during manufacture, shipping and storage and secondly, consumers were slow to recognise the advantages of the adhesives. In the 1970s, however, these shortcomings were addressed and the adhesives were marketed to a much greater success. Loctite were the most notable of the companies to enter the cyanoacrylate market, introducing the adhesives as “superglues”.

Cyanoacrylates are attractive as adhesives because of their ease of application and stability during storage. They cure rapidly at room temperature forming a strong bond between many different substrates.

Cyanoacrylate adhesives are used in the assembly and repair of automobiles and in the electronics industry where they are used in the manufacture of printed circuit boards. They are also used in the manufacture of jewellery and cosmetics packaging.

The cyanoacrylate adhesives were found to have very strong tissue bonding capabilities. Their use during the Vietnam war by American troops as clinical sutures and hemostatic agents has led to their development as medical adhesives in hospitals, replacing sutures in the sealing of wounds following surgery [41].

1.4.2 Cure Chemistry

These adhesives are based on the very reactive cyanoacrylic acid ester. In a stark contrast to all other reactive adhesives, the cyanoacrylate adhesives consist, almost entirely, of the pure monofunctional monomer, which means that they can homopolymerise rapidly at room temperature. Therefore, the cyanoacrylate adhesives do not require large amounts of co-reactants.

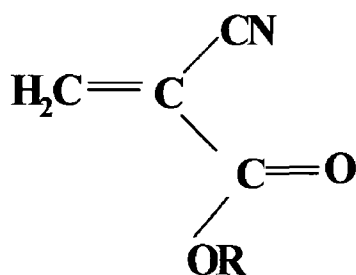


Fig. 1.10 The structure of the cyanoacrylate monomer. R denotes an alkyl group (e.g. methyl, ethyl, isopropyl).

Their reactivity stems from the strong electron withdrawing capabilities of the cyano and carbonyl groups, which makes the double bond very susceptible to attack by a weak base, e.g. water or an alcohol.

The basis of the curing process is, therefore, an exothermic, anionic polymerisation mechanism. Any anion found on the surface of the materials to be bonded together will initiate the polymerisation process (Fig 1.11) [39, 40]. This is in contrast to the curing mechanism of anaerobic adhesives which cure via a free radical polymerisation mechanism.

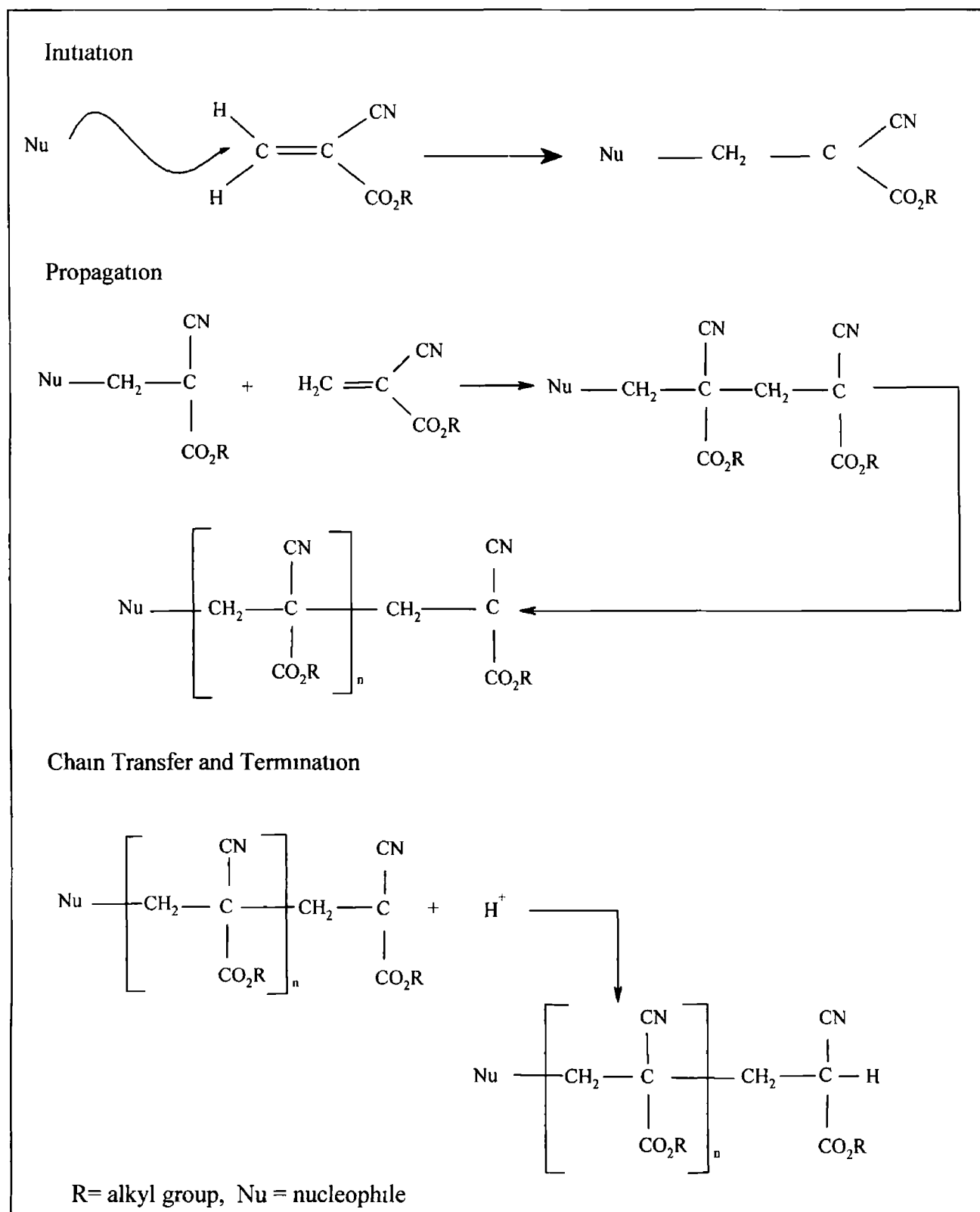


Fig 1 11 Cyanoacrylate adhesives cure in an anionic polymerisation mechanism [42]

Initiation of polymerisation begins when a nucleophile (Nu) (generally an anion, such as a hydroxide ion) attacks the β – carbon atom of the cyanoacrylate monomer to form the monomeric anion. During propagation this anion attacks another monomer to generate a dimeric species. The chain is propagated by reaction of this species with more monomer until a high molecular weight polymer chain is formed.

The processes of chain transfer and termination inhibit propagation. In chain transfer the polymeric chain attacks another species (e.g. water or weak acid) to form an inert polymer and a new anion. This new anion initiates the growth of a new polymer chain. However, if a strong acid is introduced, the anion will be protonated and polymerisation will be terminated [42].

Acids are therefore used as stabilisers in the final adhesive formulation. Generally Lewis or protonic acids, such as sulphur dioxide, aliphatic and aromatic sulphonic acids are used [43].

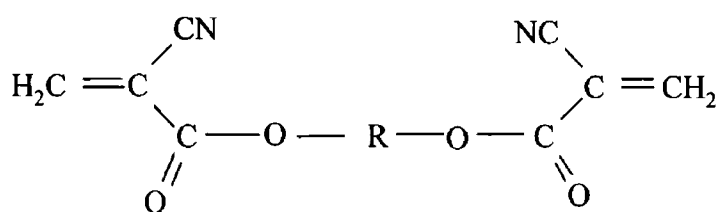
Less common modes of cyanoacrylate polymerisation are radically initiated [44] or photochemically initiated reactions [45].

1.5 Components of Cyanoacrylate Adhesives

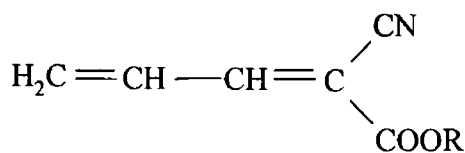
1.5.1 Monomers

Since their inception, no major changes to the composition of the cyanoacrylate adhesives have been made. This is due to the fact that small changes in the structure of the monomer can have dramatic effects on the adhesive properties. Methyl and ethyl esters are the most commonly used monomers while butyl esters are used in the synthesis of the medical adhesives. Other esters used include the biscyanoacrylates (XVI) and the 2-substituted 1-cyanoacrylates (XVII) (Fig. 1.12).

Alkyl 2-cyanoacrylates are synthesised in a Knoevenagel condensation reaction of alkyl cyanoacetate with formaldehyde in the presence of a base, e.g. piperidine. The resulting poly(alkyl-2-cyanoacrylate) is depolymerised at a temperature of 170–200°C to form the reactive monomer [41].



Biscyanoacrylate (XVI)



Cyanopentadienoate (XVII)

Fig. 1.12 Examples of other esters used in cyanoacrylate adhesives

1.5.2 Initiators

As mentioned previously, the curing process requires the presence of a base component, i.e. the initiator. On most substrates, any anion present on the surface can initiate polymerisation. However, on acidic surfaces, such as some woods, a primer containing an initiator must be applied to achieve a normal bond. Coover [46] suggested the use of alcohols on primers for unseasoned wood surfaces. Epoxides, secondary and tertiary amines and caffeine have all been used as cure initiators for cyanoacrylate adhesives [42].

1.5.3 Accelerators

Accelerators are termed “substances which increase the rate of polymerisation but are not initiators in their own right” [42]. In the cyanoacrylate adhesives the accelerators are compounded in the adhesive and do not affect their stability.

Crown ethers, e.g. 18 crown 6, are effective accelerators for cyanoacrylate adhesives used on fast, automated assembly lines. During the winter months, the relative humidity is decreased, limiting the amount of water on the surface of the materials to be bonded. The resulting slower cure speed creates severe problems for the assembly lines. Addition of crown ethers to the adhesive increases the cure speed in low relative humidity. The mechanism of action is unclear. However, it is thought that an interaction occurs between the crown ether and alkali metals on the surface of the substrate [47].

1 5 4 Inhibitors

There are two types of inhibitors used in cyanoacrylate adhesives. The first are the radical polymerisation inhibitors, of which hydroquinone and para-methoxyphenol are examples. During storage, these inhibitors protect the adhesive from polymerisation induced by radical sources such as heat and light.

Anionic polymerisation inhibitors are the second type of inhibitors. The major difficulty encountered in the attempt to commercialise cyanoacrylate adhesives was their lack of stability during storage. In an attempt to overcome this problem, sulphur dioxide was used in the manufacturing process and in the final adhesive formulation. Other gases used include boron trifluoride and sulphur trioxide. The concentration of the volatile inhibitors in an adhesive is usually under 100 ppm. However, while these 'acidic' gases are efficient stabilisers, they present a number of problems to the manufacturers. The gases are hard to handle and their exact concentration is difficult to determine. Under prolonged storage, the gases may evaporate from the adhesive. Introduction of non-volatile acidic inhibitors, such as benzene sulphonic acid and para-toluene sulphonic acid, has overcome these problems [42].

1.5.5 Other Additives

In common with other adhesives, the cyanoacrylates contain additives, principally trace levels of acids such as anionic stabilisers and phenolic stabilisers. These stabilisers offset free radical polymerisation. However, the most common additive is a synthetic organic polymer e.g. polyalkylmethacrylates or polyvinylacetate which function as thickeners. This permits grades of differing viscosity to be marketed [42].

Thickeners, plasticisers, fillers and tougheners are also found in the cyanoacrylate adhesives. Plasticisers reduce brittleness, which was a deficiency of the earlier cyanoacrylates. Commonly used plasticisers are aliphatic esters, aliphatic diesters and alkyl phthalates [42].

1.6 Analysis of Cyanoacrylate Adhesives

Literature on the analysis of cyanoacrylate adhesives is scarce. However, a number of studies have been carried out into the determination of anions present in the adhesive preparation.

Cyanoacrylate adhesives cure via an anionic cure mechanism in the presence of a weak base, typically water. Acids such as SO_2 , sulphuric acid, aliphatic and aromatic sulphonic acids are used to stabilise the adhesive preparation. When approaching the analysis of cyanoacrylate adhesives, the reactivity of the adhesive to any aqueous environment must be taken into account. Cyanoacrylate adhesives also have a limited solubility in organic solvents. Therefore, the major problem to be overcome when analysing cyanoacrylate adhesives is the sample preparation.

The preparation of cyanoacrylate adhesives for analysis by ion chromatography was investigated by Raftery [48] as part of the development of a method for the analysis of acids used as stabilisers. Initially, he employed tetrahydrofuran (THF) up to a concentration of 40% to dissolve the cyanoacrylate adhesive. However, the THF/NaOH solution which was used as the eluent did not provide a good separation of the ions because they eluted with the solvent front, probably because the concentration of THF was too high. Consequently, the cyanoacrylate adhesive was dissolved in chloroform and then extracted into citric acid. A precipitate was formed in the aqueous layer of the solution, but it did not interfere with the successful extraction of the anions. A separation of the anions in adhesive preparation was obtained using a Dionex AS-11 column and NaOH as the counter ion. Phthalic acid was used as the internal standard for the separation because it did not interfere with the analysis of the anions.

Kincaid [38] also investigated the analysis of acids in cyanoacrylate adhesives. He developed a capillary electrophoretic (CE) method for the analysis of acidic components in cyanoacrylate adhesives. The separation of 14 anions was achieved in seven minutes (Fig. 1.13).

A fused silica capillary of separation length 52 cm with an internal diameter (i.d.) of 50 μm was employed, while the anions were observed with indirect UV detection. The background electrolyte was a 10 mM chromate solution adjusted to pH 8.0 with sulphuric acid. A quaternary ammonium modifier (0.25 mM) was used to improve the resolution between the phosphate and succinate peaks and the chloride and sulphate peaks.

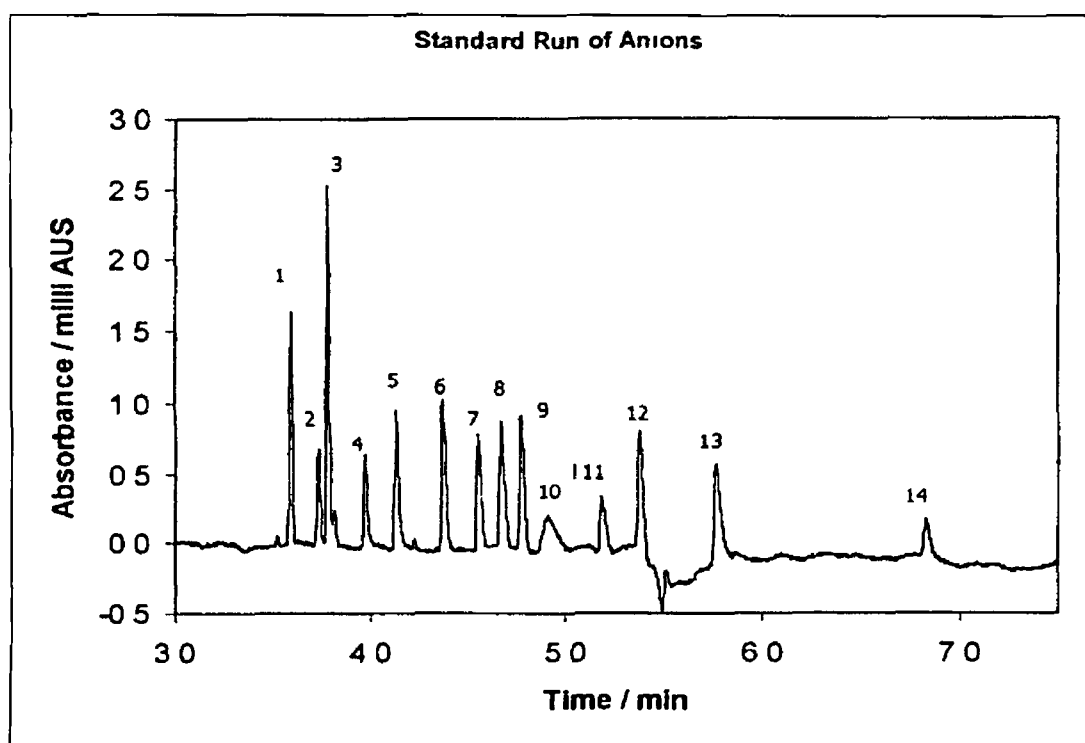


Fig. 1.13 Electropherogram showing the separation of 14 standard anions including those found in cyanoacrylate adhesives: 1 Chloride, 2 Nitrate, 3 Sulphate, 4 Oxalate, 5 Sulphite, 6 Malonate, 7 Maleate, 8 Formate, 9 Succinate, 10 Phosphate, 11 Phthalate, 12 Methylsulphonic acid, 13 Cyanoacetate, 14 Hydroxypropane sulphonic acid [38].

1.7 Conclusion

The cure chemistry of the anaerobic adhesives, while based on the radical polymerisation of the monomer, is actually quite complex. This is demonstrated by the roles the accelerators play in the curing process of anaerobic adhesives i.e. they can act as reductants of the peroxide and reduce transition metal ions to their lower oxidation state. Consequently, the exact mode of action of some of the accelerators in anaerobic adhesives remains unknown. The development of a quantitative technique to determine the role of accelerators in the curing mechanism and the products of their reactions, if any, would be very beneficial. An understanding of the role which each of the components in the anaerobic adhesive plays in the curing process would allow for the development of more efficient adhesives.

While the cure chemistry of the cyanoacrylate adhesives is seemingly simple, based on the anionic polymerisation of the monomer in the presence of a weak base, there are a number of challenges to be overcome. One particular challenge is the removal of any base present in the reaction vessel during manufacture of the pure cyanoacrylate monomer. A base catalyst is required in the first step of the manufacture of the cyanoacrylate monomer. However, acid is added thereafter to neutralise any base, which prevents premature polymerisation of the pure cyanoacrylate monomer. A quantitative analysis of the mixture in the reaction vessel would allow for the actual amount of base present in the vessel to be determined. Consequently, the correct quantity of acid required for neutralisation of the base would be added. This would increase the amount of pure monomer produced and reduce the amount of acid wasted, thereby making the process more cost efficient.

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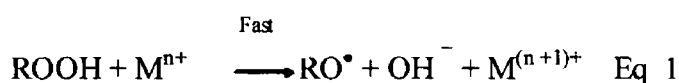
Chapter 2

The Analysis of Reducing Agents in Anaerobic Adhesives with Liquid Chromatography/Mass Spectrometry

2.1 Introduction

Anaerobic adhesives are single component liquids that remain in liquid form until confined between two metallic surfaces which exclude air, whereupon, the adhesives cure to form a strong bond. The high efficiency of the anaerobic adhesives for bonding metals stems from a redox catalysed decomposition of a hydroperoxide by the metal ions. Free radicals are formed which initiate the polymerisation of methacrylic monomers in the absence of oxygen.

The metal substance on which the anaerobic adhesive is applied plays a crucial role in initiation of polymerisation. Equation (1) represents the fundamental step for the formation of hydroperoxy radicals while a second reaction between the hydroperoxide and the metal ions, in their higher oxidation, can also occur (equation (2)). The latter reaction regenerates the metal ion in its low valence state. A stationary concentration of each ion is established when the rates of equations (1) and (2) are equal, however, the contribution of equation (2) depends on the nature of the metal [1]



Anaerobic adhesives meet the demands of their industrial application having a long shelf life (up to one year) and a high rate of polymerisation (half times greater than 30 seconds). Their stability is achieved by the addition of cure inhibitors, such as hydroquinone and pyrogallol, and by the use of hydroperoxides, which have a high stability at room temperature [2]. High rates of polymerisation are generally attained by the use of room temperature accelerators, such as organic acids and bases.

Early research found that when tri-alkylamines were added to the adhesive formulation they increased the cure speed without having a detrimental effect on the overall stability of the adhesive [3] It was assumed that the amine protonated the hydroperoxide ions, however, this only produced an increase in cure speed on copper and iron surfaces, not on cadmium - or zinc- plated metals [4]

Further research identified a co-accelerator combination of saccharin and diarylalkylamines, which produced a synergistic increase in polymerisation rates on all metal surfaces [5] The importance of the diarylalkylamines to the cure speed of the anaerobic adhesives was emphasised by the decrease in cure speed caused by the gradual autooxidation of the diarylalkylamines during storage It was also discovered that the hydroperoxide would only react extensively with the amine at elevated temperatures The presence of weak acids, e.g. saccharin and maleic acid, was critical in allowing the reaction to take place at room temperature [4]

Further research on triaryl and trialkylamines led to the development of adhesives containing 1-acetyl – 2 – phenylhydrazine (APH) and 1,2,3,4 tetrahydroquinoline (THQ)

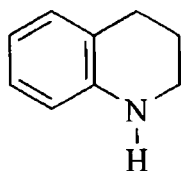
2.2 Aims of Research

The purpose of this project was to elucidate the products of decomposition of two reducing agents found in anaerobic adhesives, 1,2,3,4 tetrahydroquinoline (THQ) (Fig 2 1 (I)) and dihydropyridine (DHP) (Fig 2 1 (II)), with liquid chromatography/mass spectrometry (LC/MS) Identifying these products could assist in understanding the loss in performance of the adhesives with time

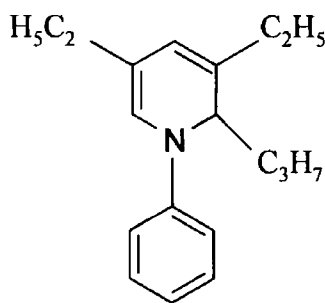
Initially, a liquid chromatographic method was developed which was compatible with mass spectrometric detection This involved preliminary investigations into the decomposition of THQ and DHP

The ratio of elemental copper and iron to CHP, acid and reducing agent, which would yield the greatest decomposition of the reducing agents to form products was also determined

Finally, LC separation followed by MS detection was carried out to ascertain the products of the decomposition of THQ and DHP



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



n-phenyl-2-propyl-3,5-diethyl-1,2, dihydropyridine (II)

Fig 2 1 Two reducing agents used in anaerobic adhesives

2.3 Liquid Chromatography/ Mass Spectrometry (LC/MS)

The combination of liquid chromatography (LC) with mass spectrometry (MS) offers the separation power of chromatography with the identification ability of mass spectrometry. When these properties are combined the result is an extremely powerful analytical technique.

2.3.1 Liquid Chromatography and Mass Spectrometry

In LC, separation of analytes is based on their selective distribution between a liquid mobile phase and a stationary phase. The sample is injected through an injector port into the mobile phase stream delivered by a high-pressure pump and transported through a column where separation takes place. The separation is monitored by a flow through detector.

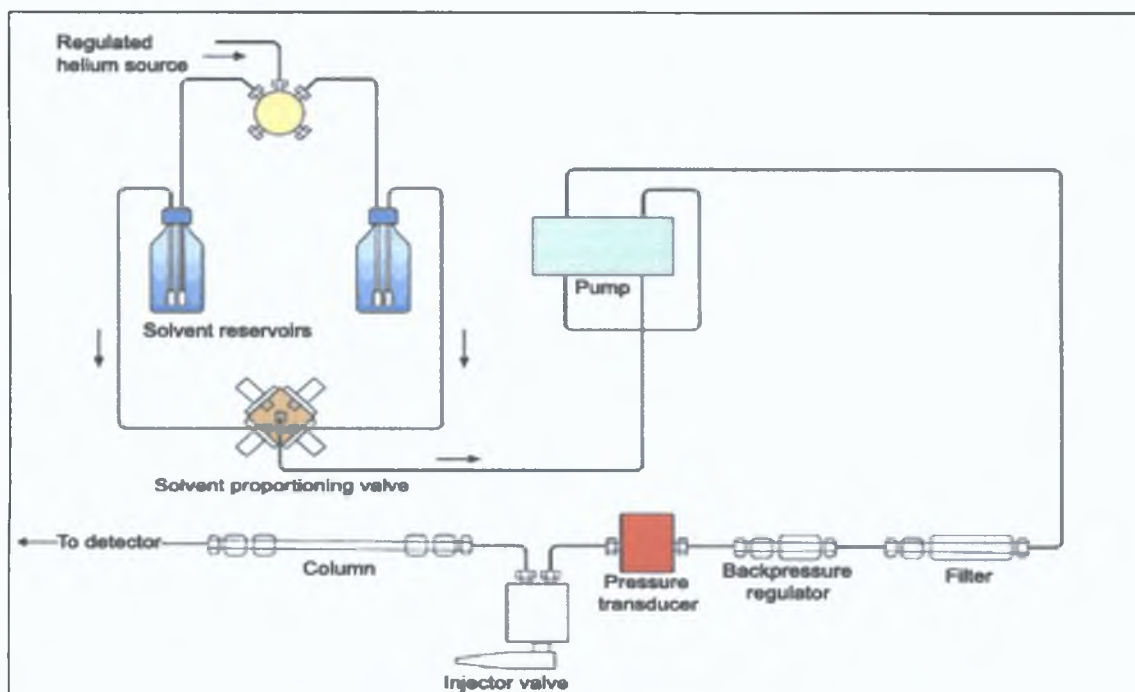


Fig. 2.2 Schematic of the components of a HPLC system [6].

Many different separation techniques are available in LC of which reversed phase chromatography is the most commonly used (Table 2.1).

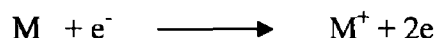
LC Separation Mechanism	Description	Mobile Phase	Stationary Phase
Adsorption	selective adsorption/desorption on a solid stationary phase	<i>Normal phase</i> Apolar organic solvent with organic modifier	Silica gel, alumina, bonded-phase material
		<i>Reverse phase</i> aqueous buffer with organic modifier e.g. MeOH or ACN	bonded-phase material silica with octadecylsilane (C18)
Partition	selective partition between two immiscible liquids	liquid, mostly non-polar	liquid, physically coated on a porous solid support
Ion-exchange	difference in ion-exchange properties	aqueous buffers	cationic (SO ₃) or anionic (quaternary ammonium compounds) exchange resin and bonded-phase materials
Ion-pair	formation of ion-pair and selective partition or sorption of these ion-pairs	aqueous buffer with organic modifier and ion-pairing agent	reverse phase bonded-phase material
Size exclusion	differences in molecular size, or more explicitly, the ability to diffuse into and out of the pore system	non-polar solvent	silica gel

Table 2.1 The characteristics of various LC techniques [7]

The basis of mass spectrometry is the production of ions, which are subsequently separated or filtered according to their mass / charge (m/z) ratio and detected. The resulting mass spectrum is a plot of the relative abundance of ions as a function of m/z ratio.

Ions are produced by a number of mechanisms, two of the most common of which are electron impact (EI) and chemical ionisation (CI) [7]. Both of these are hard ionisation techniques, suitable only for the analysis of volatile and thermally stable analytes.

In electron impact ionisation, an analyte vapour is bombarded by energetic electrons. Most of the electrons are elastically scattered, however, some cause electronic excitation of the analyte molecules upon interaction, while a few cause the removal of an electron from the analyte molecules. It is the latter two collisions that produce a molecular ion (M^+).



The molecular ion has a m/z ratio, which corresponds to the molecular weight of the analyte. EI is performed under high vacuum to prevent intermolecular collisions. Only a low number of negative ions are produced in EI, therefore it is restricted to the analysis of positive ions (Fig 2.3).

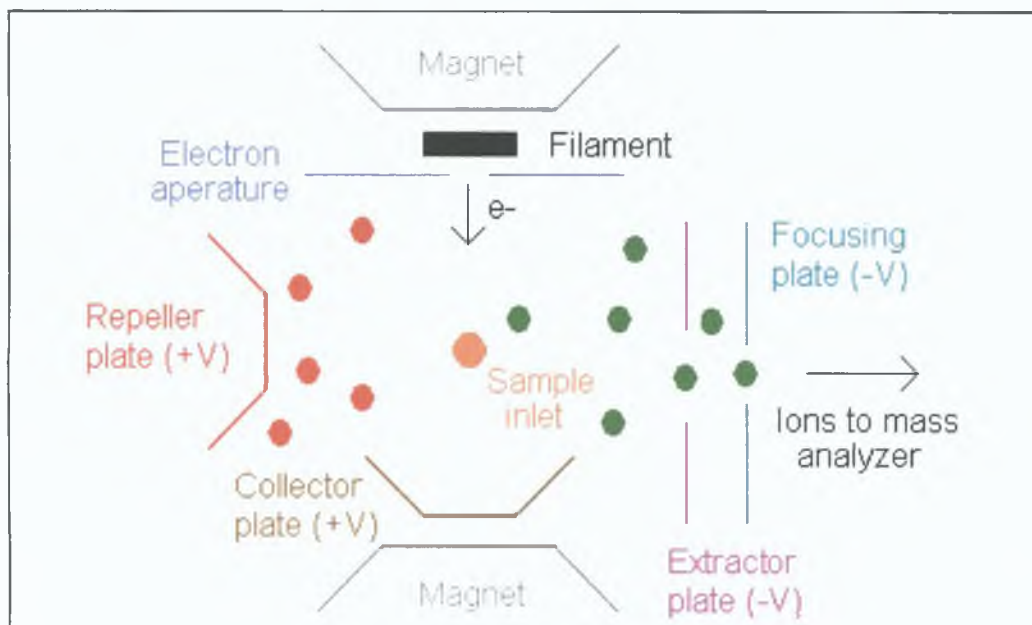


Fig. 2.3 Schematic of electron impact ionisation source. The positive ions (green) are drawn by the negatively charged plate towards the mass analyser while the negative ions (red) are drawn away from the opening to the mass analyser [8].

In contrast to electron impact ionisation, the ion in chemical ionisation (CI) is produced in a chemical reaction that occurs between a reagent gas ion and the analyte. The reagent gas ion is produced by bombardment of the reagent gas by energetic electrons followed by a series of ion-molecule reactions.

An example of this is the use of ammonia as the reagent gas. Upon its electron ionisation, the protonated ammonia (NH_4^+) is formed. This reacts with the analyte molecules in a proton transfer reaction.

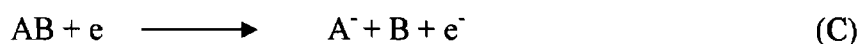
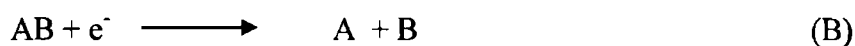


The resulting ion has a low internal energy, the mass of which leads to the molecular weight of the analyte.

Negative ions are produced in abundance in CI by proton transfer or abstraction



or by electron capture, where negative ions are formed by associative resonance (A), dissociative resonance (B) or ion-pair production (C)



The instrumentation required for chemical ionisation is similar to the EI chamber because the reagent gas is produced by electron impact ionisation. However, the chamber in CI is maintained at high pressures, typically between 1 Pa and atmospheric pressure (10^{-3} Pa).

Other ionisation techniques include fast atom bombardment (FAB), where the analyte is dissolved in a solvent (e.g. glycerol), ^{252}Cf plasma desorption (PD), field desorption (FD), laser desorption (LD), thermospray and electrospray.

In thermospray the sample is dissolved in a volatile buffer and passed through a heated vaporizer tube. Part of the liquid in the tube evaporates and expands at the wall of the tube. The solution becomes nebulized as a result of the disruption of the liquid by the expanding vapour. Prior to the onset of partial evaporation inside the tube a significant amount of heat is transferred to the solvent which assists later on in the desolvation of the droplets. Nonvolatile molecules are retained in the droplets of the mist generated as a result of nebulisation.

As a result of continuous solvent evaporation from the droplets, a high local field strength is generated, which allows the charged species to evaporate from the droplets. The charged species are comprised of analyte ions and of buffer ion clusters. The buffer ion clusters equilibrate with the solvent vapour but the analyte ions are sampled and sent to the mass analyser [9].

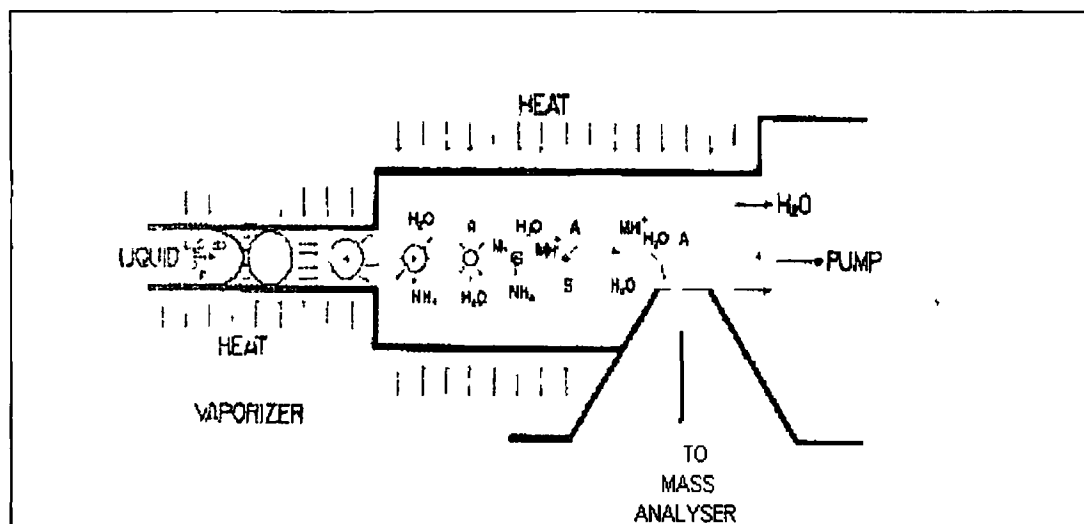


Fig 2.4 Schematic of the thermospray ionisation mechanism, MH^+ , A analyte ions, NH_4^+ and H_2O buffer ion clusters [7]

The ionisation process in electrospray is not fully understood. However, a generally accepted mechanism is outlined as follows. The electrospray process begins when a solution of the analyte is passed through a capillary which is held at a high potential. As the solution passes through the tip of the capillary it experiences the electric field associated with the maintenance of the tip at high potential. Taking the example of a tip at positive potential, positive ions accumulate at the surface of the liquid. As a result, the liquid is forced downward to produce a cone of liquid. This is known as the 'Taylor cone'. At a high enough electrical field the liquid is drawn into a filament. At the point at which the surface tension is exceeded by the applied electrostatic force the liquid filament is forced into droplets.

As the droplets traverse the pressure gradient towards the analyser their diameter is reduced by solvent evaporation and 'fission'. Fission of the droplets occurs at the point at which the magnitude of the charge is sufficient to overcome the surface tension holding the droplet together. This is known as the Rayleigh limit. Continual reduction in droplet size continues via solvent evaporation and fission until only a single ion is contained in each droplet [10] (Fig 2.5)

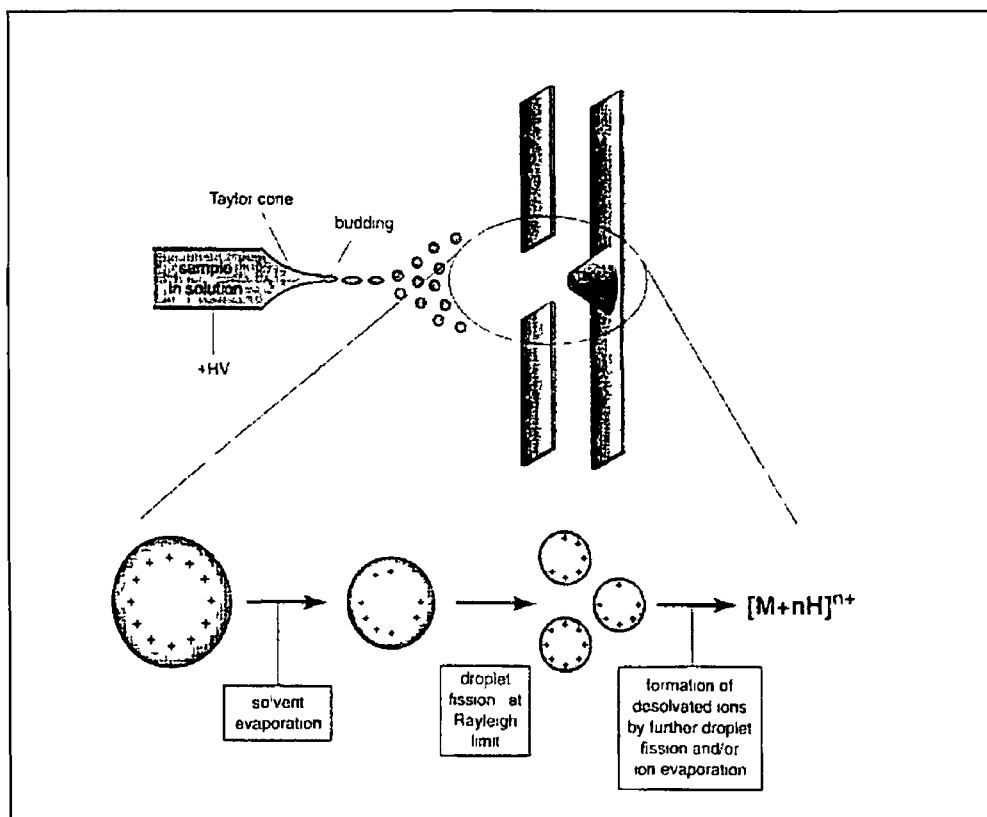


Fig 2.5 [10] Production of droplets during electrospray ionisation [10]

In FAB, the analyte is dissolved in a compatible solvent e.g. glycerol and brought as a continuous thin film onto a metal target. This is subsequently presented to a beam of high energy particles which ionise the analyte [9]. Similar to FAB is secondary ion mass spectrometry (SIMS) where ions instead of atoms are used to ionise the analyte.

Ionisation is accomplished in PD by dissolving the analyte in a volatile solvent, which is deposited on a nitrocellulose target material. This is then bombarded with ^{252}Cf fission fragments [7].

In field desorption the sample solution is placed on an FD emitter which is activated to produce microneedles on the surface. The ions are produced when high local electrical fields at the tip of the microneedles enable electrons to tunnel from the sample molecules into the field ionization emitter. Ions are also desorbed under these conditions. FD spectra exhibit cationized and molecular ion molecules with little fragmentation [7].

Laser desorption (LD) is generally used for the analysis of very high molecular-weight compounds, such as proteins with masses of 200kDa. The ions are produced by bombarding the sample solution with photons [7]. The energy from the photons is transferred to the dissolved analyte molecules, which subsequently become desorbed and analysed as protonated or cationized molecules.

Beside the molecular ion, other ions are produced during ionisation, including fragment ions. A fragment ion is produced when a molecular ion (or another fragment) loses a radical or neutral group. Fragment ions can be used to elucidate the structure of a compound. Other peaks that can be observed on a mass spectrum include the contribution by isotopic impurities e.g. natural chlorine exists for 75% of ^{35}Cl isotopes and for 25% of ^{37}Cl isotopes. Background peaks are also seen due to air and oil leaks in the system and solvent impurities.

2 3 2 Interfacing Liquid Chromatography with Mass Spectrometry

The major problem when combining LC with MS is the incompatibility between the techniques. The major restrictions to interfacing the two techniques are

- 1 The flow rate incompatibility as expressed by the requirement to introduce 1ml/min of liquid effluent from a conventional LC column to the high vacuum of the mass spectrometer
- 2 The solvent composition incompatibility as a result of the frequent use of non-volatile mobile phase additives in LC separation development
- 3 The ionisation of non-volatile and/or thermally labile analytes [9]

The requirements of an interface are laid out in Table 2 2. Initially, the problems of sample introduction and ionisation of non-volatile and thermally labile analytes were approached separately. One of the earliest LC/MS interfaces involved the introduction of sample via a moving belt interface [11] followed by ionisation by electron impact or chemical ionisation (section 2 3 1).

LC Operation	No restriction on solvents
	Gradient elution
	Buffers, volatile and non-volatile
	Ion-pair reagents
	Flow rates up to 2ml/min (= 200-2000cc/min gas)
	Free choice of LC column dimensions
Interface Operation	Enrichment of sample to solvent
	High transfer efficiency
	No additional peak broadening, no loss in efficiency
	No uncontrolled chemical modification of the analytes
	Low volatility samples vaporised
MS Operation	High vacuum in mass analyser, hence flow rate = 20ml/min
	Free choice of ionisation method
	Free choice of CI reagent gas
	Both positive and negative mode
	Low interference from solvents and solvent impurities
General	Low cost
	Operational simplicity
	Low detection limits
	Quantitation

Table 2.2 Operating characteristics desired from an LC/MS interface Adapted from [12]

The moving belt interface consists of an endless continuously moving Kapton ribbon, which transports the LC column effluent from the column outlet towards the MS ion source. During transport the mobile phase is removed via gentle heating and evaporation under reduced pressure in two pumped vacuum chambers. The analyte is desorbed into the ion source by flash evaporation at the tip of the moving belt interface. The analyte is now in a gaseous state and is susceptible to EI or CI (Fig 2.6)

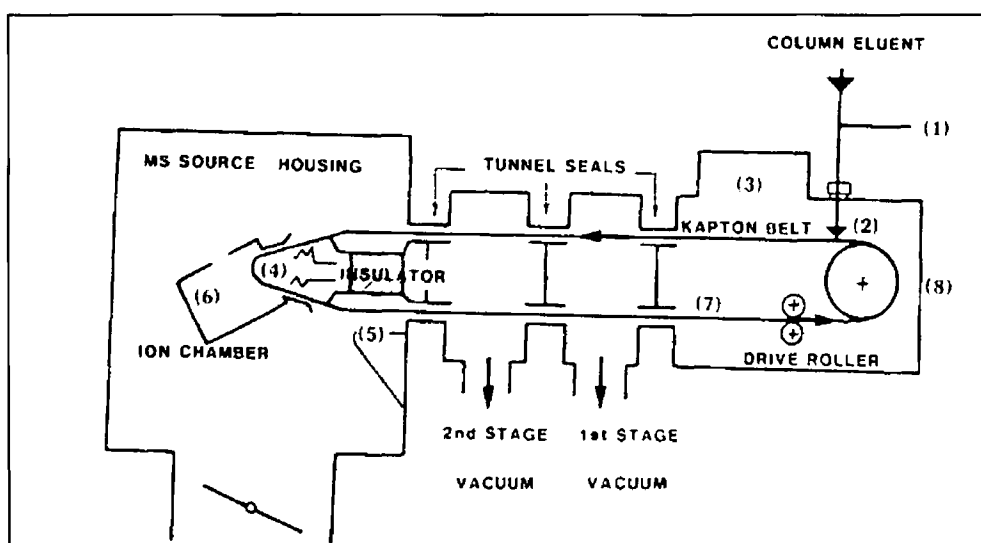


Fig 2.6 Schematic of a moving belt interface 1 Drain line, 2 Simple contact depositor, 3 Solution preheating, 4 Sample heater, 5 Clean-up heater for EI/CI modes, 6 Ionisation source, 7 Belt cleaner, 8 Probe aligning mechanism Reproduced from [11]

The moving belt was an indirect sample introduction technique, however, direct liquid introduction (DLI) [13, 14] evolved to become a more popular technique for sample introduction on LC/MS. In DLI the LC effluent stream is split and only the amount of solvent tolerable by the MS is injected [15]

The column effluent is nebulised by the disintegration into small droplets of a liquid jet formed at a small diaphragm. The solutes are analysed using CI, with the reverse phase solvent as the reagent gas, following their desolvation in a desolvation chamber (Fig 2.7). The major problem with DLI interfaces is that the diaphragm frequently becomes clogged. Another drawback is that the liquid jet often changes direction, causing peak broadening and distortion in the mass spectrum of an analyte.

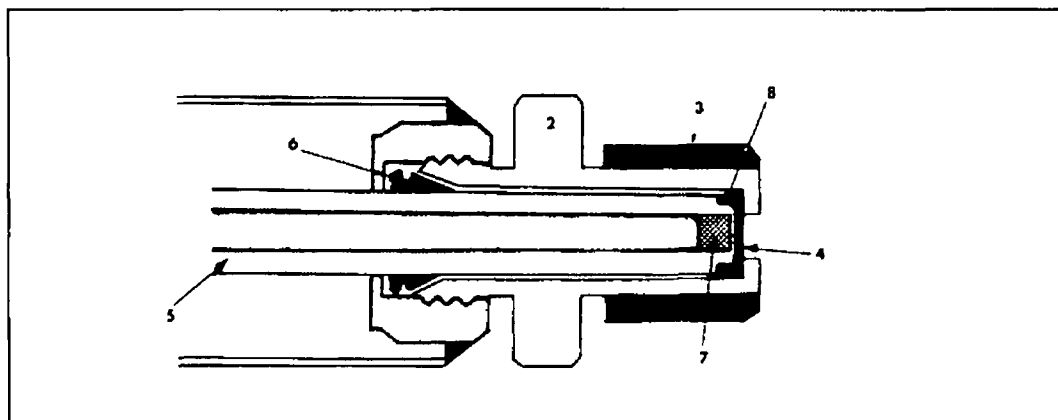


Fig 2.7 Diagram of a DLI interface 1 DLI probe, 2 Probe head, 3 Insulator, 4 Diaphragm, 5 Packed microbore column, 6 Vespel ferrule, 7 0.5µm porosity filter and 8 PTFE seal. Reproduced from [7]

With the advent of DLI, it was concluded that nebulisation was an important step in both the transfer of analytes from the LC to the MS and in their transition from the liquid phase to the gas phase. All the early research on LC/MS was based on the assumption that ionisation must follow vaporisation of the intact neutral analyte molecules. Arpino and Guichon [16] recognised that the LC effluent was not only important in transferring the analytes to the MS but also in assisting in their ionisation.

By combining sample introduction with soft ionisation techniques it is possible to divert a large fraction of the mobile phase from the mass spectrometer and make the transformation of non-volatile and fragile species from solute - in - liquid to ions - in - vacuum ready for mass analysis

To the forefront of these interfaces are techniques based on atmospheric pressure ionisation (API) [17] These techniques are soft ionisation processes well suited for the analysis of large and small, polar and non-polar, thermally labile compounds The information provided by these API techniques provides sensitive and accurate molecular weight and fragmentation information

2.3.2.1 Atmospheric Pressure Ionisation – Electrospray (API-ES)

API-ES [10] is a three - step process comprising of nebulisation, desolvation and ionisation

Nebulisation

HPLC effluent is pumped through a nebulising needle, which is held at ground potential The resulting spray travels through a cylindrical electrode, which is at high potential A strong electric field is produced due to the difference in potential between the needle and the electrode The surface of the liquid becomes charged by this field and forms a ‘Taylor cone’ A surface charge density is created on the liquid which produces coulomb repulsion forces sufficient to overcome the surface tension so that the liquid is forced to break into increasingly smaller droplets to eventually form a fine spray

Desolvation

The charged droplets are attracted towards a capillary-sampling orifice. In a counterflow of nitrogen gas, the droplets continue to shrink in size until they reach a stage where droplet fission occurs due to the magnitude of the droplet charge being sufficient to overcome the surface tension of the liquid. All uncharged material is carried away to waste.

Ionisation

The mechanism by which the analytes become ionised in API-ES is not fully understood [18], however, the most generally accepted route is that the charged droplets continue to shrink via solvent evaporation and fission until only one ion exists in each droplet, whereupon they are desorbed into the gas phase and carried to the mass analyser.

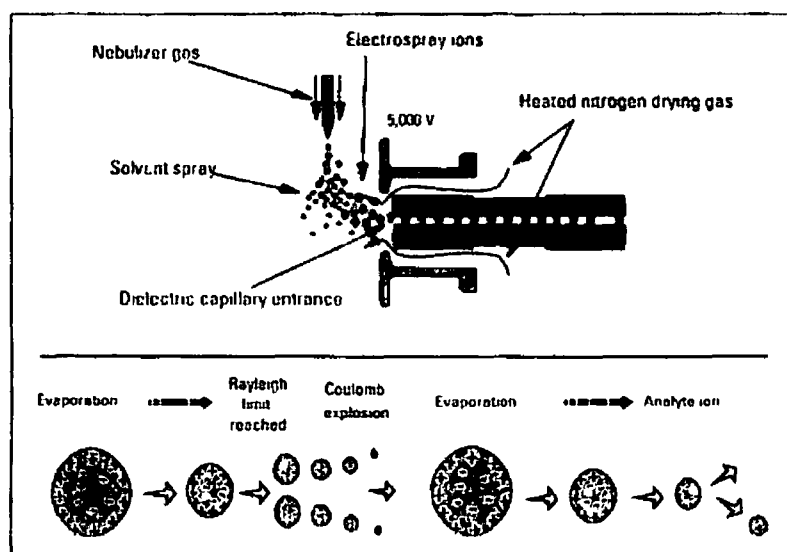


Fig 2.8 Diagram of API-ES showing the nebulisation/ionisation process [19]

2.3.2.2 Atmospheric Pressure Chemical Ionisation (APCI)

API-ES is ideally suited to LC/MS applications in that it allows for gentle ionisation of thermally labile organic compounds with minimum fragmentation. Atmospheric pressure chemical ionisation, on the other hand, allows for fragment ions to be generated.

The nebulisation and desolvation of ions in APCI is similar to that in API-ES, however, APCI nebulisation occurs in a hot vapourisation chamber (typically 250°C – 400°C). The spray droplets rapidly evaporate in the heated chamber resulting in gas phase HPLC solvent and analyte molecules.

The gas phase solvent molecules are ionised by the discharge from a corona needle (Fig. 2.9). A charge transfer from the ionised solvent reagent ions to the analyte molecules occurs, resulting in the formation of analyte ions. The analyte ions are then transported through the ion optics to the filter and detector.

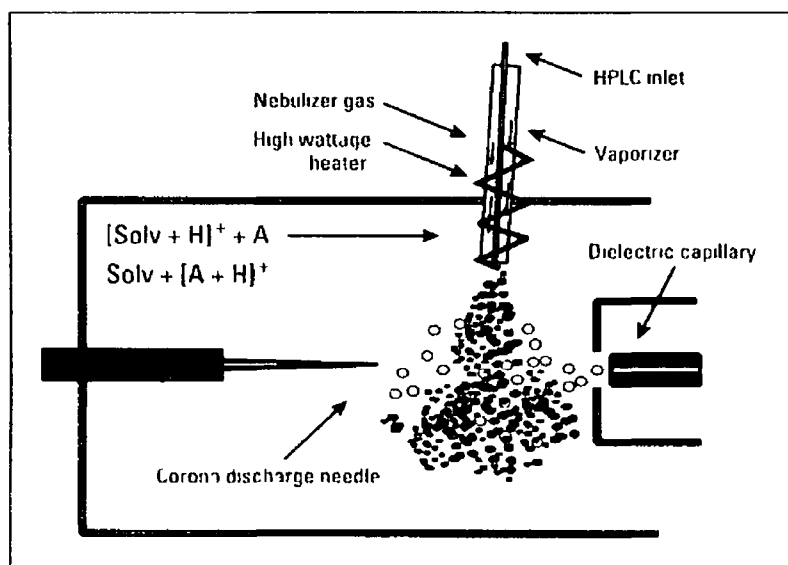


Fig. 2.9 Diagram of APCI Reproduced from [19]

2.3.3 Mass Analysers

Four analysers are available for the analysis of ions in LC/MS sector, quadrupole, time of flight (TOF) and fourier transform ion cyclotron resonance (FT-ICR). In TOF mass analysis, a pulsed beam of ions is accelerated by a potential, V . The time required to reach a detector (t) placed at a distance (d) is measured. The time is related to the m/z ratio by

$$t = d \sqrt{\frac{m}{2zev}} \quad \text{Equation 3[7]}$$

where z = charge of ion, m = mass of ion, e = elementary charge of ion and v = velocity of ion

However, the most commonly used mass analysers are the sector and quadrupole. In a single focusing sector instrument, the ions, with mass (m), elementary charge (ze) and a kinetic energy are introduced into a magnetic field (B), while the acceleration of the ions towards a slit is determined by their kinetic energy at a voltage (V). In order to transmit ions to a detector, the magnetic force is counter-balanced by a centrifugal force. The m/z ratio of an ion is given by

$$\frac{m}{z} = \frac{B^2 r^2 e}{2v} \quad \text{Equation 4[7]}$$

where r is the radius of curvature of the path through the magnetic field. By varying B or V , ions can be detected at a fixed position behind a slit as a function of time.

Often, an electrostatic analyser (ESA) is added to a single focusing sector instrument, forming a double focusing sector instrument. Processes in the ion source generally lead to a large distribution of kinetic energies. An ESA selects only for the ions with particular kinetic energy and discards the rest, thus improving immensely the resolution but decreasing the output signal of the instrument.

The geometry of the instrument must take this decrease in signal into account in order to achieve both high resolution and high ion transmittance. The maximum m/z value of a sector instrument is quite low (approx 2400 daltons), therefore, a viable alternative is the quadrupole mass analyser.

A quadrupole mass analyser is actually a powerful mass filter. It consists of four hyperbolic, or circular, rods that are placed in parallel in a radial array. Opposite rods are charged at positive or negative DC potential at which an oscillating radio-frequency voltage is superimposed. This oscillating voltage overwhelms the DC field. When the ions of certain m/z ratio are introduced into the quadrupole at a low voltage they oscillate in a plane perpendicular to the rod length.

When the oscillations are stable the ions are transmitted to the ion detector. To transmit ions of varying m/z ratio the quadrupole changes DC potential and radio frequency voltage.

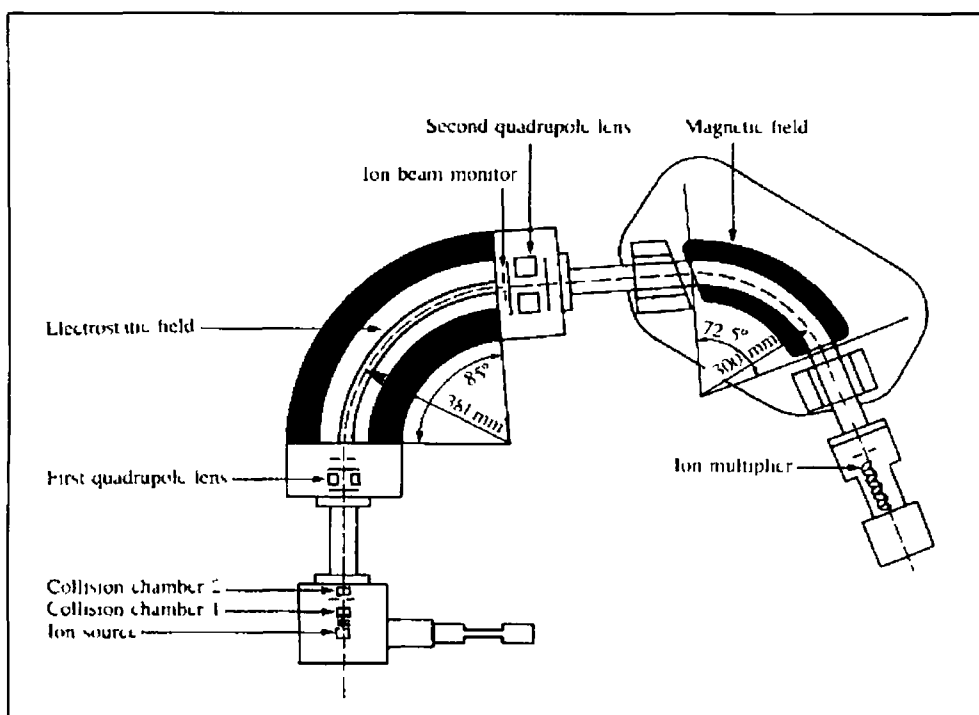


Fig 2.10 Mass spectrometer with a double-focusing mass analyser. Reproduced from [20]

Quadrupole mass analysers are commonly operated in either of two modes scan mode and selective ion monitoring (SIM) mode. In scan mode the instrument detects signals over a mass range during a short period of time. This is appropriate for qualitative analysis or for investigative analysis where the masses of all analytes are not known in advance. In SIM, only a few m/z ratios are monitored. Consequently, the quadrupole devotes more time to sampling each of the m/z values resulting in a significant increase in sensitivity. SIM is generally employed for targeted compound analysis, where the SIM ion sampling choices can be time-programmed to match the elution time of the analytes of interest.

A development of the quadrupole is the octopole in which eight rods of opposing potential are employed. The quadrupole and octopole mass analysers are widely used in LC/MS instruments because of the ease of voltage control. A study carried out by Choi et al. [21] found that the quadrupole gave the greatest sensitivity in comparison with TOF and FT-MS when operated in the selective ion monitoring mode (SIM). However, higher resolution mass spectra through a broader mass range were obtained with the TOF and FT mass analyser.

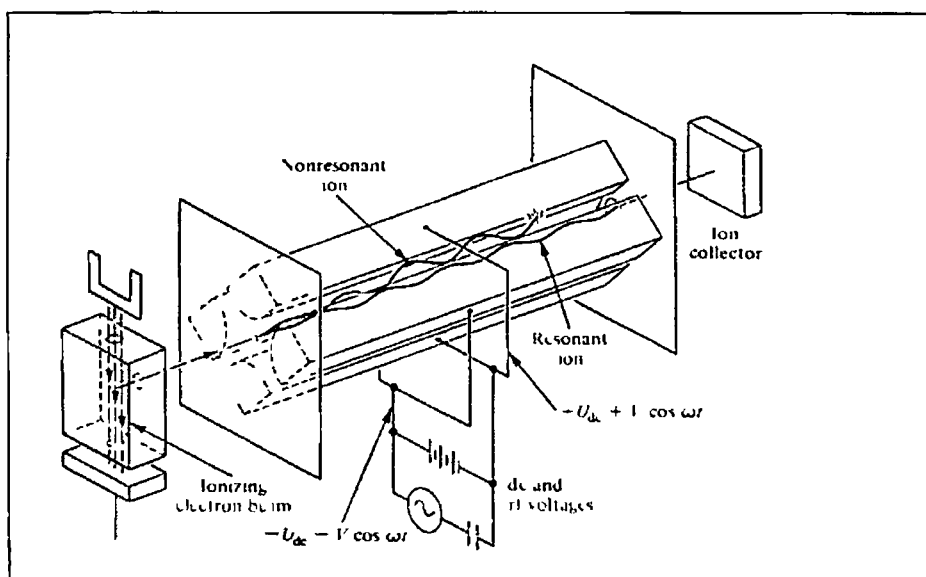


Fig 2 11 Diagram of a quadrupole mass analyser Reproduced from [20]

2 3 4 Ion Detection

Electron multipliers are generally used for detection of ions in LC/MS analysis. In an electron multiplier the ion beam strikes a conversion dynode. This is a metal plate that converts impinging ions into electrons. The currents created are then further multiplied by the “cascade” of ions down a series of dynodes. Analogue detection is accomplished by conversion of the output current into a voltage suitable for digitisation.

2.4 Experimental Detail

2.4.1 Reagents and Apparatus

The sources of chemicals were as follows

Chemical Name	Supplier	Purity
1,2,3,4 Tetrahydroquinoline	Sigma-Aldrich	99%
Dihydropyridine	Loctite (Irl)	not supplied
Cumene hydroperoxide	Loctite (Irl)	not supplied
Saccharin	Loctite (Irl)	not supplied
Maleic Acid	Loctite (Irl)	not supplied
Copper (II) sulphate pentahydrate	Riedel de Haen	99%
Copper powder 200 mesh	Sigma-Aldrich	99%
Iron (III) nitrate 9 – hydrate	AnaLar (BDH)	98%
Iron granules 10 – 40 mesh	Sigma- Aldrich	99 99%
Acetic Acid	AnaLar (BDH)	100%
Acetonitrile	Labscan	HPLC grade & very high purity for LC/MS
Triethylamine	Sigma-Aldrich	99%
Sodium dihydrogen orthophosphate 1- hydrate	AnaLar (BDH)	99%

The HPLC instrument used was a Beckman System Gold equipped with a 116 photodiode array (PDA) detector and a 118 solvent delivery system. An injection loop of 20 μ l was employed. All chromatograms were recorded with System Gold chromatographic software loaded onto a Gateway P5-133 personal computer.

An ESQUIRE LC/MS from Hewlett Packard - Bruker Daltronics was used. Fig 2.12 shows a diagram of the components of an LC/MS interface. The ESQUIRE LC/MS is equipped with an octopole mass analyzer and a PDA detector. The mass analyser can be operated in both scan mode and selective ion monitoring (SIM) mode. Ionisation was accomplished via atmospheric pressure ionisation electrospray (API – ES) or atmospheric pressure chemical ionisation (APCI), both of which are described in section 2.4.

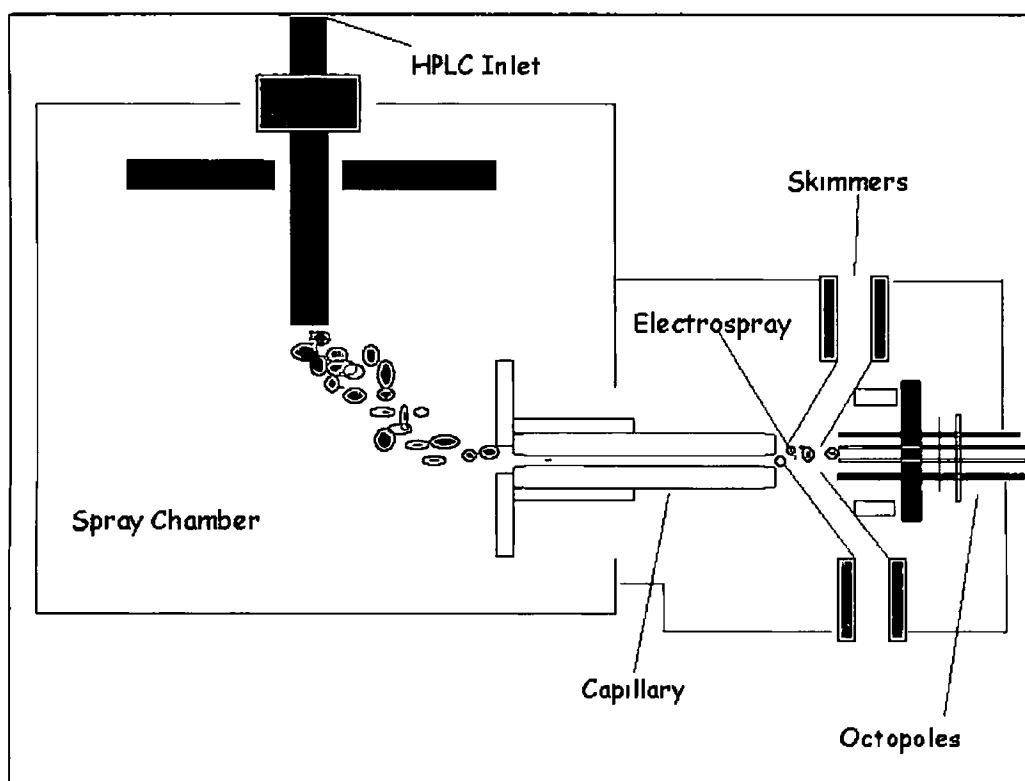


Fig. 2.12 Schematic of an LC/MS interface equipped with an octopole mass analyser

2 4 2 Method

2 4 2 1 Introduction

The HPLC method developed by Kincaid [22] for the analysis of the reducing agents in anaerobic adhesives cure systems used a Lichrosorb μ -Bondapak, 10 mm, 300 mm x 3.9 mm column with a mobile phase composed of 40% acetonitrile (ACN) 60% buffer. The buffer used was a 50 mM phosphate solution containing 5% triethylamine (TEA) and adjusted to pH 4.5 with ortho-phosphoric acid. The mobile phase was filtered through a Millipore filter and degassed by sonication for 20 minutes before use. A flow rate of 1.2 ml/min was found to be sufficient to separate all cure components. The column was maintained at ambient temperature.

2 4 2 2 Selection of Conditions Suitable for LC/MS Analysis

The aim of this analysis was to adapt the method used by Kincaid [22] for LC/MS analysis of the reducing agents in anaerobic adhesives. Consequently, for this analysis, a column was selected that would allow for the easy transition from liquid chromatographic conditions to mass spectrometric conditions. An ACE C18, 5 mm, 250 mm x 4.6 mm column was selected on the basis that it has the capability to separate acidic and basic molecules, is relatively inexpensive and is available in narrower bores for scaling down to LC/MS conditions. All solutions were prepared as stock solutions, of concentration 250 mM, in acetonitrile and then dissolved in 40% acetonitrile 60% 18 M Ω de-ionised water to give a final molar ratio of reducing agent to cure system component (i.e. CHP, maleic acid or saccharin) of 1:1 or 4:1. The metal salt stock solutions were prepared in 18 M Ω de-ionised water. They were also 250 mM in concentration. The final concentration of the reducing agent in the solutions was 0.75 mM. All solutions were filtered prior to injection.

In Figure 2.13 the analysis of THQ cure system with the ACE C18 column is presented. The analysis was performed with the same mobile phase as used by Kincaid [22]. A good separation of the components was achieved with this column.

It should be noted that when CHP is separated it is detected as two peaks on the chromatogram: cumyl alcohol and acetophenone. These are the last peaks to elute on the chromatograms of both the DHP and THQ cure systems. The decomposition of CHP was probably caused by the acidic environment of the mobile phase [22, 23].

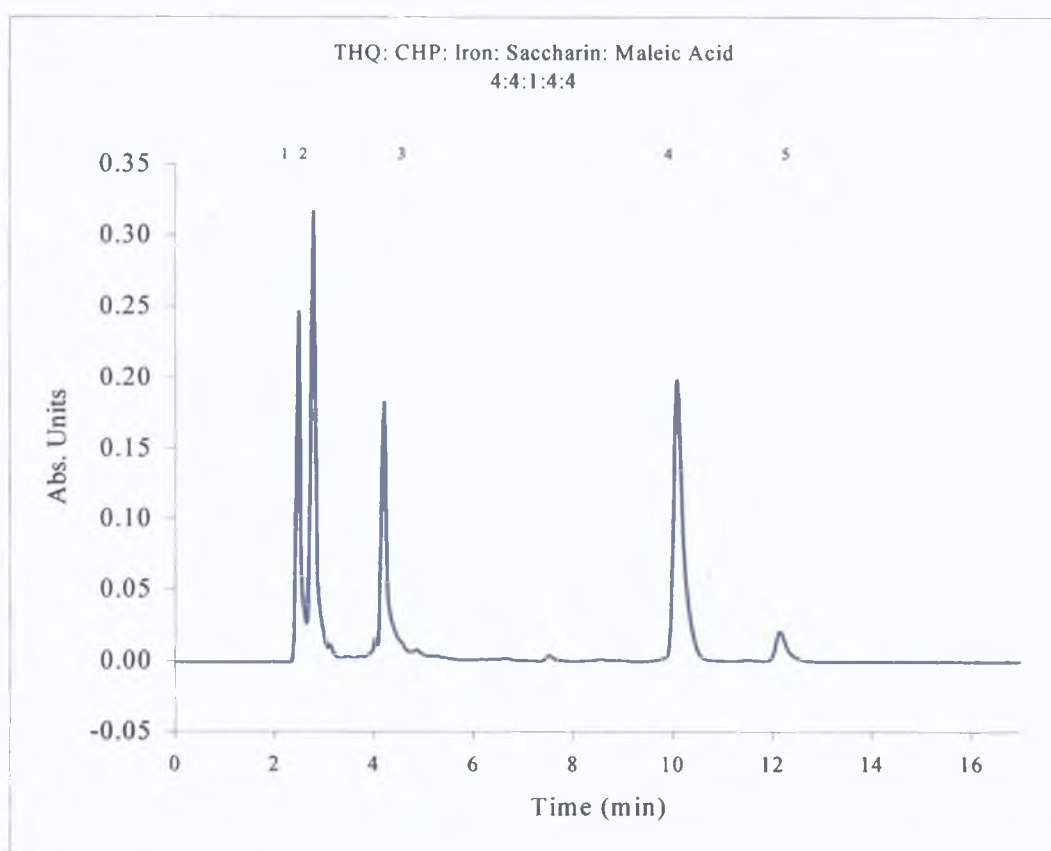


Fig. 2.13 Chromatogram of THQ cure system. Mobile phase: 40% ACN : 60% buffer (Buffer: 50 mM phosphate, pH 4.5, 5% TEA); Column: ACE C18 5 mm, 250mm x 4.6 mm; Flow Rate: 1.0 ml/min; Detection : 254 nm; Temperature: ambient.

1. Maleic acid; 2. Saccharin; 3. THQ; 4. , 5.CHP.

A separation of the components of the DHP cure system was also carried out on the new column (Fig. 2.14). DHP cannot be observed in Fig. 2.15 because it has a very poor UV absorbance. Its presence on the chromatogram can be detected by spiking a solution with a large concentration of DHP. It elutes after the two CHP peaks. However, it can be seen from Fig. 2.14 that a good separation of the components of the DHP cure system was obtained.

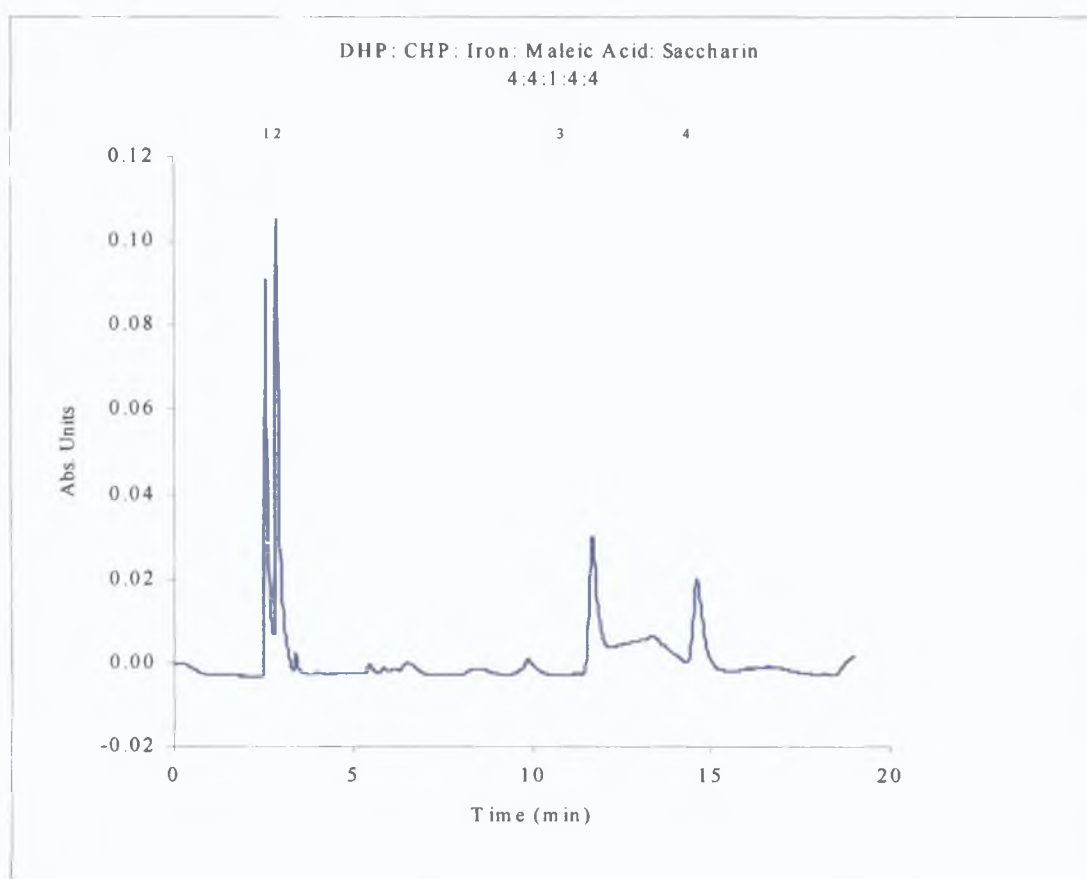


Fig. 2.14. HPLC Analysis of DHP cure system. Mobile phase: 40% ACN : 60% buffer (Buffer: 50 mM phosphate, pH 4.5, 5% TEA); Column: ACE C18 5mm, 250 x 4.6 mm; Flow Rate: 1.0 ml/min; Detection : 254 nm; Temperature: ambient.

1. Maleic acid; 2. Saccharin; 3.,4. CHP.

2.4.2.3 Initial Studies

Initially, the aim was to develop a mobile phase which was compatible with both LC/MS detection and the ACE C18 column. It was proposed that the phosphate component of the mobile phase developed by Kincaid [22] could be replaced with a dilute acetic or formic acid solution. These buffers would not form salt deposits in the MS and, therefore, interfere with its operation. Accordingly, the buffer used was a 1% acetic acid solution, from which a mobile phase of 35% acetonitrile: 65% buffer was prepared. The mobile phase was filtered and degassed by sonication for 20 minutes prior to use. Fig. 2.16 shows a chromatogram of THQ cure system separated with this mobile phase.

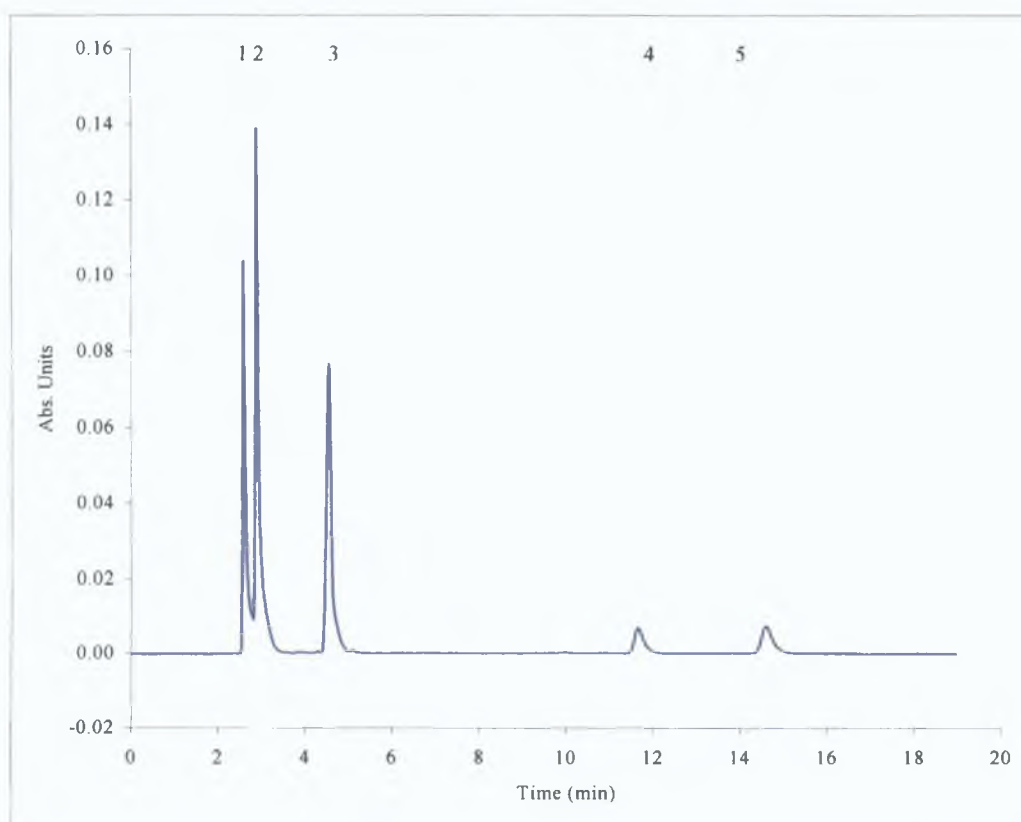


Fig. 2.15. Chromatogram of THQ cure system. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 2.5 mm; Flow Rate: 1.0 ml/min¹; Detection : 254 nm; Temperature: ambient. 1. Maleic acid; 2. Saccharin; 3. THQ; 4., 5. CHP

As with the method developed by Kincaid [22], all solutions were prepared as stock solutions, of concentration 250 mM, in acetomtrile They were then dissolved in 35% acetonitrile 65% 18 MΩ de-ionised water to give a final molar ratio of reducing agent to component of 1 1 or 4 1 The solutions were not prepared in the mobile phase This is particularly important in the case of DHP because it has been shown to undergo air-oxidation over a prolonged period of time in the presence of acetic acid to form a pyridinium salt [24] (Fig 2 16) The metal salt stock solutions were prepared in 18 MΩ de-ionised water They were also 250 mM in concentration The final concentration of the reducing agent in the solutions was 0 75 mM All solutions were filtered prior to injection

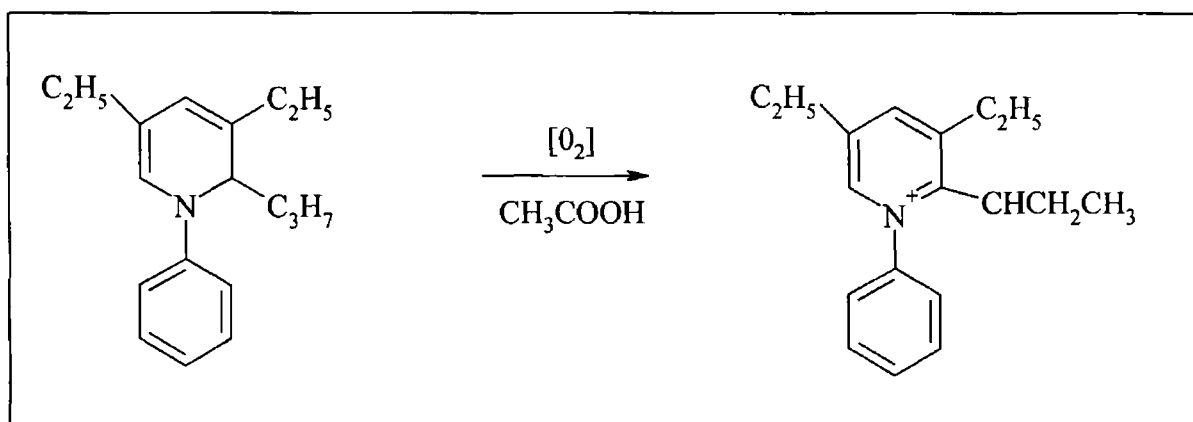


Fig 2 16 Air oxidation of DHP in the presence of acetic acid to form a pyridinium salt [24]

2 4 2 4 Monitoring of Products Generated during the Decomposition of Reducing Agents

To prepare the molar ratios of reducing agent to components, solutions were prepared from stock standards of all components as outlined in section 2 4 2 3 The solutions containing mixtures of components in concentrations of %v/v were prepared by adding the correct volume of each component to the volumetric flasks

When metal salts were added to the solutions, controls were prepared, which were the same solutions containing no metal salts The controls and the cure systems solutions were run simultaneously in order to make comparison between the two

2 4 2 5 LC/MS Determination of the Products Formed due to Decomposition of the Reducing Agents

Solutions of the copper powder and iron filings, in the concentrations of 5% w/v, were prepared in methanol The other cure components were added to the metal solutions in the following concentrations (%w/v) All solutions were prepared in acid washed volumetric flasks

Solution 1 THQ Saccharin Maleic acid CHP (1% 2% 1% 1%)

Solution 2 DHP Saccharin Maleic acid CHP (1% 2% 1% 1%)

Solution 3 THQ Saccharin CHP (1% 2% 1%)

Solution 4 DHP Saccharin CHP (1% 2% 1%)

Solution 5 THQ Maleic acid CHP (1% 1% 1%)

Solution 6 DHP Maleic acid CHP (1% 1% 1%)

Control solutions of each of the cure components in the absence of metals were also prepared All solutions were sonicated for 30 minutes after preparation and filtered through a 0 45 μm filter prior to injection

2 4 2 6 LC/MS Conditions

An ACE C18 5mm, 250 mm x 2 5 mm column was employed at a flow rate of 0 3 ml/min The mobile phase was the same as that used for the HPLC analysis (35% ACN 65% acetic acid) It was filtered and degassed by sonication for 20 minutes prior to use All samples were filtered though a 0 45 μ m filter prior to introduction to the LC/MS

API-ES in the positive polarity mode was used at a scan range of m/z 50 – 2200 API-ES has been used previously for the analysis of DHP – related compounds [25] and for the analysis of heterocyclic amines [26], to name but two examples Other conditions include the sheath gas (nitrogen) at 70 psi (1 psi = 6894 76 Pa) and the trap drive was set to 24

The solutions analysed were the same as those prepared in section 2 4 2 5 All solutions were filtered through a 0 45 μ m filter before injection and the injector was rinsed with ACN before each injection

2.5 Results

2.5.1 Introduction

The generation of ions in atmospheric pressure ionisation is affected by a number of parameters including mobile phase additives, solution pH, flow rate and solvent composition [27]

Non-volatile buffers, such as phosphate are incompatible with mass spectrometry because they can form salt deposits, which interfere with the operation of the instrument. A more subtle reason to avoid the use of non-volatile buffers is that they can affect the process of ion formation in API-ES and APCI [27]. Generally, for most compounds, the best sensitivity is obtained in APCI and electrospray when ion formation occurs by protonation. However, this can only occur in the absence of cations. Therefore, in the presence of non-volatile buffers, multiply cationised species are observed, which increases the complexity of mass spectra obtained and decreases sensitivity. In general, volatile buffers, such as dilute solutions of acetic or formic acid (1%-2%), are utilised.

The type of solvent used also plays an important role. Their properties: surface density, conductivity, viscosity, dielectric constant, were found to be very important parameters in the success of the API-ES process [28]. Jemal and Hawthorne [29] studied the positive and negative ion electrospray response of nucleotides in mobile phases that contained methanol or ACN at neutral or low pH and with or without ammonium acetate. The authors found that the positive ion response was consistently higher in a methanolic mobile phase under all conditions studied.

The flow rate in LC/MS is affected by the requirement for the introduction of mobile phase from the LC, which flows at approx. 1.0 ml min^{-1} , into the vacuum of the MS. This incompatibility is generally overcome by the use of a narrow bore column.

This brings about a decrease in the flow rate of the LC mobile phase without any increase in analysis times (Equation 5)

$$V_R = t_R F_c \quad \text{Equation 5}$$

where V_R is defined as the retention volume, t_R is the retention time, while F_c is the volumetric flowrate and is defined in equation 6 [20]

$$F_c = \frac{\pi d_c^2}{4} \times \varepsilon_{tot} \times \frac{L}{t_m} = \frac{V_{col} \varepsilon_{tot}}{t_M} \quad \text{Equation 6}$$

where d_c is the column bore, L is the column length, ε_{tot} denotes the total porosity of the column packing and V_{col} is the bed volume of the column [20]

Mobile phase additives, e.g. triethylamine (TEA) cannot be used in LC/MS analysis because they suppress the ionisation of some compounds and can also produce unwanted peaks in the mass spectrum, e.g. TEA has a peak at m/z 101 in both the positive and negative ion mode in API-ES

Careful manipulation of the mobile phase composition is required in order to obtain baseline resolution without the use of ion-pairing reagents. Some groups have employed TEA in their mobile phase and then utilised post-column addition of propionic acid to suppress the effects of the ion-pairing reagent in the mass spectrometer [18]

The use of ammonium acetate to buffer the mobile phase can also improve the resolution between LC peaks and, as a result, produce clearer mass spectra. Ammonium acetate is a non-volatile buffer, but it can be used in LC-MS because ammonium ions form only weak complexes. Therefore, ammonium acetate produces fewer multiply cationized species than other non-volatile buffers, resulting in less complex mass spectra.

The preparation of the LC/MS mobile phase compatible with LC/MS detection and all other conditions is outlined in methods section 2.4.2.3.

2.5.2 HPLC Study of the Decomposition of THQ

The effect of copper and iron on the cure chemistry of the anaerobic adhesives has been investigated by many groups and shown to be an important factor in generating a good cure profile, as in chapter 1, section 3. This is demonstrated by the difference in the decomposition rates of THQ in the absence and presence of metals (Fig. 2.17 – 2.19). This investigation into the decomposition rates of the reducing agents had been carried out by Kincaid [22]. However, it was decided to perform the investigation again using the new mobile phase. The knowledge gained from this analysis could then be used when preparing the solutions for the determination of the products of decomposition of the reducing agents. The percentage decomposition of THQ was determined by following the procedure used by Kincaid [22]. A solution of the reducing agent of interest was prepared at a given concentration and the area of the peak in the chromatogram determined. The other components of the cure system were added and the area of the reducing agent peak determined again.

The relative reduction in the concentration of reducing agent was calculated as the difference between the initial peak area of the reducing agent peak and each successive reading

The relative decrease could then be expressed as percentage decomposition of the reducing agent. The percentage decomposition of reducing agent versus time (time being the age of the solution in question) was plotted for each THQ cure system. Generally, the solutions were monitored over a ten to fourteen day period. All instrumental conditions and solution preparations were as outlined in section 2.4.2.2.

The results obtained compare well to the previous studies carried out by Kincaid [22]. The decomposition of THQ is significantly higher in the presence of the metal salts, with copper sulphate causing the fastest rate of decomposition (Fig. 2.18). However, in the absence of metals, when both acids were present in the solution, the decomposition rate was approx. 75% of THQ decomposed after 2 days (Fig. 2.17).

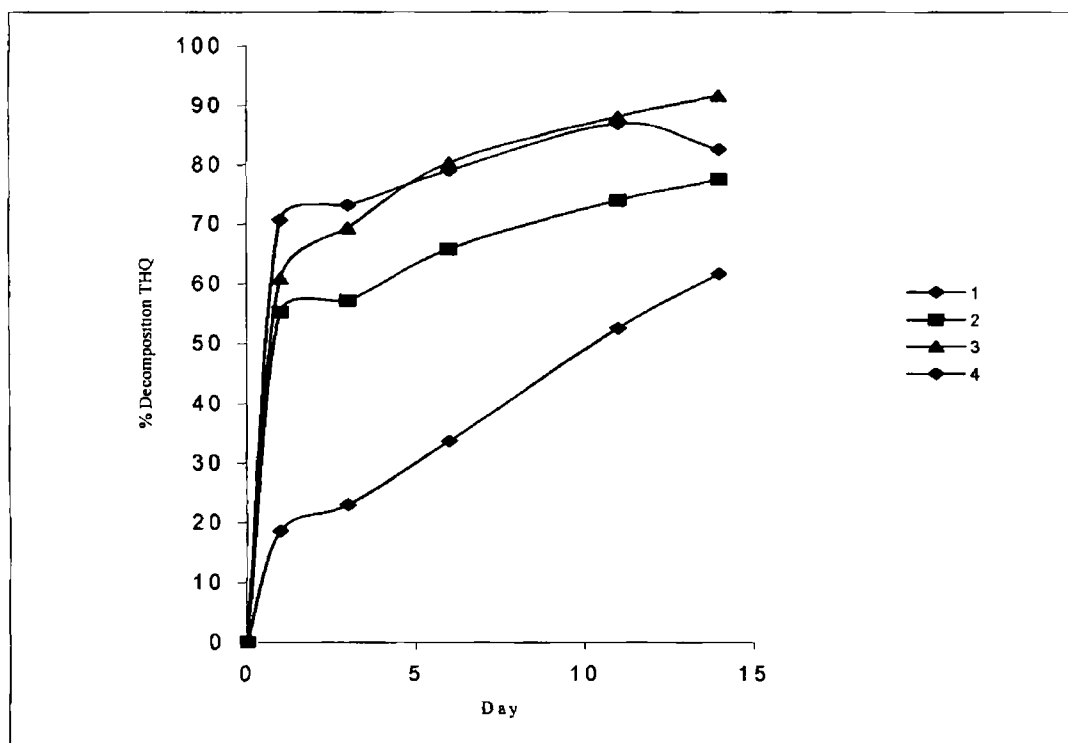


Fig 2.17 Decomposition of THQ in the absence of metals

Key 1 THQ:CHP (1:1)

2 THQ:CHP:MA (1:1:1)

3 THQ:CHP:SAC (1:1:1)

4 THQ:CHP:MA:SAC (1:1:1:1)

The decomposition of THQ in the presence of copper is faster than in the presence of iron with almost 100% decomposition of the reducing agent after 2 days (Fig. 2.19). In the absence of acids the decomposition of THQ in the presence of iron (Fig. 2.18) is much slower than its decomposition in the solution containing copper and no acids. The decomposition of DHP could not be monitored because no DHP peak was observed at a concentration of 0.75 mM at 254 nm. Consequently, the decrease in the concentration of DHP could not be monitored with the procedure used by Kincaid [22].

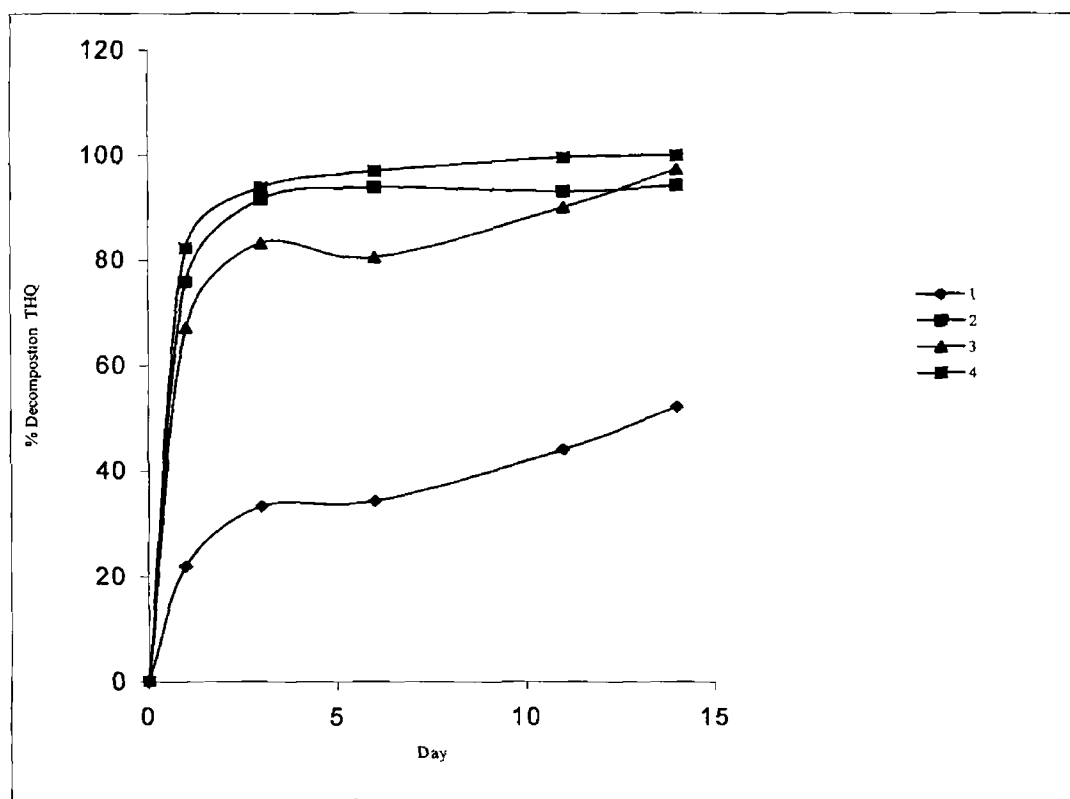


Fig 2.18 Decomposition of THQ in the presence of iron nitrate

Key 1 THQ CHP iron nitrate (1 1 1)

2 THQ CHP iron nitrate maleic acid (1 1 1 1)

3 THQ CHP iron nitrate saccharin (1 1 1)

4 THQ CHP iron nitrate maleic acid saccharin (1 1 1 1 1)

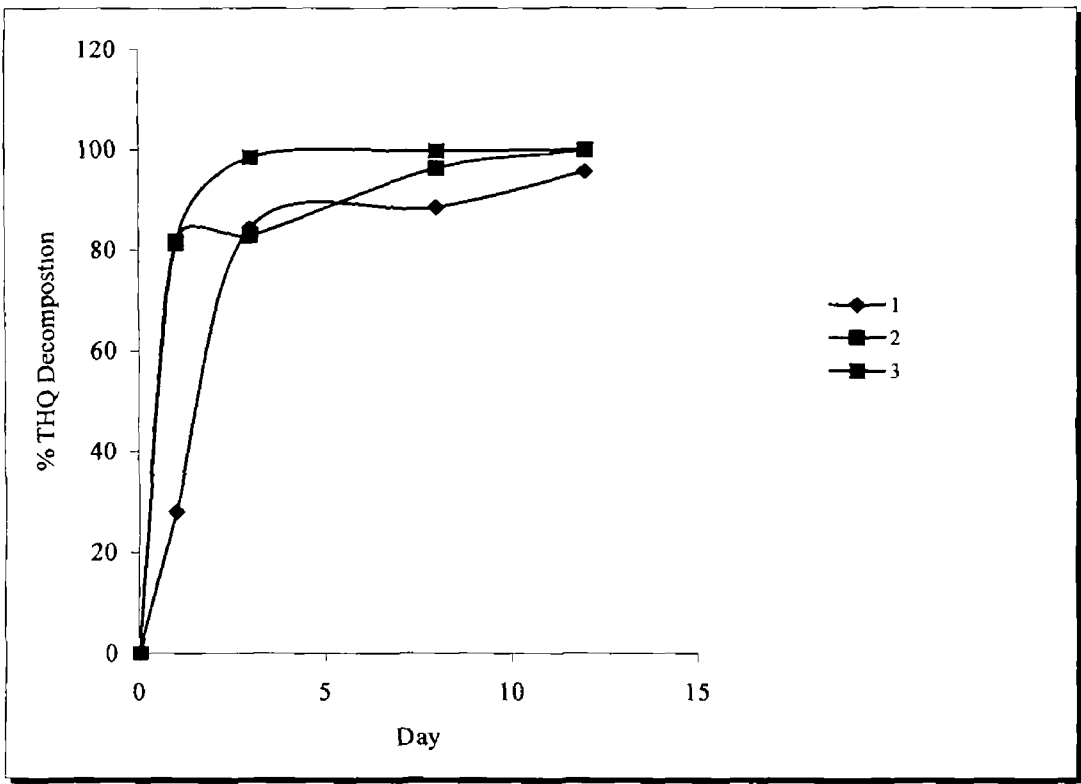


Fig 2 19 Decomposition of THQ in the presence of copper sulphate

- Key 1 THQ CHP copper sulphate (1 1 1)
- 2 THQ CHP copper sulphate maleic acid (1 1 1 1)
- 3 THQ CHP copper sulphate maleic acid saccharin (1 1 1 1 1)

2 5 3 Monitoring of Products Generated during the Decomposition of Reducing Agents

2 5 3 1 Products formed due to Decomposition of THQ in the Absence of Metals

The aim of this project was to determine if any products were formed as a result of the decomposition of the reducing agents and, if so, to identify them. While monitoring the decomposition of THQ, new peaks were observed on the chromatograms of all THQ cure systems as decomposition of the reducing agent progressed. Different ratios of reducing agents to other components in the cure system were prepared in order to generate the greatest amount of products possible. The preparation of these solutions is described in section 2 4 2 3.

In the absence of metals the decomposition of the reducing agents was slower and, as a result, fewer products were formed in smaller amounts. Firstly, in the absence of metals, in Figure 2 20, by day 12 a product of the decomposition of THQ can be seen to form at approximately 8 minutes. In Figure 2 21 a number of other products formed between THQ and saccharin can be seen as small peaks (peak 2, peak 3 and peak 4) on the chromatogram.

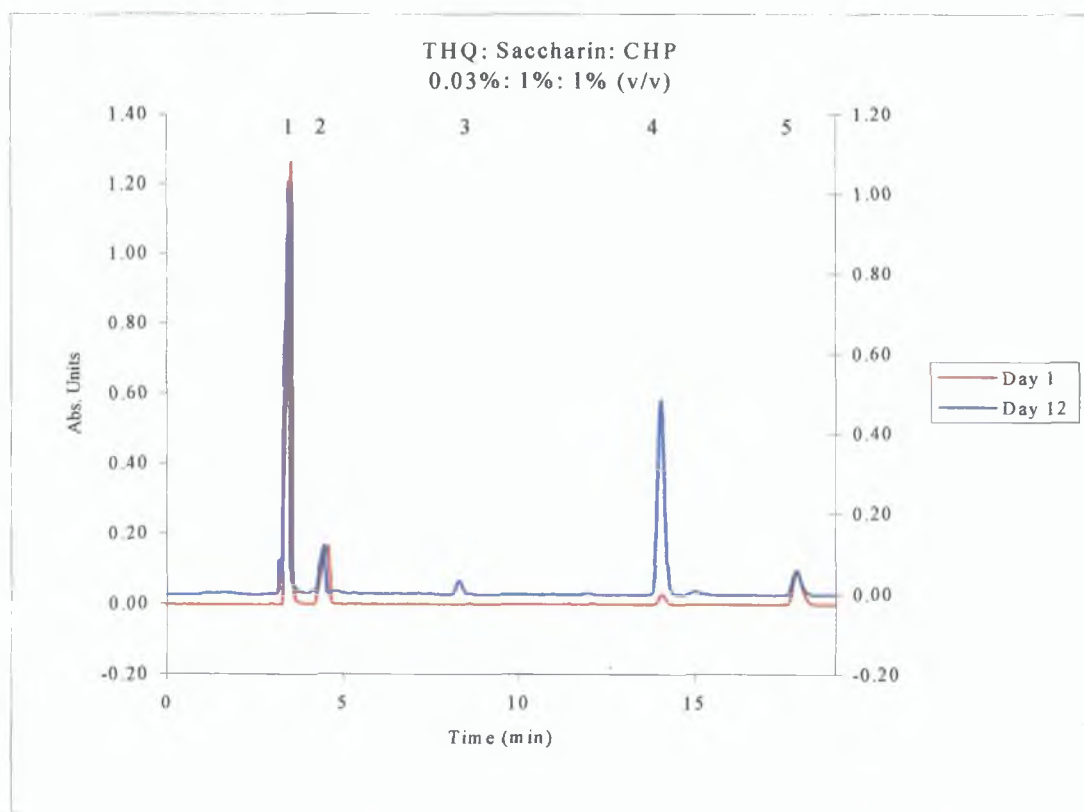


Fig. 2.20. Chromatogram of THQ cure system. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Saccharin; 2. THQ; 3. Unknown; 4, 5. CHP.

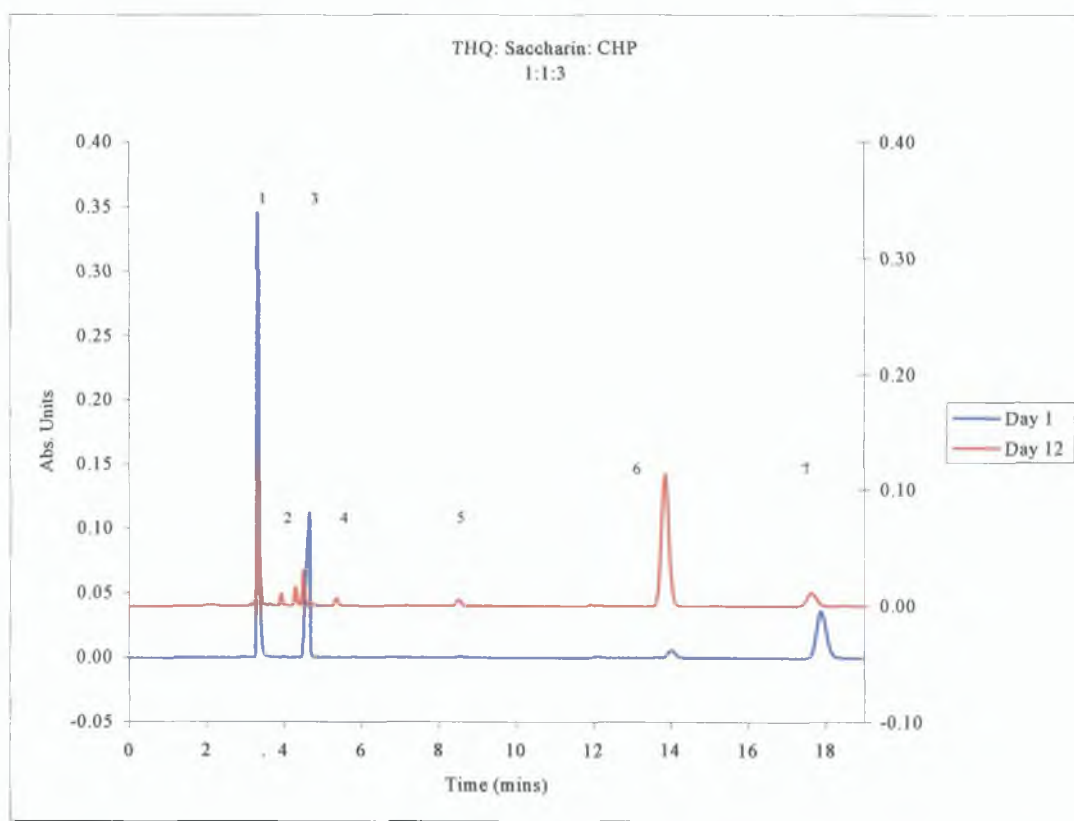


Fig. 2.21. Chromatogram of THQ cure system (concentration of THQ: 0.75 mM). The numerous products formed can be seen as peaks on the chromatogram of the components on day 12. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Saccharin; 2. Unknown; 3. THQ; 4. Unknown; 5. Unknown ; 6,7. CHP.

In Figure 2.22 the chromatogram of the cure system containing both acids can be seen. A peak appears between the two acids (Fig. 2.22a). This peak may be related to the presence of the maleic acid because it is not present in Figure 2.20 or Figure 2.21, where the chromatograms are of the THQ cure system containing only saccharin. It is also clear that the decomposition of THQ is very slow because, even after 12 days, a peak for THQ is still present on the chromatograms, indicating that it has not been completely decomposed.

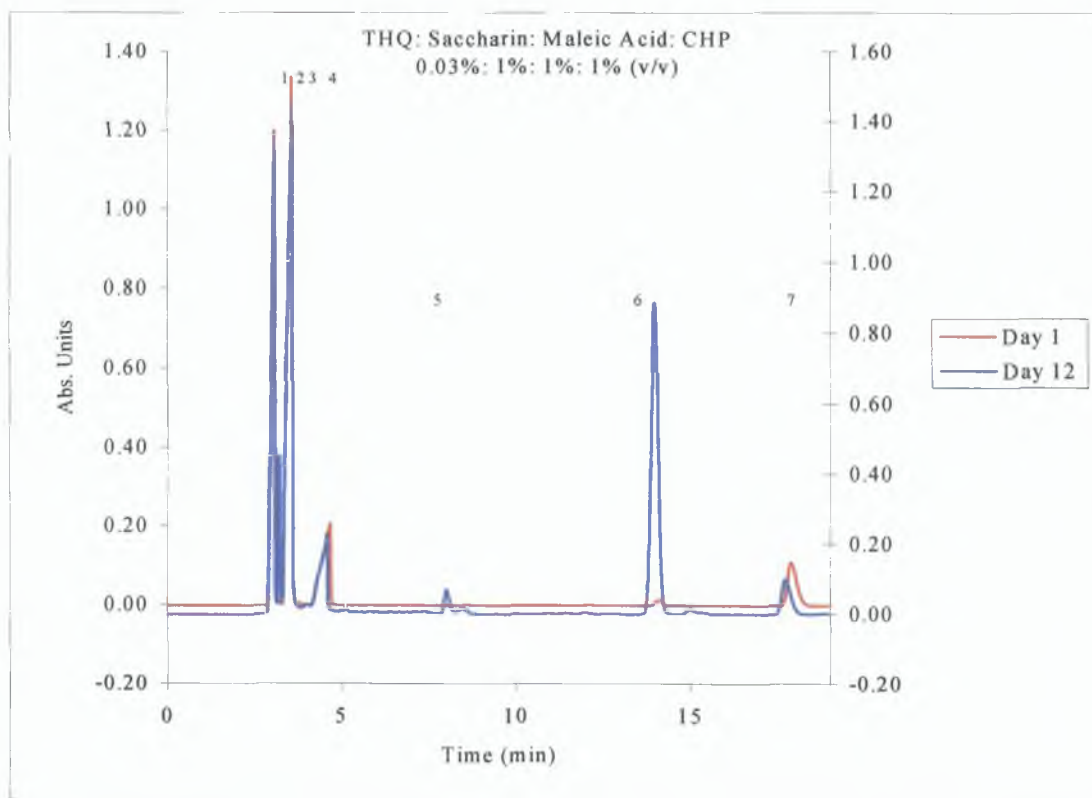


Fig. 2.22. Chromatogram of THQ cure system. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Maleic acid; 2. Unknown; 3. Saccharin; 4. THQ; 5. Unknown; 6, 7. CHP.

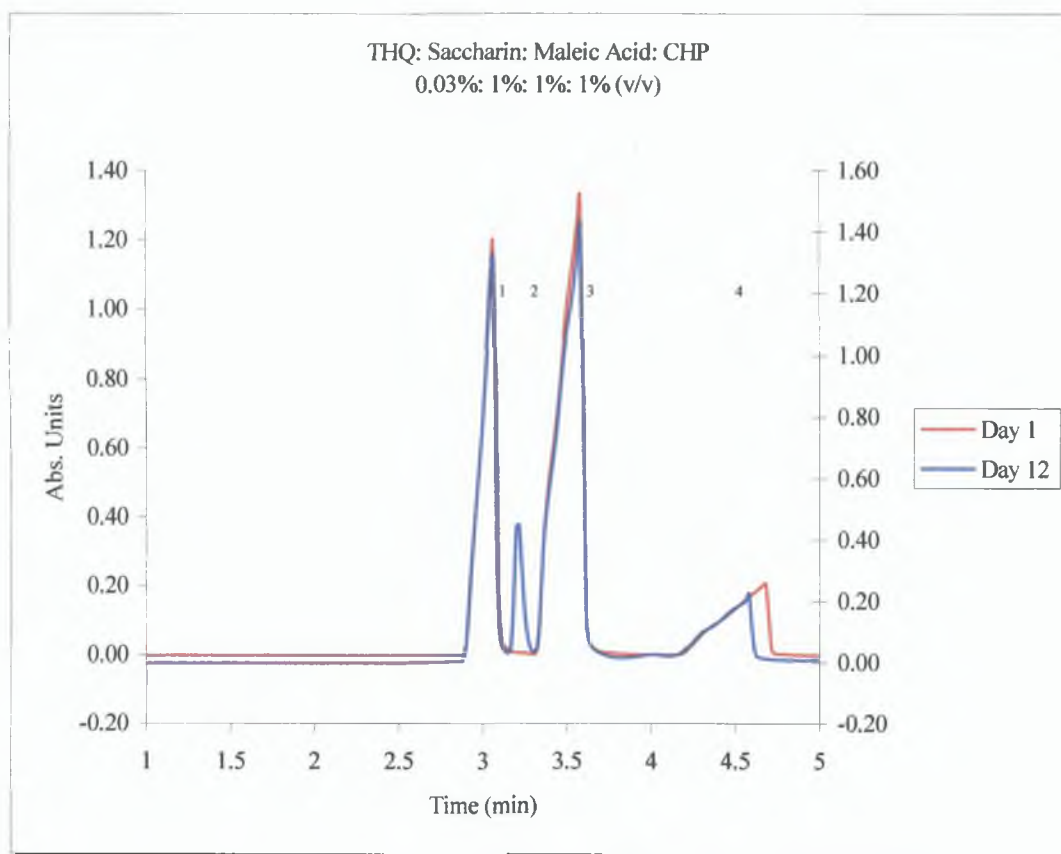


Fig. 2.22a. Zoom of Fig. 2.22 showing the unknown product formed between saccharin and maleic acid. Conditions as for Fig. 2.22. 1. Maleic acid; 2. Unknown; 3. Saccharin; 4. THQ.

2.5.3.2 Products formed due to Decomposition of DHP in the Absence of Metals

As mentioned, the decomposition of DHP could not be quantified. However, peaks were observed on the chromatograms of the DHP cure systems over the period of the analysis. In the absence of metals the formation of products in the DHP cure system is slow. This can be seen in Figure 2.23, where only a couple of peaks with small responses can be observed.

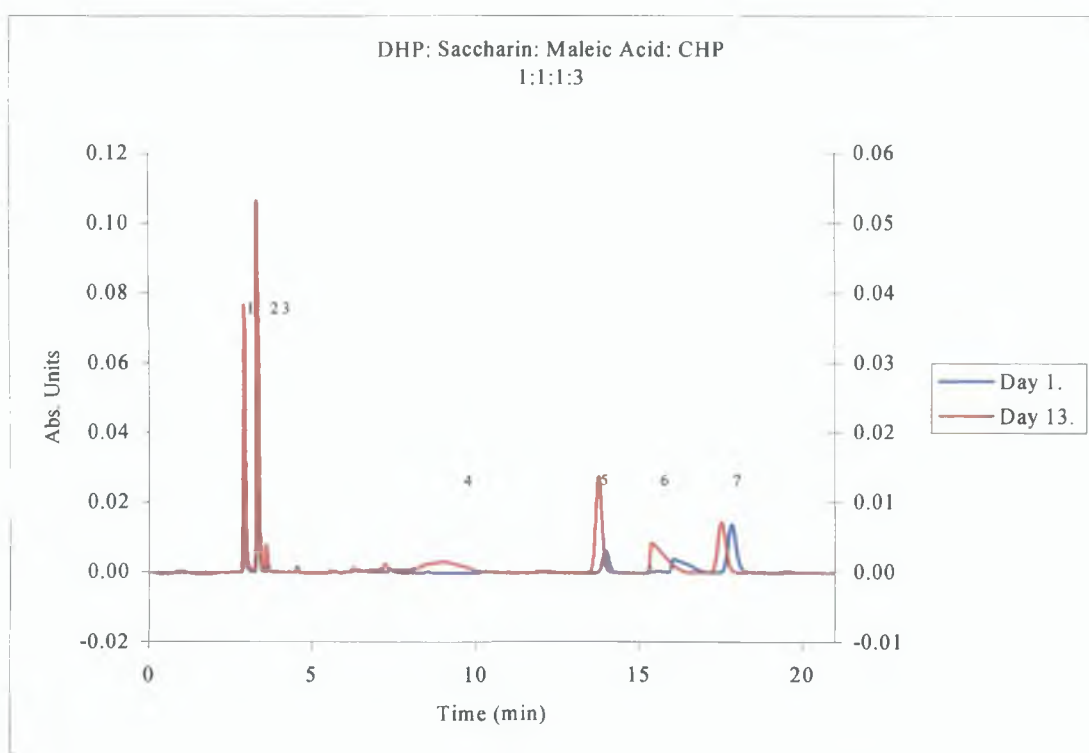


Fig. 2.23. Chromatogram of DHP cure system showing a peak was formed between the CHP peaks. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Maleic acid; 2. Saccharin; 3. Unknown; 4. Unknown; 5, CHP; 6. Unknown; 7. CHP.

2.5.3.3 Products formed due to Decomposition of THQ in the Presence of Copper

In contrast, the presence of metals caused the decomposition of both reducing agents to occur significantly faster and a large number of peaks were observed on the chromatograms of the cure systems, even after one day. Comparing the decomposition of THQ in the presence of copper acetate and both acids with the control solution (contains no copper acetate), it can be seen that THQ has completely decomposed after one day and 2 products have been formed (Fig. 2.24). These can be seen more clearly in Figure 2.24a.

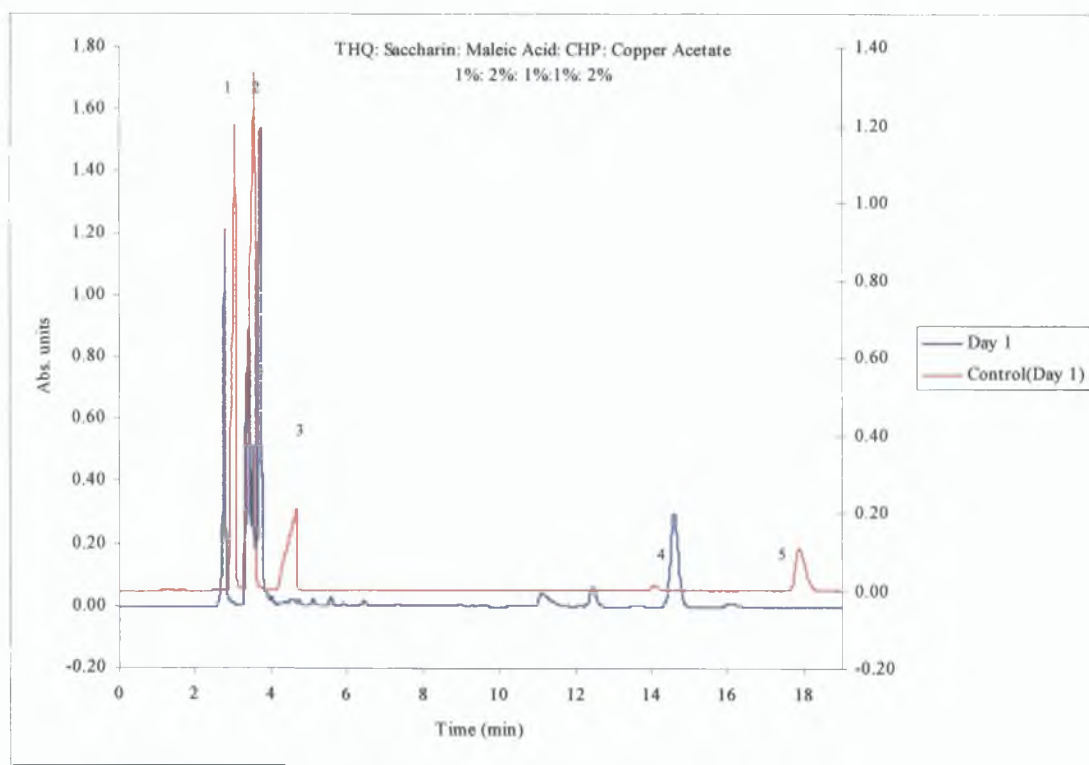


Fig. 2.24. Chromatogram of THQ cure system with copper acetate. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. Control: 1. Maleic acid; 2. Saccharin; 3. THQ; 4, 5. CHP.

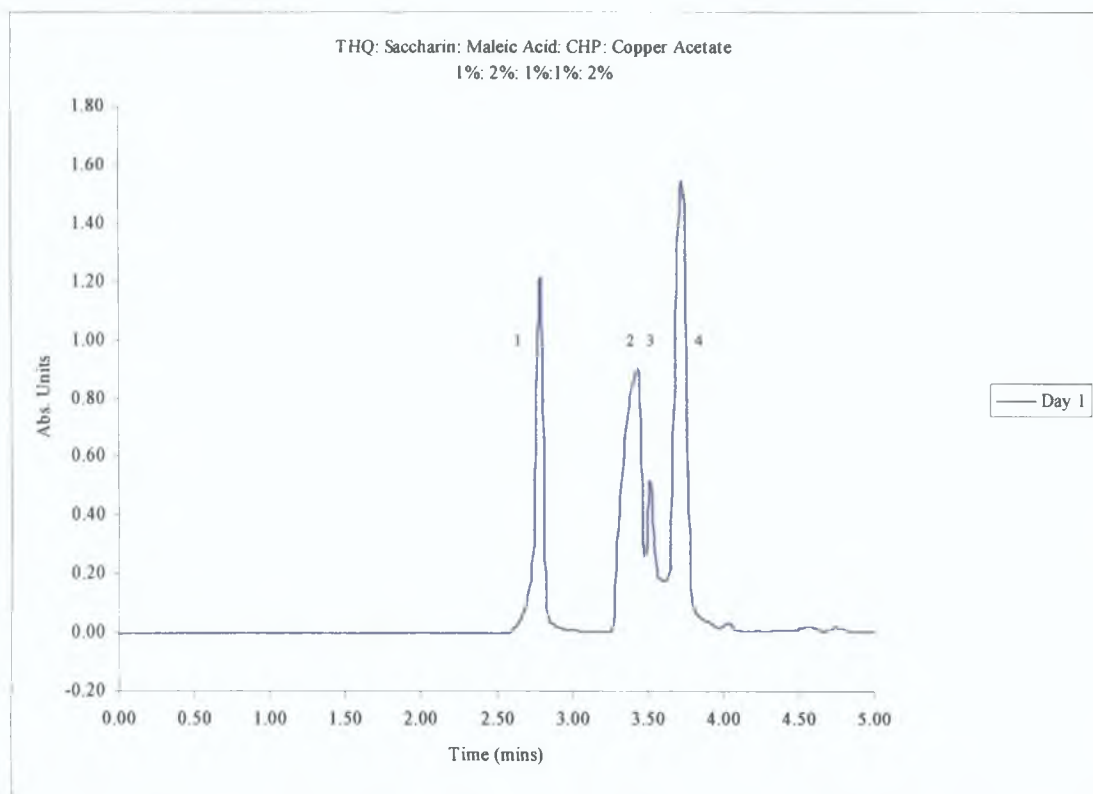


Fig. 2.24a. Zoom of chromatogram of THQ cure system with copper acetate showing the main products being formed. Conditions as for Fig. 2.24. 1.Unknown ; 2. Maleic acid; 3. Unknown; 4. Saccharin.

Figure 2.25 shows the decomposition of THQ to form a number of products by day 3 in the presence of copper acetate and saccharin (blue chromatogram). Comparing this chromatogram with the chromatogram of the control solution after three days (Fig. 2.25, red chromatogram), it can be seen that the THQ is almost completely decomposed in both cure systems. However, in the presence of copper acetate more peaks are present and the formation of the peak near saccharin is accelerated (Fig. 2.25a). The peak eluting at nearly the same time as saccharin is probably a derivative of saccharin. This peak would have to be more clearly resolved in order to determine its identity by LC/MS.

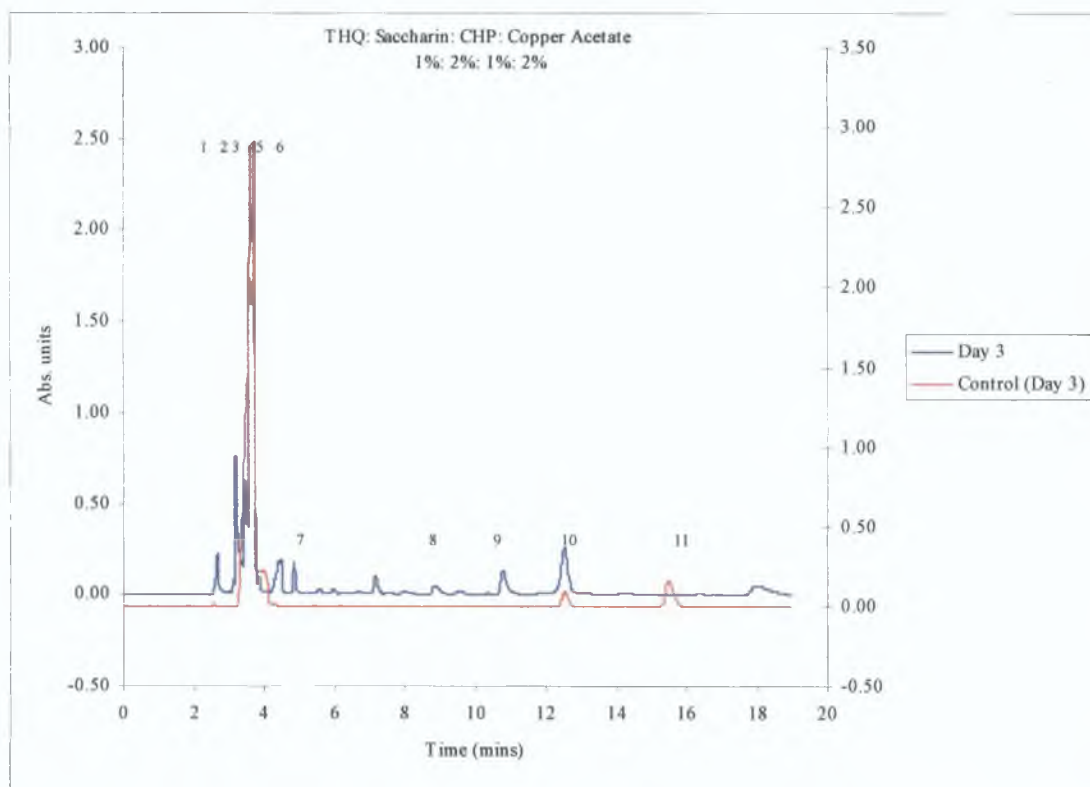
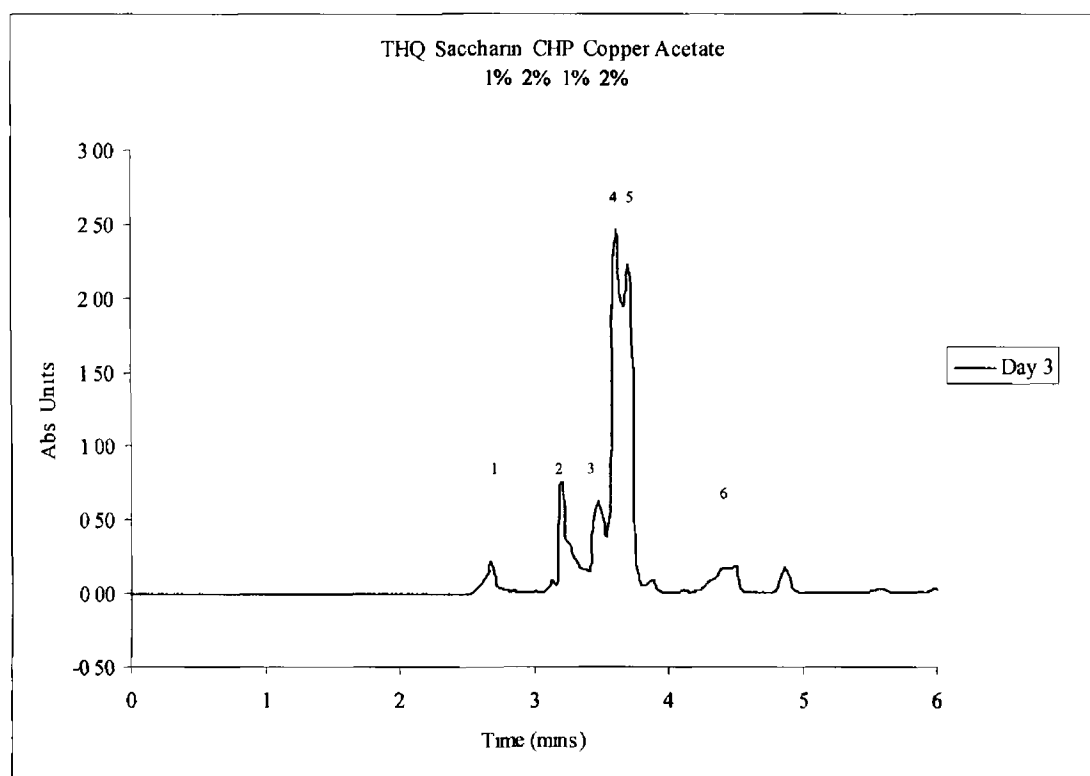


Fig. 2.25. THQ cure system containing saccharin as the only organic acid. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Unknown; 2. Unknown; 3. Unknown; 4. Unknown; 5. Saccharin ; 6. THQ; 7. Unknown; 8. Unknown; 9. Unknown; 10, 11. CHP.



2.25a Zoom of THQ cure system containing saccharin as the only organic acid
Conditions as for Fig 2.25 1 Unknown, 2 Unknown, 3 Unknown, 4 Unknown, 5
Saccharin, 6 THQ

In the presence of maleic acid and copper acetate the decomposition of THQ is not as vigorous as in the presence of saccharin and the metal salt (Fig 2.26, blue chromatogram). However, comparing this system to the control solution containing no metal (Fig 2.26, red chromatogram), it is obvious that the presence of the metal in the maleic acid sure system increases the decomposition of the reducing agent.

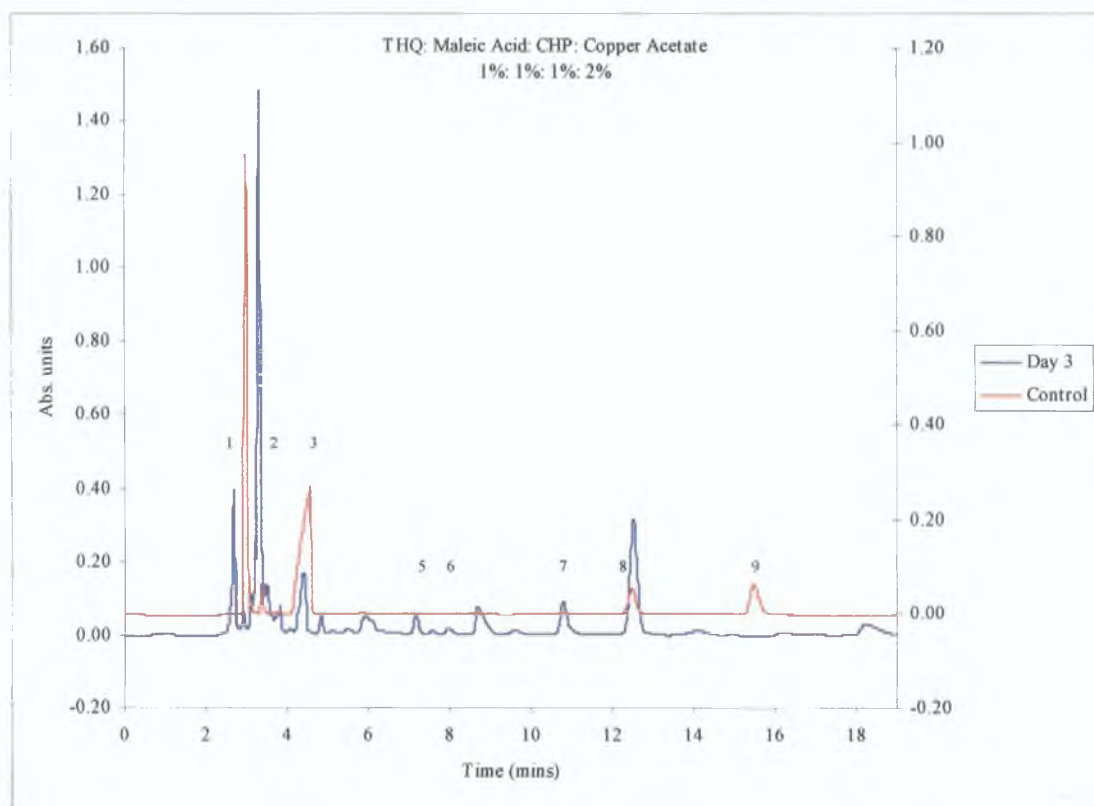


Fig. 2.26. THQ cure system containing maleic acid as the only organic acid. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.8ml/min; Detection : 254 nm; Temperature: ambient. 1. Unknown; 2. Maleic acid; 3. THQ; 4. Unknown; 5. Unknown; 6. Unknown; 7. Unknown; 8, 9. CHP.

Observing the control solutions containing one acid (Fig. 2.25 and Fig. 2.26), it can be seen that in the presence of saccharin (Fig. 2.25), the decomposition of THQ, with the formation of products, was seen in a peak that began to form close to saccharin. However, in contrast, the decomposition of THQ in the presence of maleic acid was considerably slower (Fig. 2.26), showing practically no decomposition of the reducing agent after three days. This observation is supported by the decomposition studies carried out in the absence of metals (Fig. 2.17).

While it is impossible to determine the reasons for the difference in the rates of THQ decomposition in the presence of saccharin and maleic acid within the confines of this analysis, this observation is also supported by the work carried out by Kincaid [22]. He found that the decomposition of THQ in the absence of metals was greatest when saccharin and CHP were in the solution, but lowest in the presence of maleic acid and CHP. The difference in the influence of the acids over the decomposition of reducing agents has been noted by a number of authors [30, 31, 32, 33]. It has been postulated that a protonated peroxide is more reactive than an unprotonated one [30]. Maleic acid, being a stronger acid than saccharin, causes a greater protonation of the peroxide, thereby lowering the energy required to cleave the peroxide bond and increasing the rate of CHP decomposition to form free radicals. This leads, in theory, to greater decomposition of reducing agent [31]. However, while maleic acid is thought to protonate CHP to a greater extent than saccharin, it has also been shown to have a greater negative effect on the reducing agents by causing a decrease in their reductive potential i.e. their ability to promote homolysis of the peroxide [30]. In contrast to Beaunez and co-authors [30], Moane [31] found that maleic acid caused a decrease in the rate of peroxide decomposition, which may lead to a decrease in the decomposition of the reducing agent.

It is beyond the scope of this analysis to determine the reasons for the difference between the rates of decomposition of THQ in the presence of maleic acid and saccharin. The part that the acids play in the chemistry of the anaerobic adhesives is obviously complex. The introduction of metals to the cure system further increases the complexity of the role of acids in the cure system.

In the presence of copper acetate, the formation of the peak near saccharin was accelerated and a number of smaller peaks can be seen on the chromatogram also (Fig 2 25) On addition of copper acetate to the solution containing maleic acid, the decomposition of the THQ was faster than in the absence of the metal salt and a number of peaks appear on the chromatogram (Fig 2 26) As outlined in chapter 1 section 3, investigators [31,33] have noted the important role that saccharin plays in cure systems containing metals Raftery [32] postulated that acids were involved in the liberation of metal ions from a substrate surface Saccharin leads to greater levels of liberated metals than maleic acid George [33] also found that the acids interacted with copper to make more cations available for the decomposition of CHP This, in turn, leads to the decomposition of the reducing agent

2.5.3.4 Products formed due to Decomposition of THQ in the Presence of Iron

In comparison with the THQ solution containing copper salt (Fig. 2.24), the solution containing iron nitrate demonstrates a slower formation of products (Fig. 2.27). Also, there is a large peak due to CHP, indicating that in the presence of iron nitrate, CHP does not decompose as quickly as in the presence of copper.

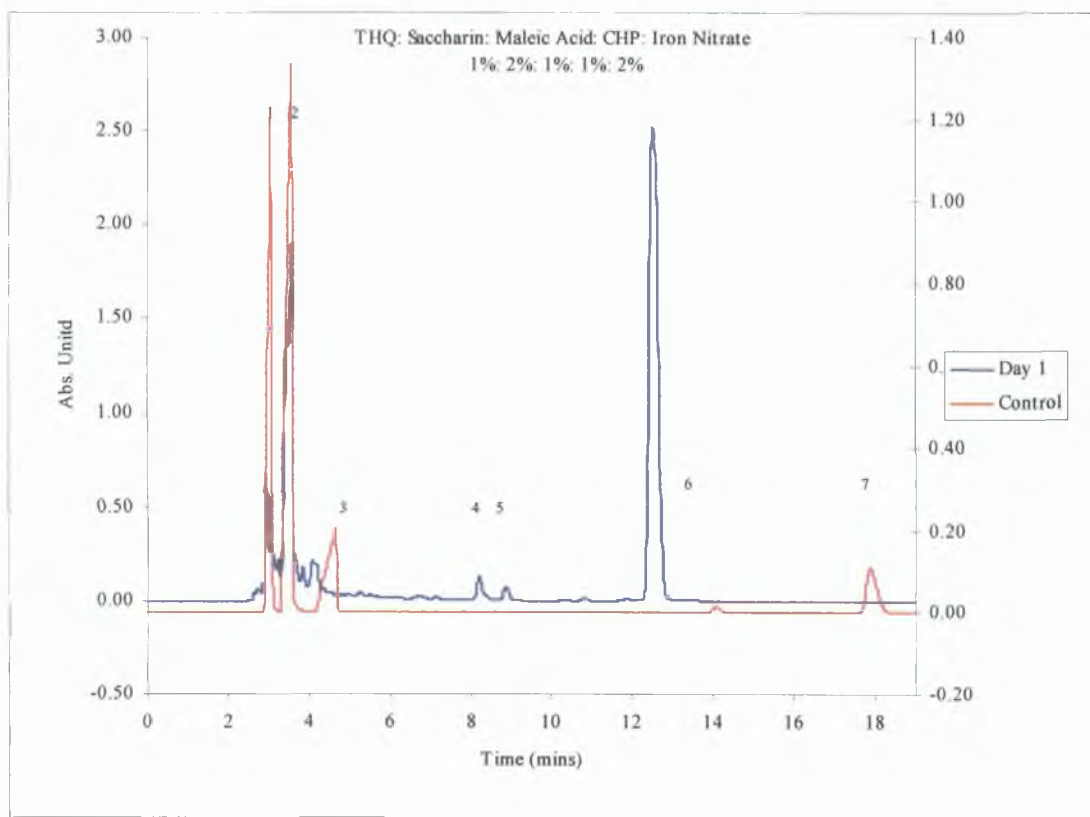


Fig. 2.27. THQ cure system with iron nitrate. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 mlmin⁻¹; Detection : 254 nm; Temperature: ambient. 1. Maleic Acid; 2. Saccharin; 3. THQ; 4. Unknown; 5. Unknown; 6, 7. CHP.

Comparing Figure 2.28 (blue chromatogram) with the chromatogram of the control solution (Fig. 2.28: red chromatogram) it can be seen that the decomposition of the reducing agent in the THQ – iron nitrate solution containing saccharin as the sole acid is almost complete after three days. However, the saccharin and CHP peaks remain (Fig. 2.28).

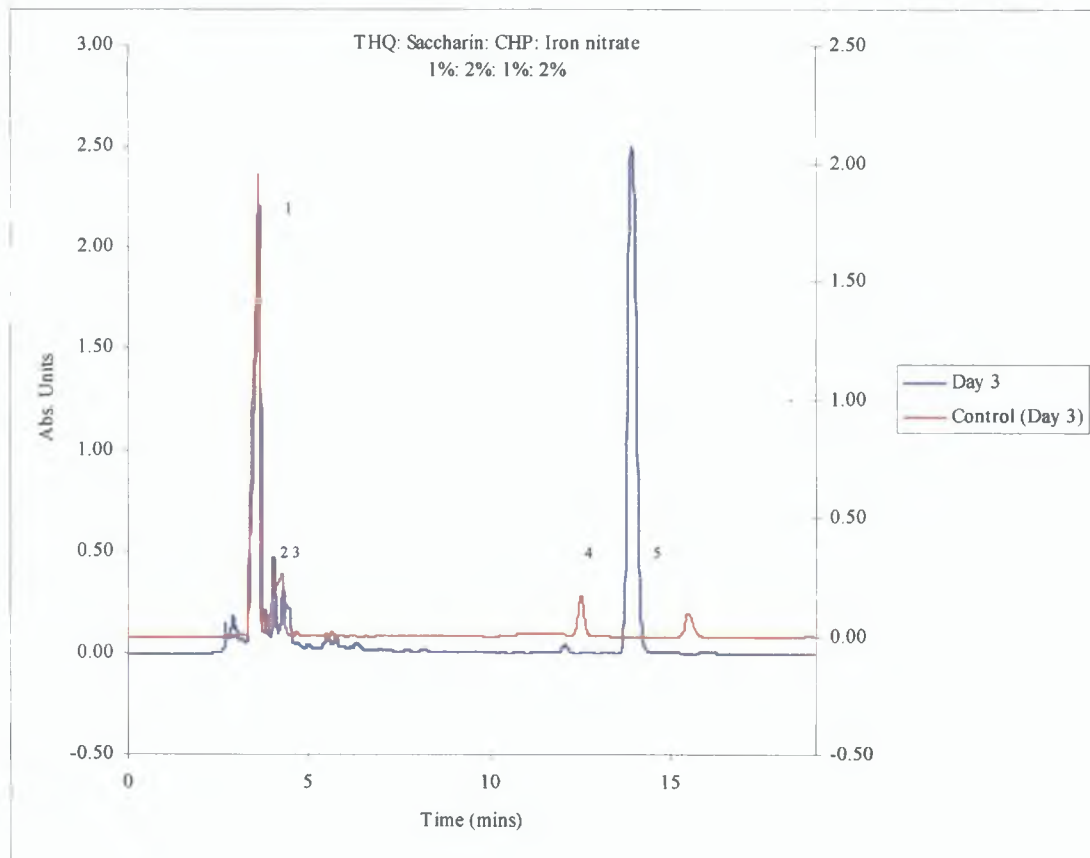


Fig. 2.28. THQ cure system with saccharin as the organic acid. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Saccharin; 2. Unknown; 3. THQ; 4,5.CHP.

A similar situation to the THQ –iron nitrate solution containing saccharin (Fig. 2.28) appears to occur in the solution containing maleic acid. The THQ is decomposed (Fig. 2.29) and maleic acid is partially decomposed. However, the CHP does not seem to have been decomposed at all.

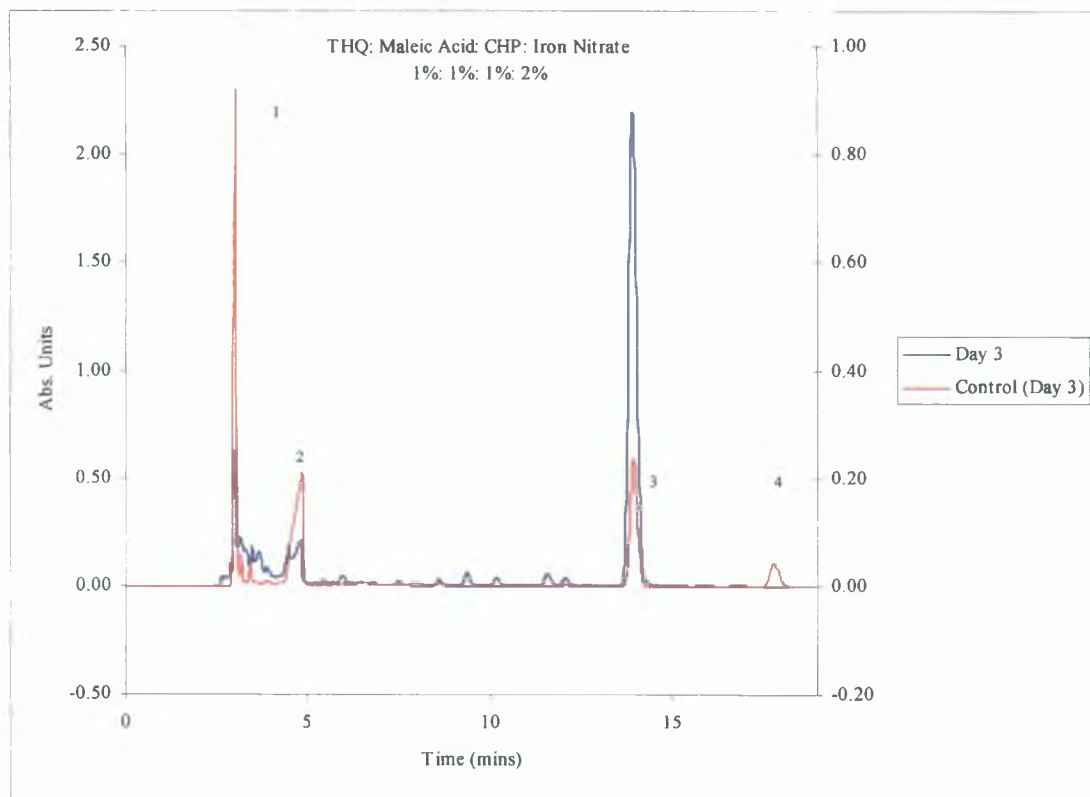


Fig. 2.29. THQ cure system with maleic acid. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Maleic acid; 2. THQ; 3,4. CHP.

2.5.3.5 Products formed due to Decomposition of DHP in the Presence of Copper

DHP is decomposed with the subsequent formation of products near maleic acid and saccharin (Fig. 2.30 and Fig. 2.30a) in the presence of copper acetate. However, in common with THQ-iron nitrate solutions, a large peak, corresponding to CHP, is present in the chromatograms. The unknowns are present at the same retention times as the unknowns in the THQ-copper sulphate cure system containing both organic acids. Therefore, it is possible that they are decomposition products of saccharin and maleic acid.

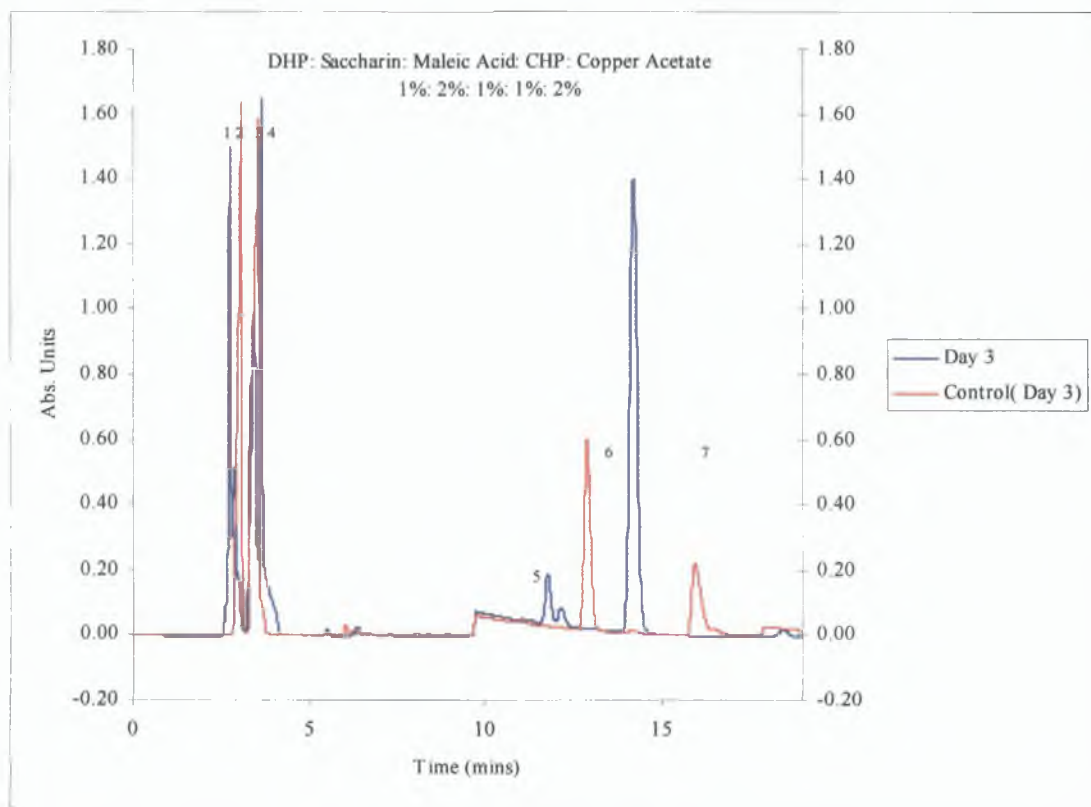


Fig. 2.30. DHP cure system with copper acetate. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Unknown; 2. Maleic Acid; 3. Unknown; 4. Saccharin; 5. Unknown; 6, 7. CHP.

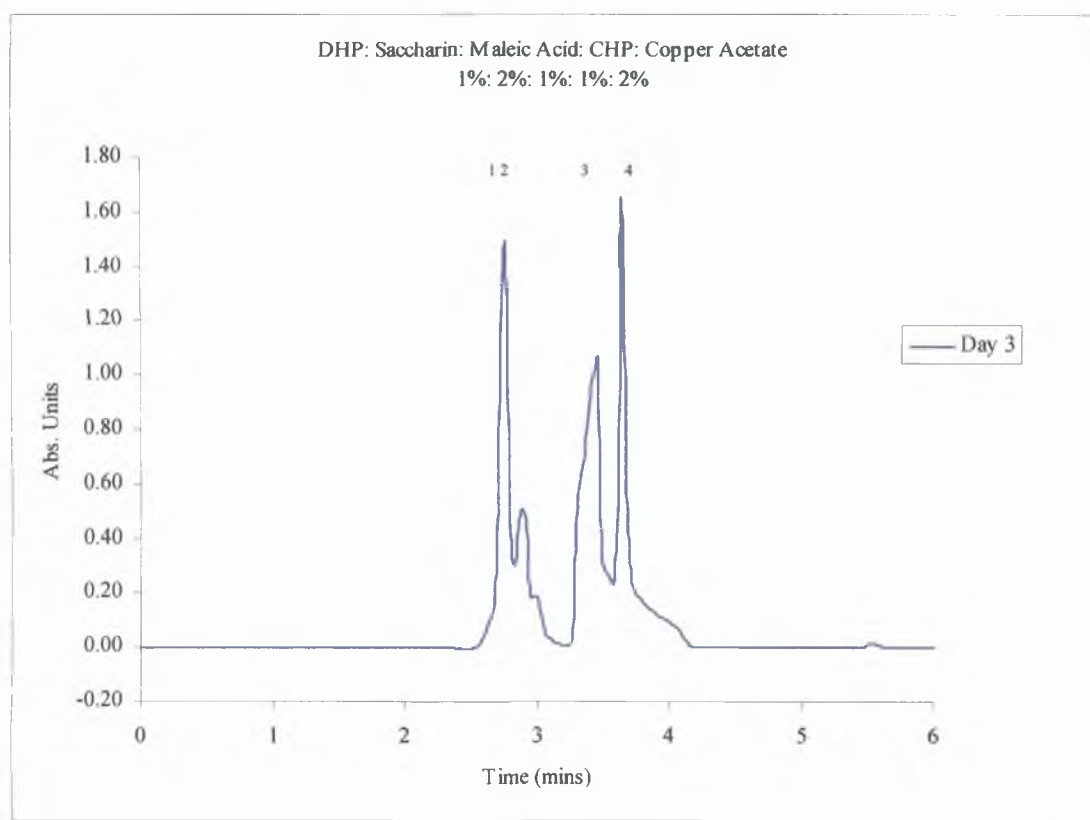


Fig. 2.30a. Zoom of DHP/copper acetate cure system. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 mlmin^{-1} ; Detection : 254 nm; Temperature: ambient. 1. Unknown; 2. Maleic Acid; 3. Unknown; 4. Saccharin.

In the DHP solutions containing saccharin only, a large peak is produced at a retention time of approximately 2.8 minutes (Fig. 2.31). This is very close to the elution time of maleic acid. However, no maleic acid was present in the solution. A peak with a similar retention time is also present in the chromatograms of the DHP-maleic acid solution (Fig.2.32). It was difficult to predict the identity of this peak and it was hoped that LC/MS could be used to determine its identity. In addition, the formation of peaks near saccharin is greatly accelerated in the solution containing the copper acetate.

This can be seen by comparing the chromatogram of the cure system (Figure 2.31: blue chromatogram) with the chromatogram of the control solution (Fig. 2.31: red chromatogram).

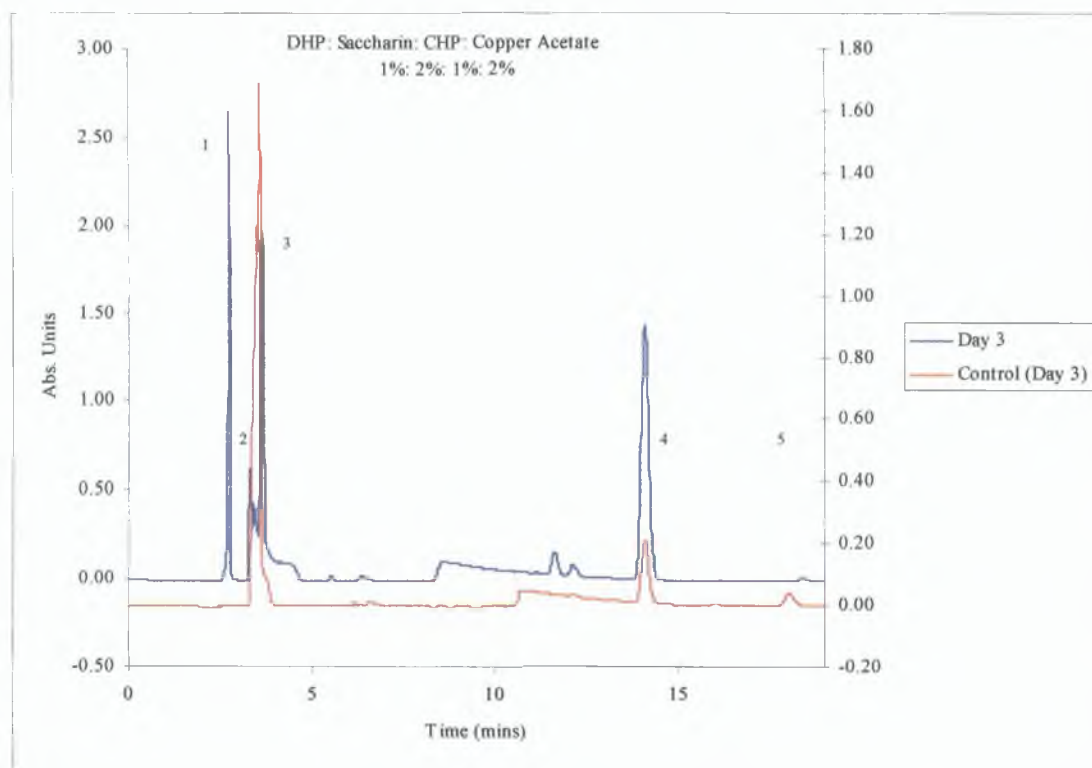


Fig. 2.31. DHP cure system with saccharin as the organic acid. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Unknown; 2. Unknown; 3. Saccharin; 4, 5. CHP.

In Figure 2.32 it can be seen that the CHP is not decomposed in the DHP cure system to the same extent as it is decomposed in the THQ cure systems. In comparison to the control solution (Fig. 2.32, red chromatogram) it can be seen that two peaks were formed near maleic acid in the presence of the copper acetate.

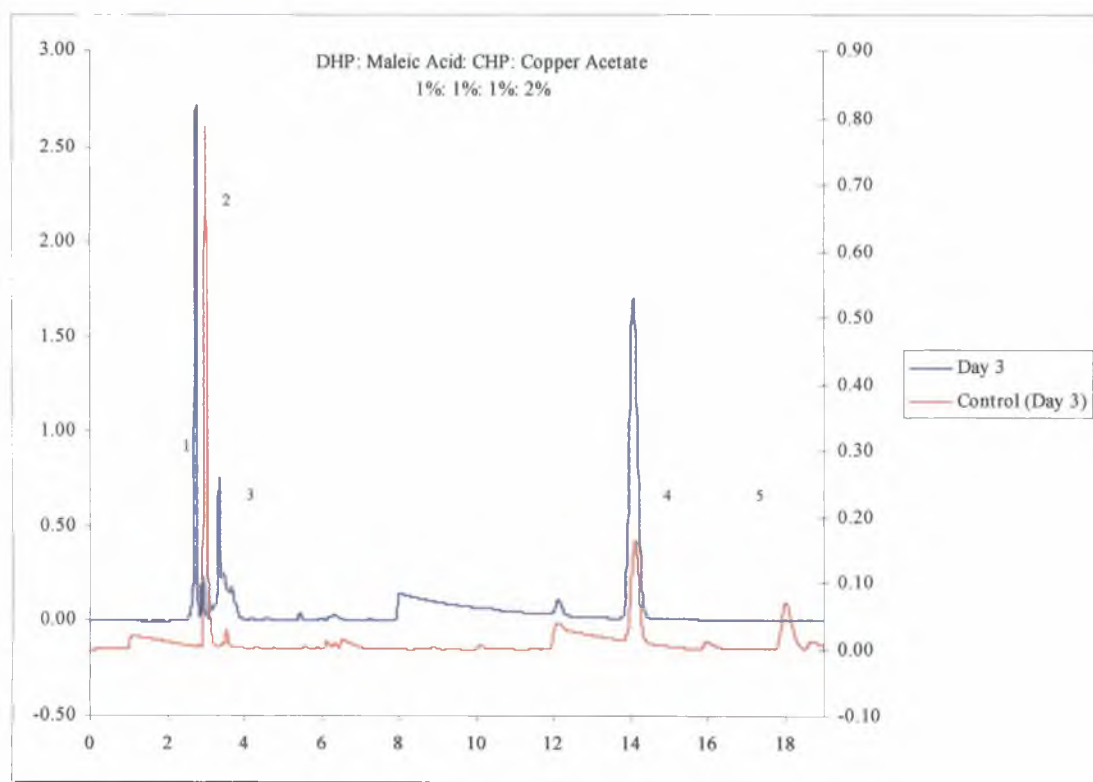


Fig. 2.32. DHP cure system with maleic acid. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Unknown; 2. Maleic Acid; 3. Unknown; 4, 5. CHP.

2.5.3.6 Products formed due to Decomposition of DHP in the Presence of Iron

The formation of products in DHP - iron nitrate solutions seems to be slower than in DHP -copper acetate solutions which is seen in Figure 2.33.

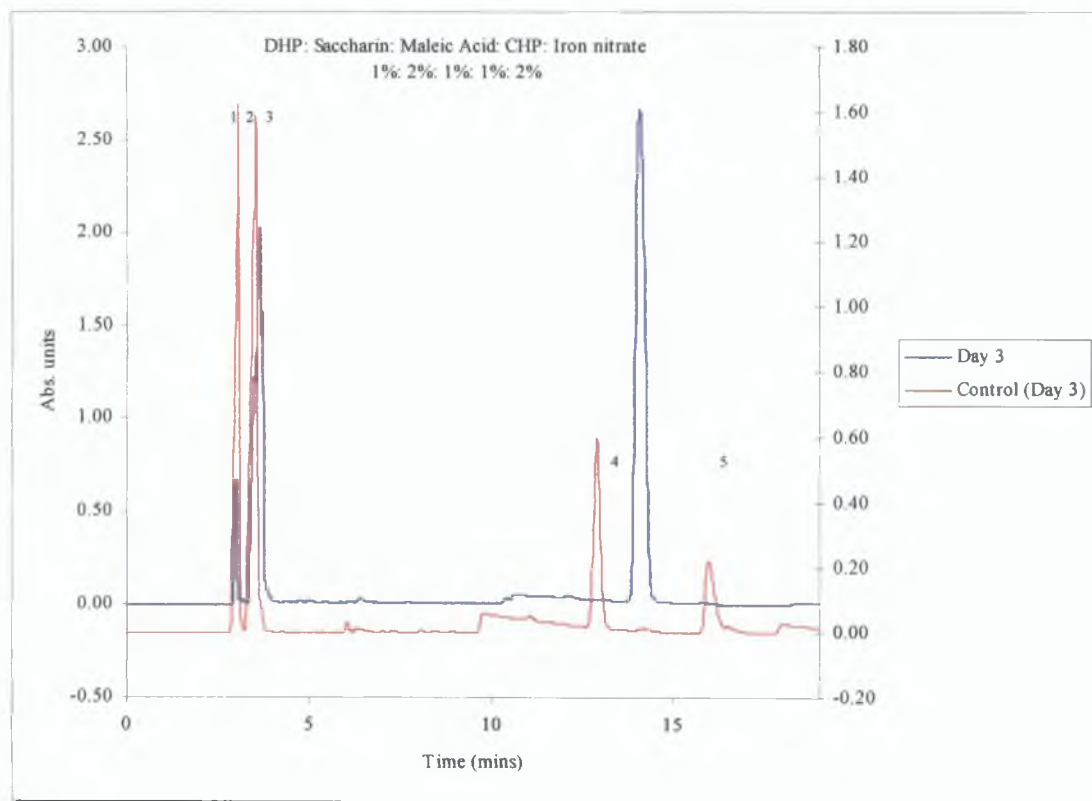


Fig. 2.33. DHP cure solution. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Maleic Acid; 2. Unknown; 3. Saccharin; 4, 5. CHP.

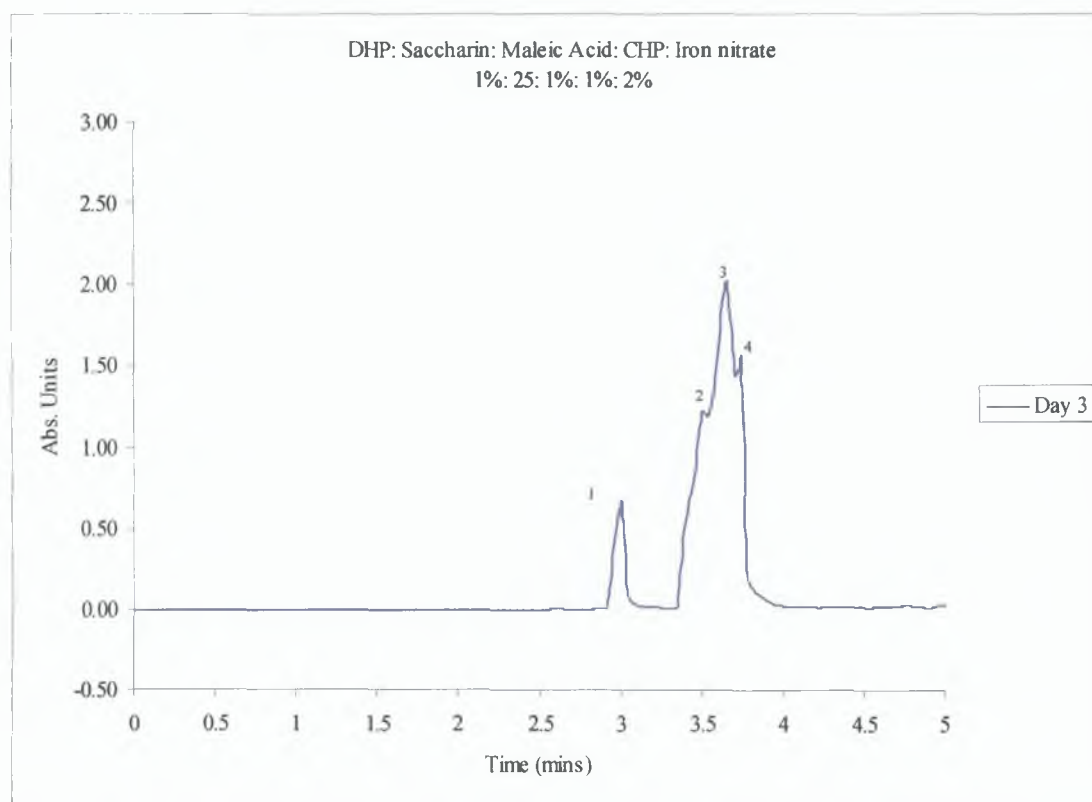


Fig. 2.33a. Zoom of DHP/iron nitrate cure solution. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Maleic Acid; 2. Unknown; 3. Unknown; 4. Saccharin.

It was interesting to note the colour changes that occurred in all the solutions over the period of time of the analysis and a precipitate formed in some of the solution also, possibly indicating the formation of a product. A blue colour formed in all solutions containing copper salt, while in the solutions containing saccharin a green-blue precipitate was formed after day 2 of the analysis. It is possible that this was copper saccharinate. George [33] observed the formation of copper saccharinate in his study of copper and saccharin solutions. A dark brown colour was observed forming in iron nitrate solutions with some precipitate formed in the saccharin – containing solutions.

2 5 4 LC/MS Determination of the Products Formed due to Decomposition of the Reducing Agents

In this study, metal salts of copper and iron were utilised to simulate the role of metals in the cure chemistry of anaerobic adhesives. Primarily, the salts were used because they had been used in previous studies [22]. As mentioned in section 2 5 1, salts cannot be used in LC/MS analysis because they form deposits on the mass analyser, which may impair its performance over time. A more suitable method for LC/MS used the metallic forms of the metals. This method was originally used by O'Dea [34] in his electroanalytical study of anaerobic adhesives. Details of the preparation of the solutions which were analysed are described in section 2 4 2 3.

Figure 2 34 shows the decomposition of THQ in the presence of copper powder. The rapid decomposition of the reducing agent leads to the formation of a number of poorly resolved products. As stated earlier, one cannot use ion-pairing reagents such as TEA, in LC/MS determinations. The resolution of the products was controlled by altering the mobile phase composition and the flow rate for some of the cure systems.

The formation of products in the DHP cure system (Fig 2 35) was much slower than in the THQ systems. As a result, the DHP solutions were analysed after three days in contrast to the THQ solutions, which were analysed on the day of preparation.

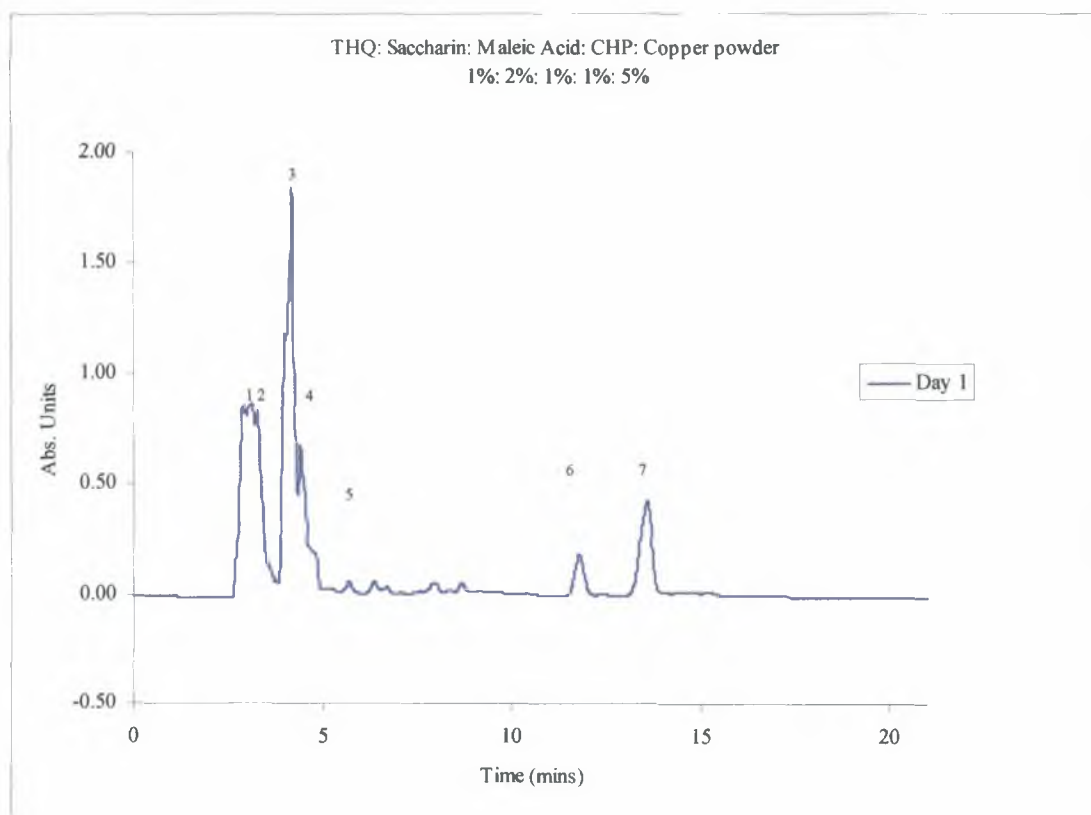


Fig. 2.34. THQ cure solution. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Unknown; 2. Unknown; 3. Saccharin; 4. Unknown; 5. Unknown; 6, 7. CHP.

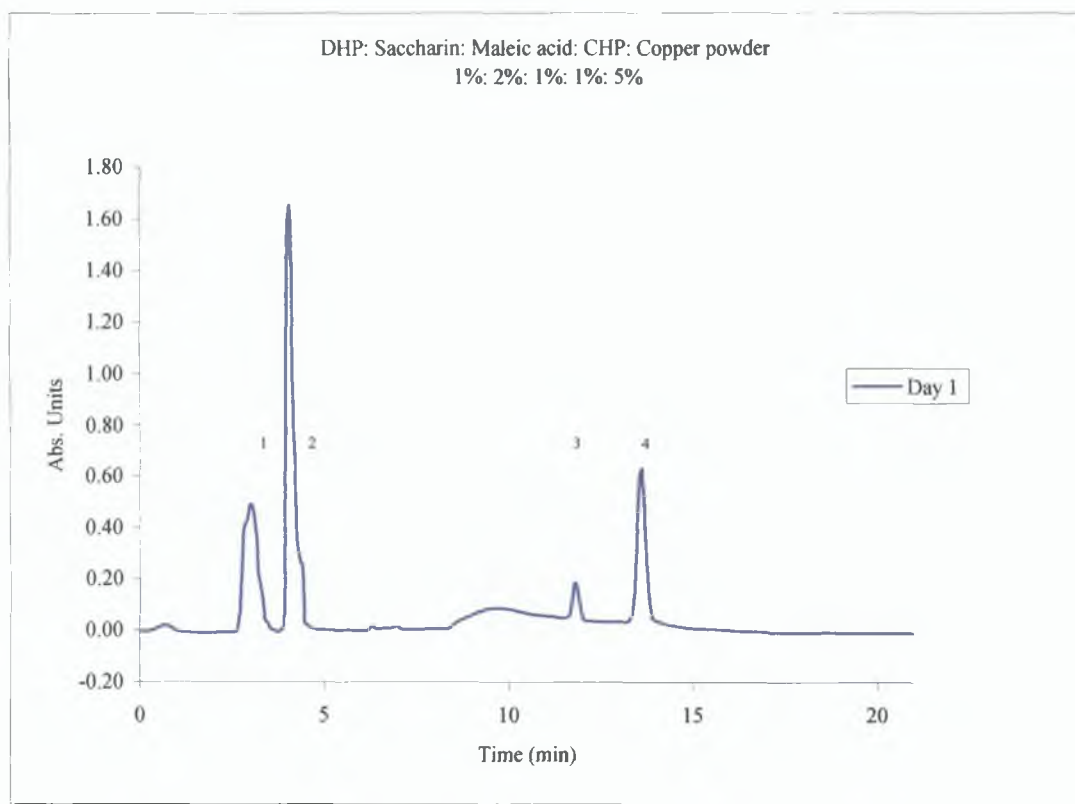


Fig. 2.35. DHP cure solution. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Maleic Acid; 2. Saccharin; 3,4. CHP.

The decomposition of THQ in the presence of iron filings was slower than its decomposition in copper powder (Fig. 2.36). The products of the decomposition of DHP can be seen in Figure 2.37. A peak can be seen forming at the same retention time as saccharin in both Figures 2.36 and 2.37. This peak is therefore not related to the either of the reducing agents and must be, therefore, be related to saccharin.

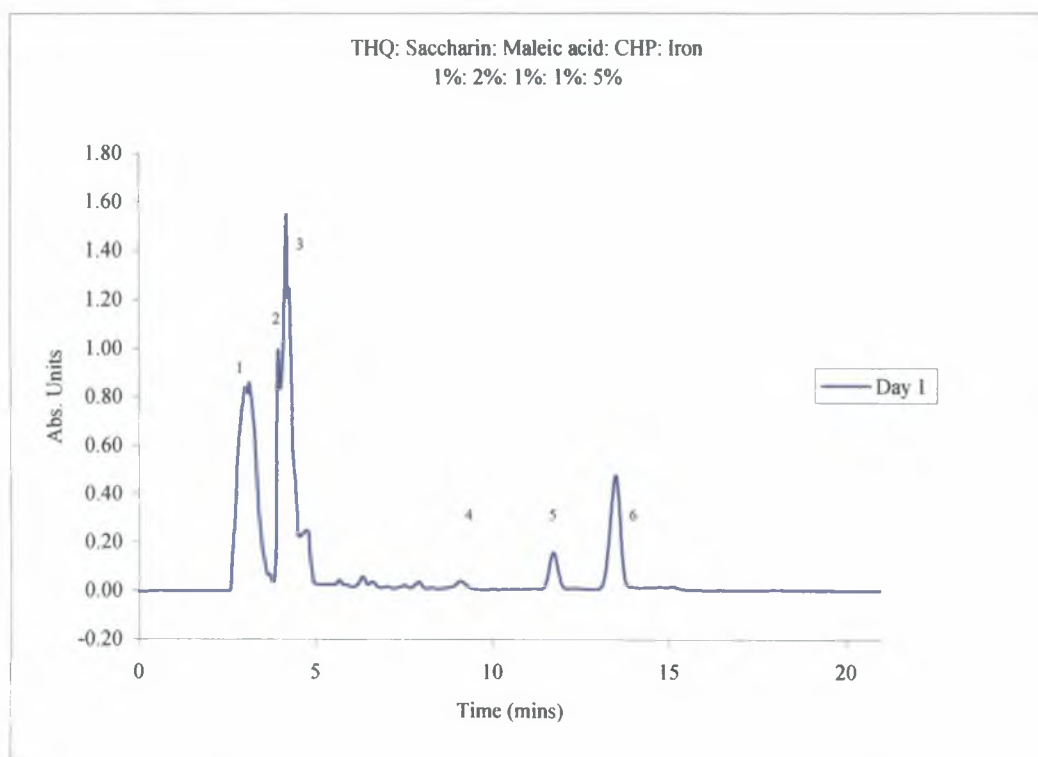


Fig. 2.36. THQ cure solution in presence of iron. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 ml/min; Detection : 254 nm; Temperature: ambient. 1. Maleic acid; 2. Unknown; 3. Saccharin; 4. Unknown; 5, 6. CHP.

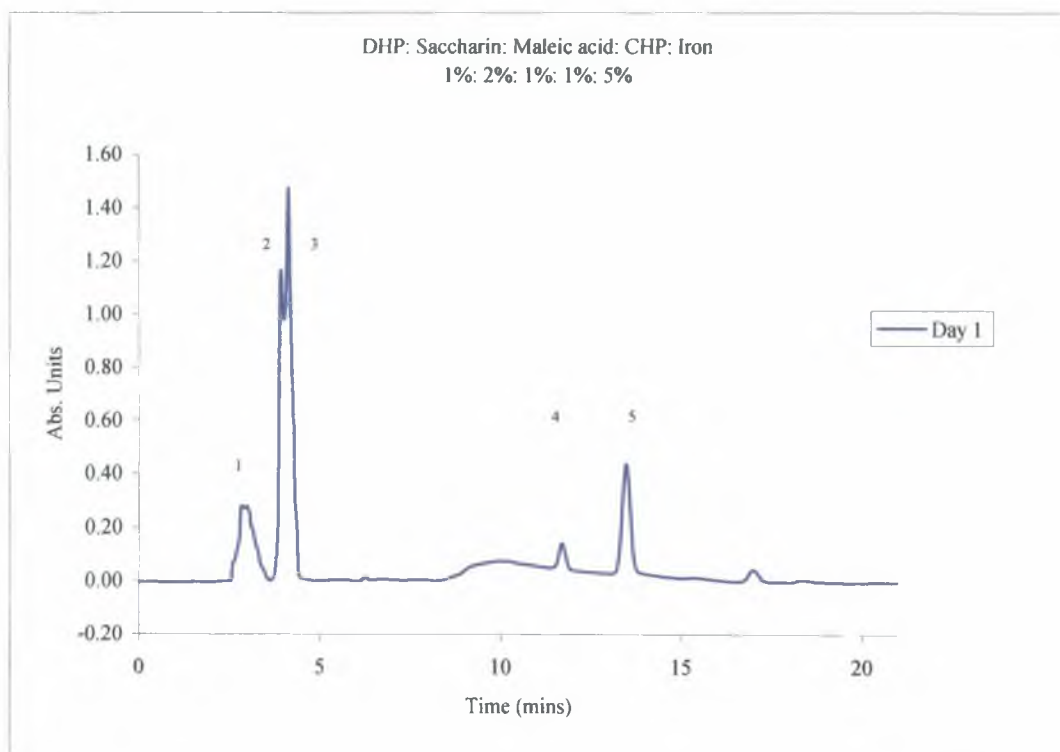


Fig. 2.37. DHP cure solution in presence of iron. Mobile phase: 35% ACN: 65% buffer (Buffer: 1% acetic acid); Column: ACE C18 5 mm, 250 x 4.6 mm; Flow Rate: 0.9 mlmin^{-1} ; Detection : 254 nm; Temperature: ambient. 1. Maleic acid; 2. Saccharin; 3. Unknown; 4, 5. CHP.

It was decided to transfer the analysis of the cure solutions to the LC/MS to attempt to identify the products formed due to the decomposition of the reducing agents. Under the conditions detailed in section 2.4.2.4, a separation of all components was achieved in 11 mins (Fig. 2.38).

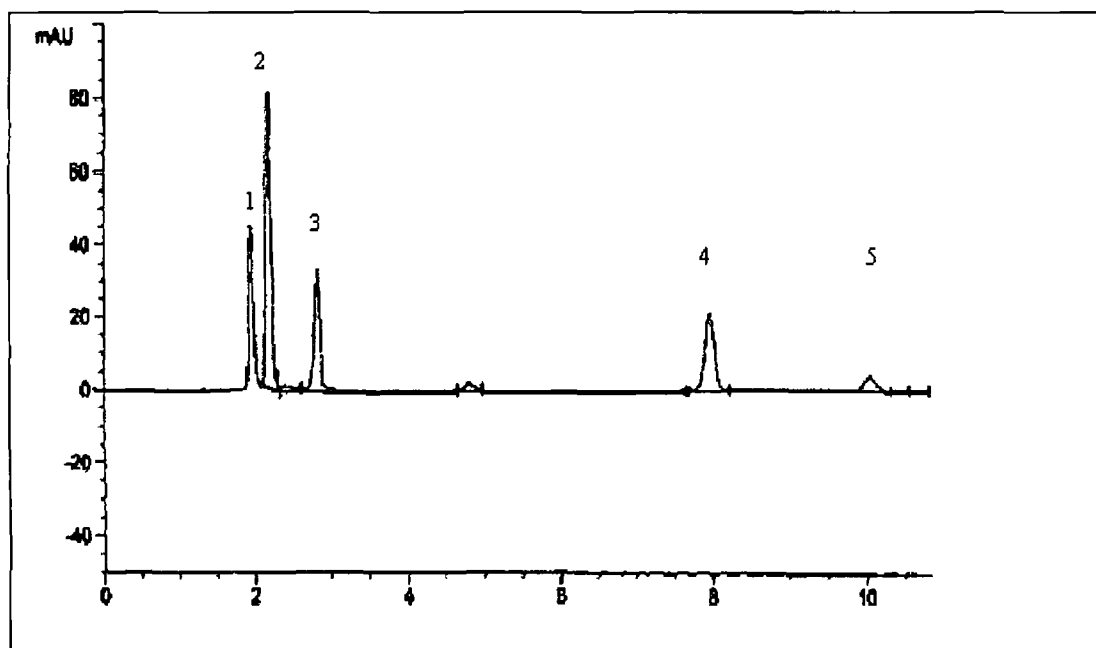
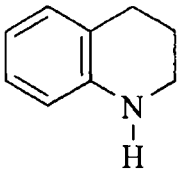
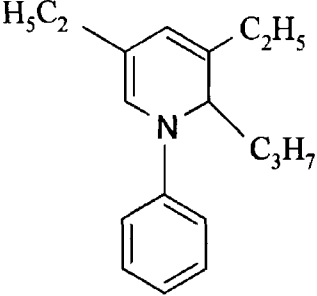
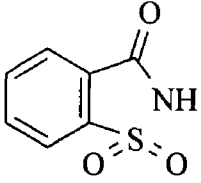
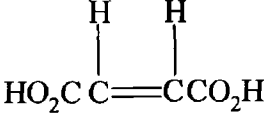
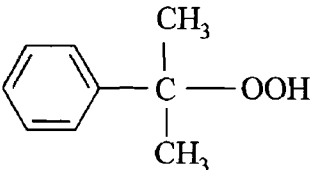


Fig 2 38 Chromatogram of THQ CHP saccharin maleic acid (1 1 1 1) Mobile phase 35% ACN 65% buffer (Buffer 1% acetic acid), Column ACE C18 5 mm, 250 x 2 5 mm, Flow Rate 0 3 ml/min, Detection 254 nm, Temperature ambient 1 Maleic Acid, 2 Saccharin, 3 THQ, 4, 5 CHP

Initially, a solution of each of the components was introduced by direct infusion into the mass analyser in order to “tune” the instrument to the masses of interest (Table 2 3) The molecular ion (M^+) is the ion formed by the removal of one or more electrons from the molecule without fragmentation of the molecular structure Direct infusion was used to determine the molecular ion for each of the components in the anaerobic cure systems In direct infusion the analyte is introduced to the flow of mobile phase in the interface of the LC/MS In the interface the analyte is ionised and sent to the mass analyser where its m/z ratio is detected and recorded by the LC/MS software

Compound	Molecular Weight	Structure	Molecular Weight (g/mol)	Retention Time*
THQ	133		133	2 84mins
DHP	253		254	12 01mins
Saccharin	183		183	2 17mins
Maleic Acid	116		116	1 98mins
CHP	152		152	7 95mins, 10 03mins

* At the following conditions mobile phase 35% ACN 65% buffer (Buffer 1% acetic acid) Column ACE C18 5 mm 250 x 2.5 mm Flow Rate 0.3 ml/min

Table 2.3 Molecular weights and structures of the components in the anaerobic cure system, which were used to “tune” the LC/MS

At this stage it was noticed that maleic acid had a signal at m/z of 99.1 (Fig 2.39). This was because it lost a hydroxyl group upon ionisation. The molecular ion (m/z 117.0) is in a lower abundance than the ion at m/z of 99.1. This is to be expected of an aliphatic compound, where the bonds are typically weaker than in aromatic compounds.

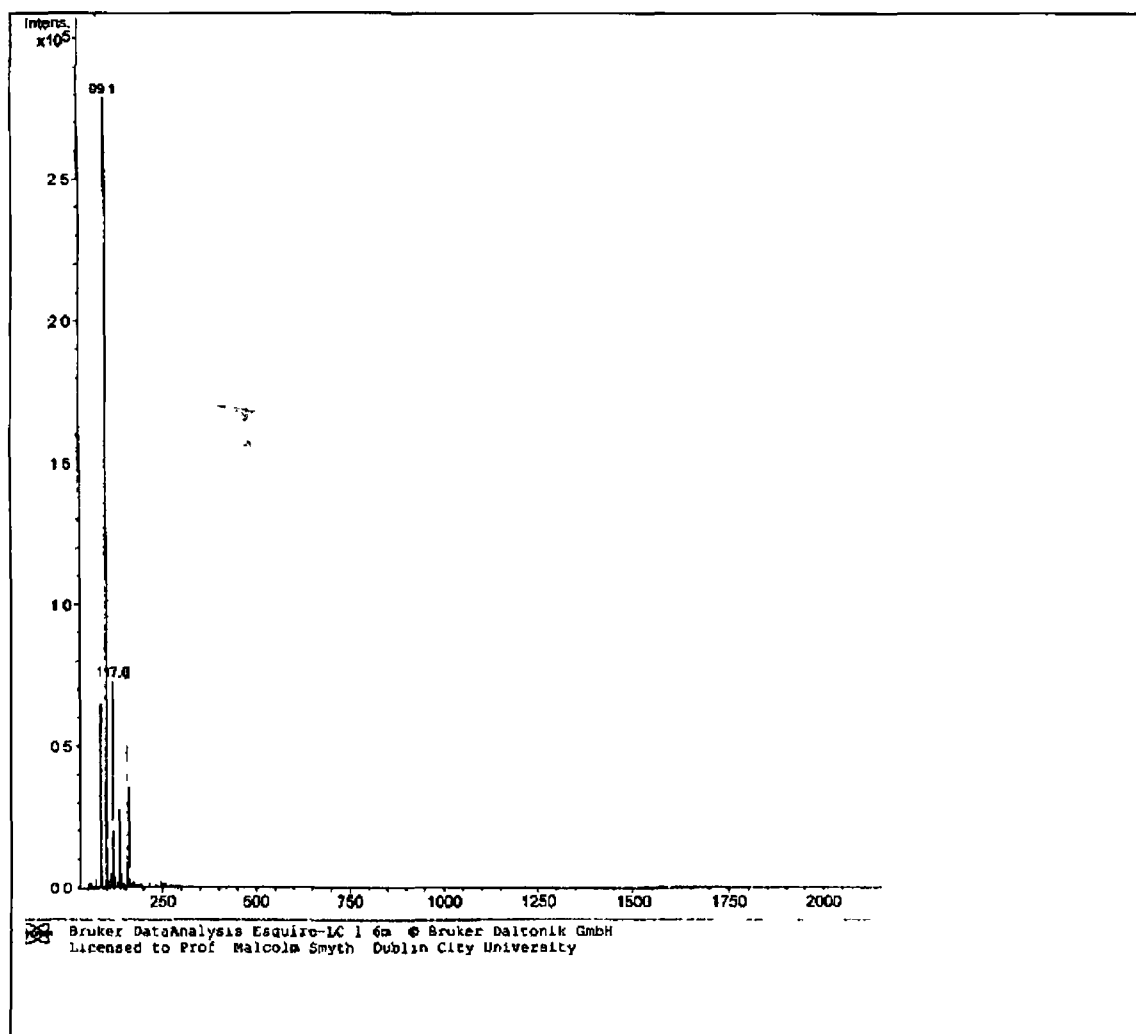


Fig 2.39 Mass spectrum of maleic acid. Obtained by direct infusion of the solution into the mass spectrometer.

Saccharin was not detected in API-ES mode. CHP was detected as a signal at m/z of 135.1 (Fig. 2.40). This corresponds to the compound having lost a hydroxyl group during ionisation. The molecular ion was in very low abundance but an ion with a m/z ratio of 175.1 was observed in the spectrum. The spectrum of the mobile phase was subtracted from the CHP spectrum and the ion of m/z 175.1 persisted, indicating that the molecular ion was not being “blocked” by the mobile phase and that the ion of m/z 175.1 was not in the mobile phase. It is possible that this ion is a contaminant which was either in the mass analyzer or in the CHP solution itself.

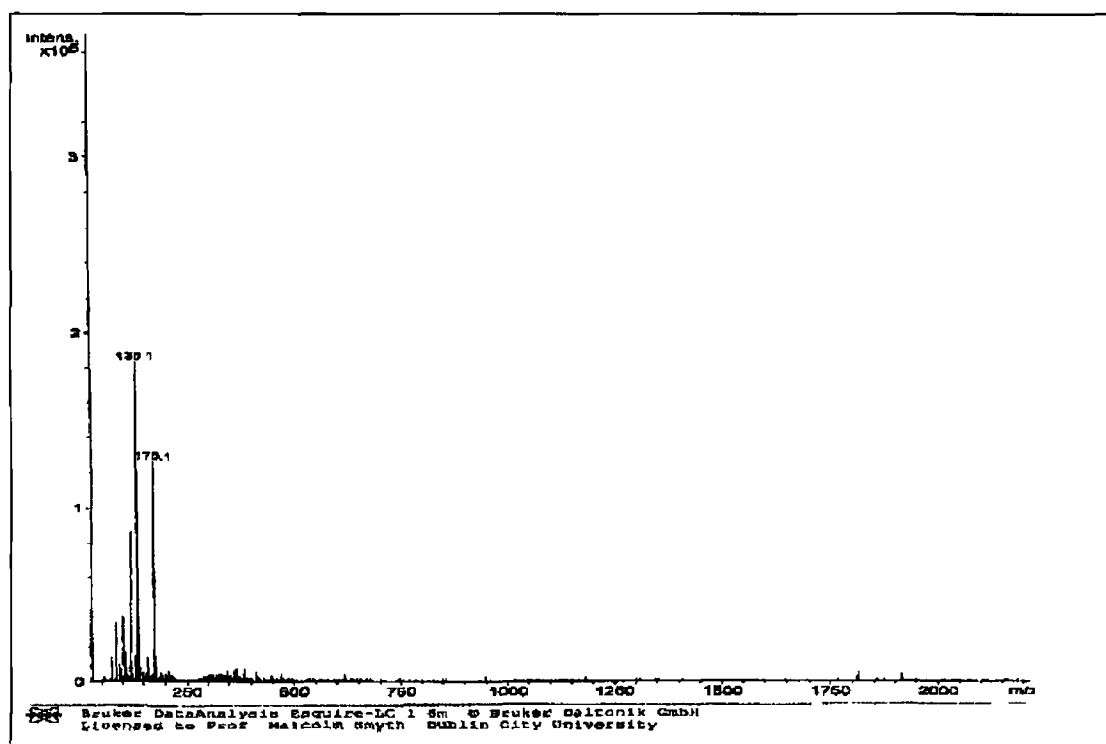


Fig. 2.40 Mass spectrum of CHP. Obtained by direct infusion of solution into the mass spectrometer.

The molecular ion in both THQ and DHP solutions was the most abundant ion present in their mass spectra (Figs 2 41 and 2 42) In fact the intensities of the molecular ion peaks are so strong that they “drown” out the signal of all other components in the cure solutions The injection line had to be washed out with ACN two or three times after each injection of the reducing agents in order to ensure that they did not persist in the analyser and affect the detection of other components

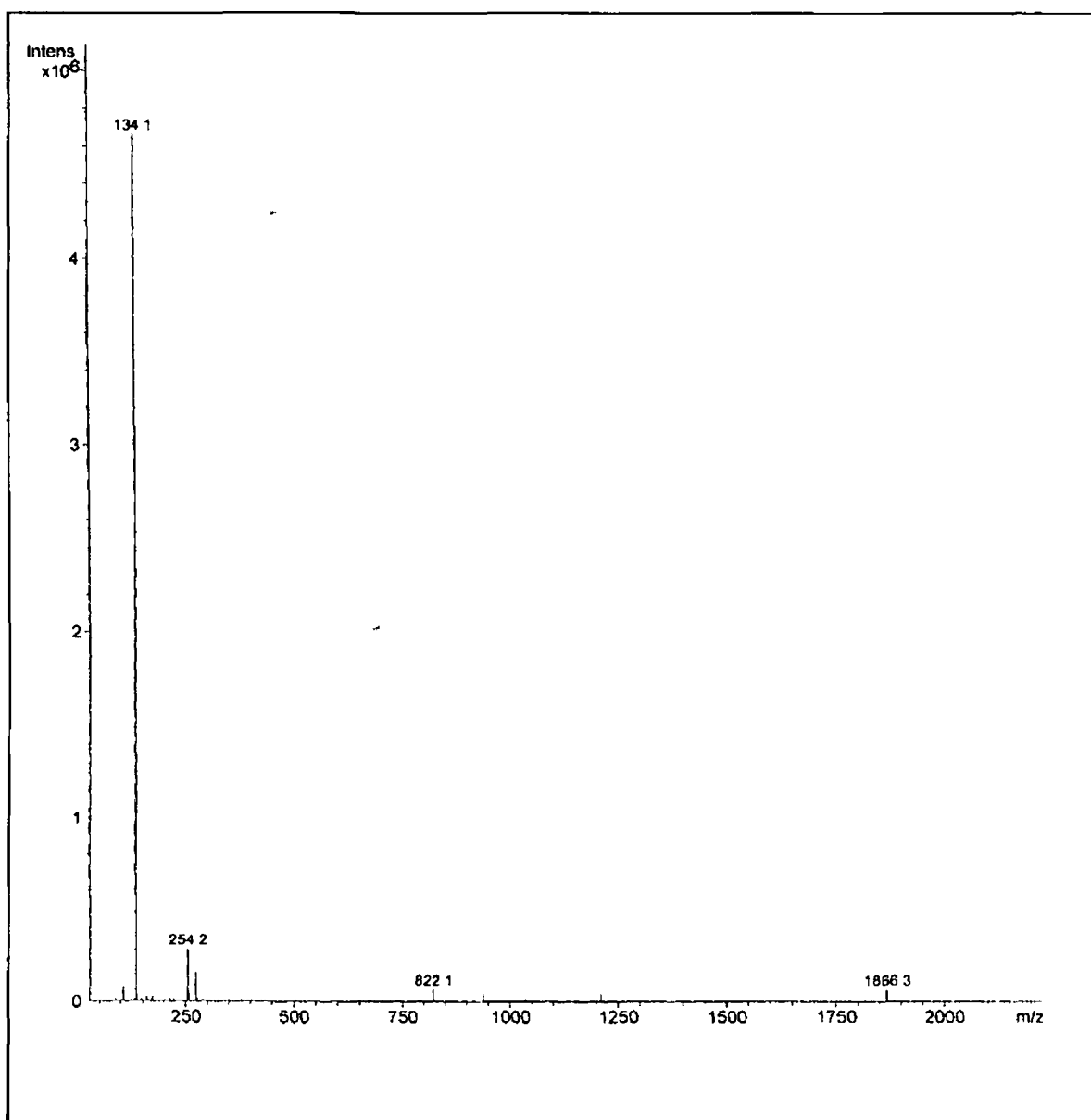


Fig 2 41 Mass spectrum of THQ Obtained by direct infusion of the solution into the mass spectrometer

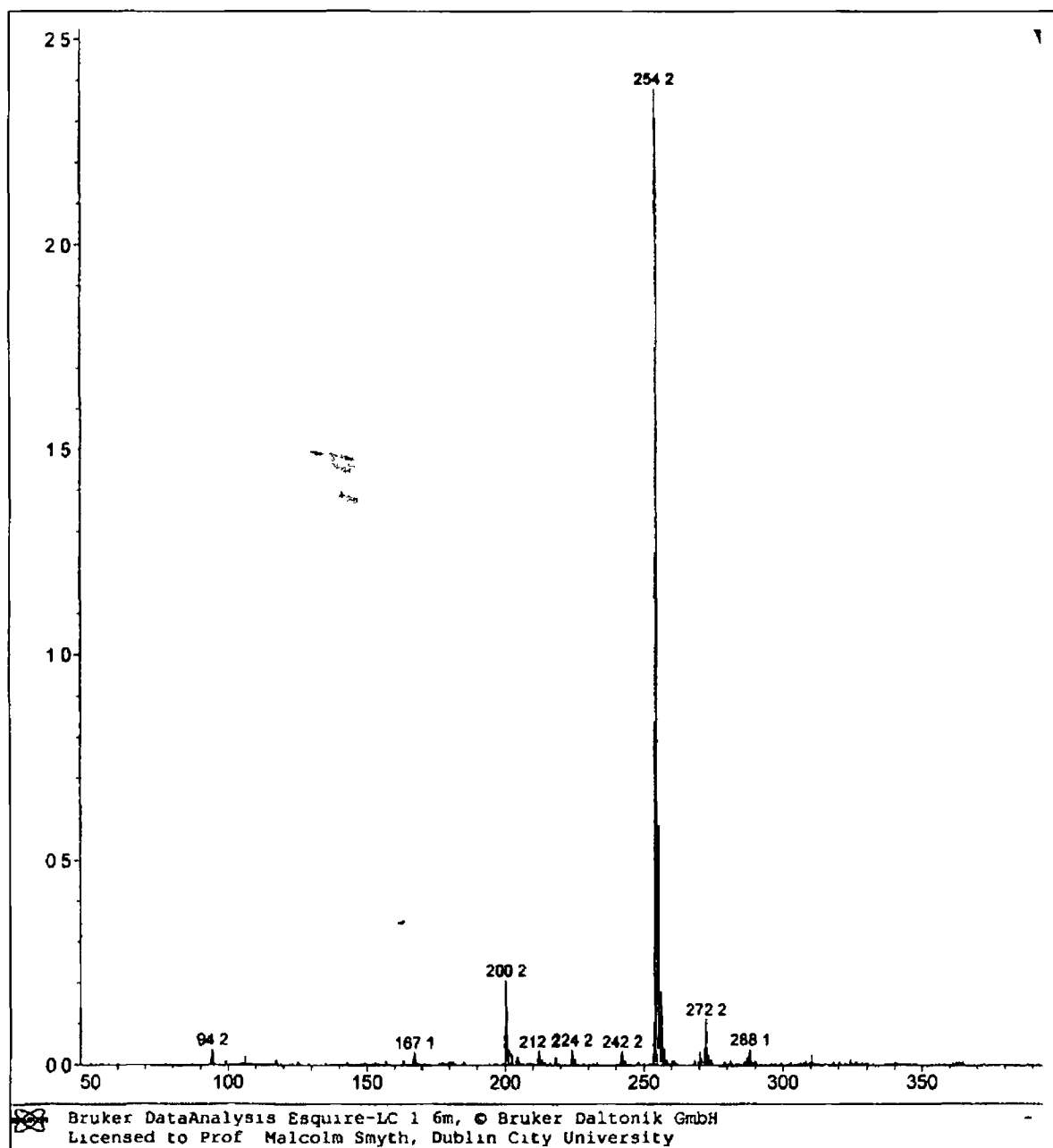


Fig 2.42 Mass spectrum of DHP Obtained by direct infusion of the solution into the mass spectrometer

After “tuning” of the mass spectrometer, the solutions containing the cure components and metals were injected onto the LC/MS. The conditions for analysis are outlined in section 2.4.2.5.

2.5.4.1 LC/MS Analysis of products formed due to the Decomposition of THQ in the presence of Copper

Firstly, a solution of THQ containing copper powder was analysed (Fig. 2.43). From this, the peaks detailed in Table 2.4 were observed.

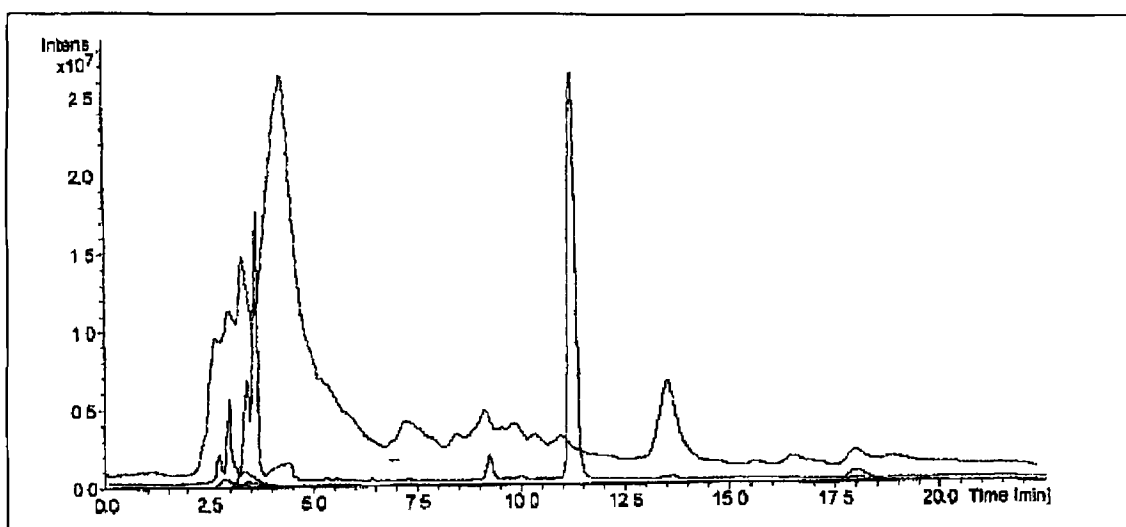


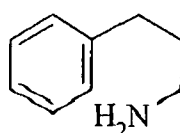
Fig. 2.43 Total ion count mass spectrum of THQ cure system with copper powder. Conditions: Column: ACE C18 5mm, 250 mm x 2.5 mm, Flow rate: 0.3 ml/min. Mobile phase: 35% ACN, 65% acetic acid. API-ES, +ve polarity, Scan range: $m/z = 50 - 2200$. Sheath gas (nitrogen): 70 psi (1 psi = 6894.76 Pa), Trap drive: 24.

m/z Ratio	Retention Time (mins)
122 1	2 63
134 1	4 27
117 1	7 06
119 1	9 92
162 2	11 25
148 1	13 56
261 2	17 22

Table 2 4 Peaks observed in the mass spectrum of THQ cure system containing maleic acid, saccharin, CHP and copper powder

The peak at a retention time of 2 89 minutes, with m/z ratio of 134 2, corresponds to the m/z of the molecular ion of THQ. From observing the chromatogram (Fig 2 43), it seems that some THQ remains in solution. The mass spectrum for this peak shows that this is a molecular ion with no fragmentation present.

One of the reactions undergone by hydroquinolines is the opening of the heterocyclic (i.e. nitrogen-containing) ring, so a probable reaction undergone by THQ is the opening of the nitrogen ring due to reduction of THQ in the presence of the acid (III).



(III)

The peak at retention time of 9.92 minutes may correspond to a cumyl cation (molecular weight 119g/mol) which is the product of the reaction between CHP and acid [38]. This peak is also present in the spectra of the THQ/CHP/maleic acid/copper and THQ/CHP/saccharin/copper cure solutions (Figures 2.45 & 2.46). In the absence of nuclear magnetic resonance (NMR) or IR information it is difficult to postulate what this may be. However, Okamoto [35] suggested a reaction pathway for CHP in acidic conditions which proposed the formation of a cumyl cation and hydrogen peroxide (Fig. 2.44). When one considers the presence of both acids in the solution, it is possible that the peak of m/z 119 could possibly be attributed to a cumyl cation.

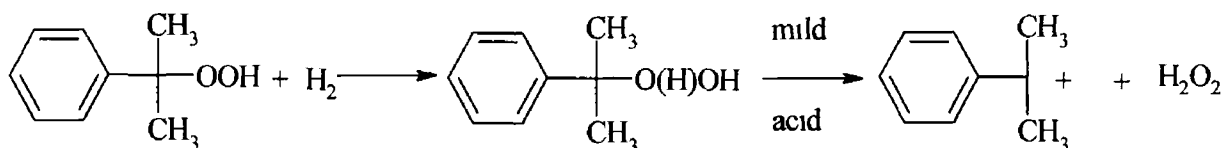
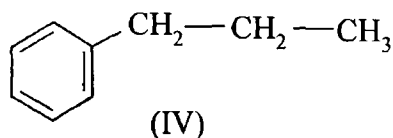
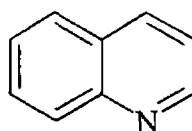


Fig. 2.44 The reaction pathway for CHP in acidic conditions proposed by Okamoto [35]

Another hypothesis is that the peak with a m/z of 119 corresponds to a decomposition product of THQ, formed following the opening of the nitrogen ring by reduction in the presence of an acid.

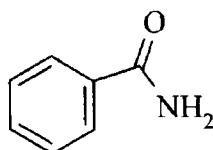


There are a number of other peaks which have been formed due to the decomposition of THQ. The primary function of THQ in the cure system is to act as the reductant to allow for the formation of hydroperoxy radicals, which will go on to initiate polymerisation of the adhesive. Therefore, it is probable that one of the products of the decomposition of THQ is quinoline (molecular weight 129g/mol) (V), which is its oxidation product.



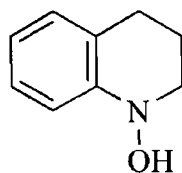
(V)

The most abundant fragment ion of the peak at 2.63 minutes has a m/z of 122.0. From the previous decomposition it was seen that the peak which eluted before maleic acid was probably a decomposition product related to saccharin because it was not present in the chromatograms of the solutions containing maleic acid only. A possible structure is given below (VI). This indicates that the saccharin is decomposed in the solution and probably reacts with the THQ to form a product.



(VI)

The peak at 13.56 minutes is very weak in the chromatogram. However, it has a strong intensity in the cure solution mass spectrum. Looking at the spectrum of this peak, only one ion is present which has a m/z of 148.1. This compound may be a side product of the decomposition of CHP (VII).



(VII)

The peak with m/z of 261.2, at a retention time of 17.22 minutes, is present in THQ solutions containing only saccharin (Fig. 2.45). The peak at 6.78 minutes has a m/z of 273.2 and is only present in the THQ solutions containing maleic acid only (Fig. 2.46). These products must have been formed as a direct result of the reduction of THQ in the presence of CHP and an acid. The formation of quinoline would allow for substitution onto the 2 or 4 position of the nitrogen-containing ring.

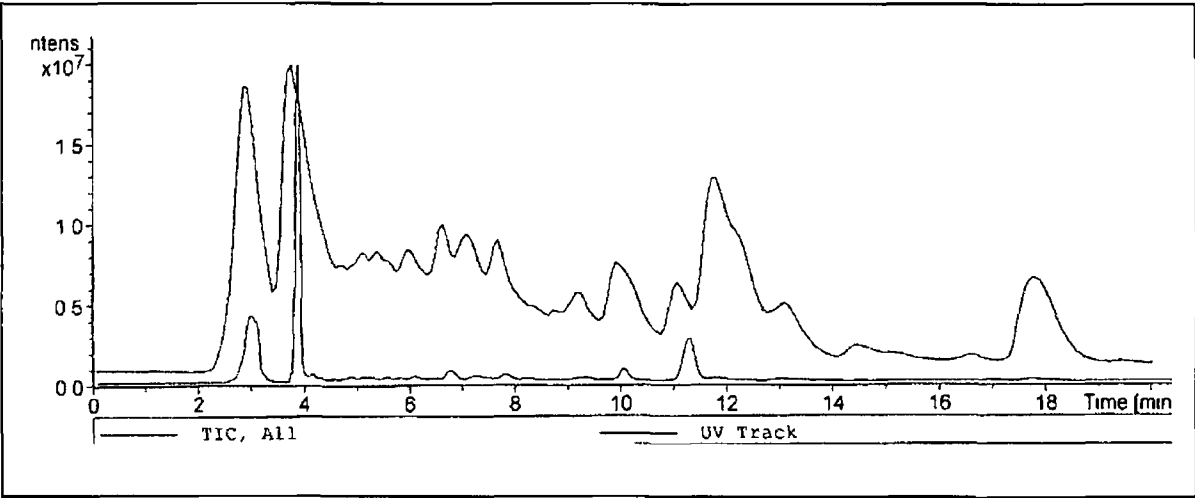


Fig 2 45 Total ion count mass spectrum of THQ/saccharin/CHP cure system with copper powder Conditions Column ACE C18 5mm, 250 mm x 2 5 mm, Flow rate 0 3 ml/min, Mobile phase 35% ACN 65% acetic acid API-ES +ve polarity, Scan range m/z = 50 – 2200 Sheath gas (nitrogen) 70 psi (1 psi = 6894 76 Pa), Trap drive 24

m/z Ratio	Retention Time (mins)
130 1	3 83
119 1	9 97
261 2	17 83
161 2	11 29

Table 2 5 Peaks observed in the mass spectrum of THQ cure system containing saccharin, CHP and copper powder

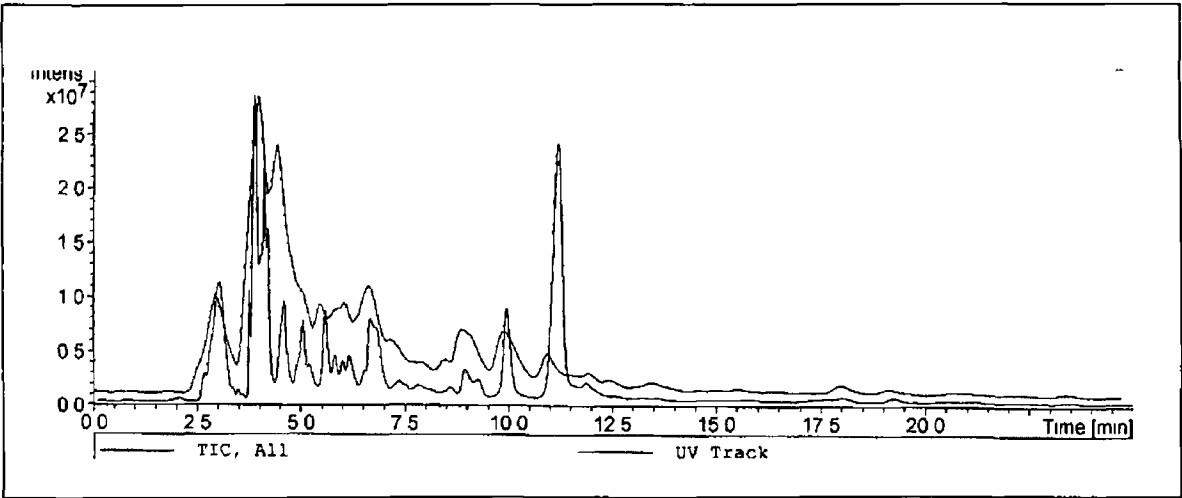


Fig 2 46 Total ion count mass spectrum of THQ/maleic acid/CHP cure system with copper powder Conditions Column ACE C18 5mm, 250 mm x 2 5 mm, Flow rate 0 3 ml/min, Mobile phase 35% ACN 65% acetic acid API-ES +ve polarity, Scan range m/z = 50 – 2200 Sheath gas (nitrogen) 70 psi (1 psi = 6894 76 Pa), Trap drive 24

m/z Ratio	Retention Time (mins)
130 1	3 83
162 1	11 25
134 1	4 28
275 0	9 92
273 2	6 68

Table 2 6 Peaks observed in the mass spectrum of THQ cure system containing saccharin, CHP and copper powder

2 5 4 2 LC/MS Analysis of products formed due to the Decomposition of THQ in the presence of Iron

The THQ/ iron cure system (Fig 2 47) has a number of peaks in common with the THQ/copper cure system spectra (Table 2 7) However, the peaks at 3 52 (m/z 264 2) and 7 67(m/z 246 1) minutes were not observed in the THQ/copper systems therefore it may be possible that the presence of iron in the solution causes the formation of different products than copper

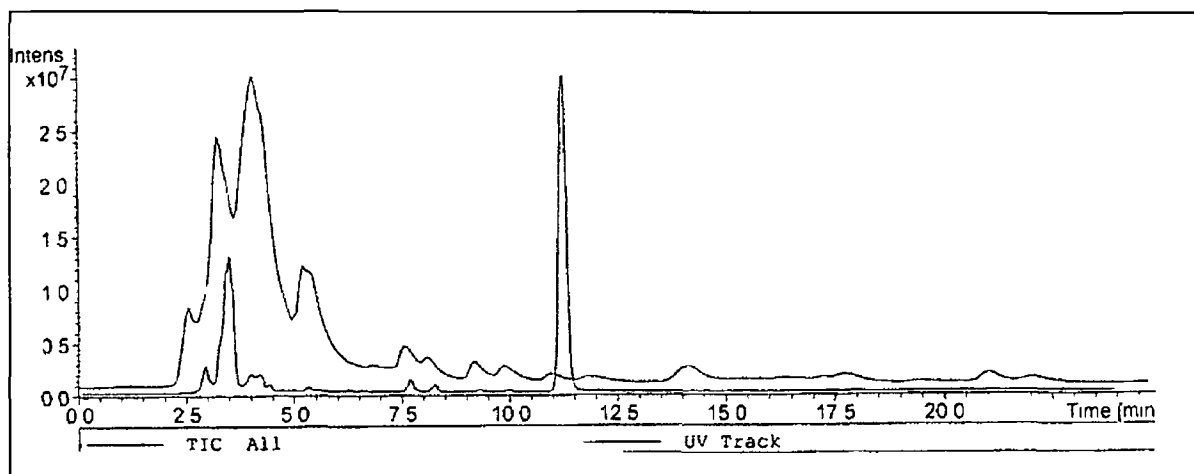


Fig 2 47 The total ion count mass spectrum of THQ/maleic acid/saccharin/CHP cure system with iron filings Conditions Column ACE C18 5mm, 250 mm x 2 5 mm, Flow rate 0 3 ml/min, Mobile phase 35% ACN 65% acetic acid API-ES +ve polarity, Scan range m/z = 50 – 2200 Sheath gas (nitrogen) 70 psi (1 psi = 6894 76 Pa), Trap drive 24

m/z Ratio	Retention Time (mins)
155 9	2 93
264 2	3 52
134 1	4 18
246 1	7 67
162 1	11 25

Table 2 7 Peaks observed in the mass spectrum of THQ cure system containing saccharin, maleic acid, CHP and iron filings

2 5 4 3 LC/MS Analysis of products formed due to the Decomposition of DHP in the presence of Copper

Only one DHP solution was analysed by LC/MS due to limited resources. Therefore, it is difficult to assign any structure to the peaks in the DHP/copper solution in figure 2 48. There were a number of products formed in the DHP solution (Table 2 8). However, there is no peak corresponding to the molecular weight of DHP (254g/mol), indicating that it had been totally decomposed in the solution.

One of the principle reactions undergone by the dihydropyridines is their oxidation to the corresponding pyridines, which are much more stable compounds. Two mechanisms have been proposed for the oxidation of dihydropyridines, by loss of a hydride ion or by the transfer of a single electron and one proton. It is the former mechanism that is the most widely accepted of the two. The 1,2, dihydropyridines can undergo a number of other reactions apart from oxidation, including a Diels-Alder reaction to form six-membered rings.

Pyridines undergo electrophilic substitution only in quite vigorous conditions but will under nucleophilic substitution readily, particularly at the 2- and 4- positions [36] The products of the decomposition of DHP must, therefore, be related to a pyridine or to a 1,2 dihydropyridines Further study of DHP solutions is required in order to elucidate the products of decomposition of this cure system

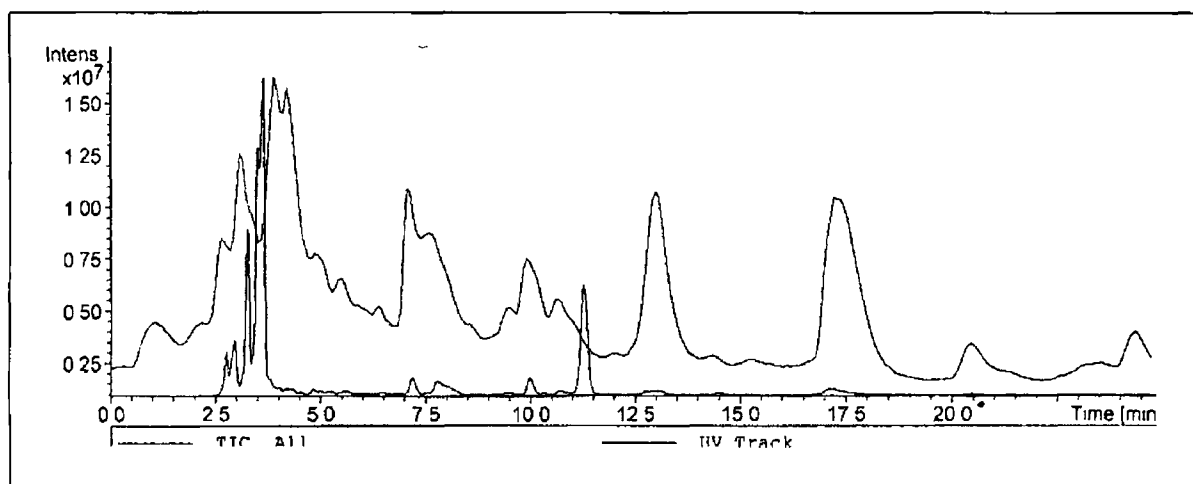


Fig 2 48 Total ion count mass spectrum of DHP/maleic acid/saccharin/CHP cure system with copper powder Conditions Column ACE C18 5mm, 250 mm x 2 5 mm, Flow rate 0 3 ml/min, Mobile phase 35% ACN 65% acetic acid API-ES +ve polarity, Scan range $m/z = 50 - 2200$ Sheath gas (nitrogen) 70 psi (1 psi = 6894 76 Pa), Trap drive 24

m/z Ratio	Retention Time (mins)
295 2	2 63
275 0	3 08
284 3	3 89
267 3	6 39
202 2	11 25
194 7	12 97

Table 2 8 Peaks observed in the mass spectrum of DHP cure system containing saccharin, maleic acid, CHP and iron copper powder

2.6 Discussion

2.6.1 HPLC Study of the Decomposition of THQ

This initial study into the determination of the speed of the decomposition of THQ in cure systems containing copper or iron was carried out using a mobile phase which was compatible with LC/MS analysis (35% ACN 65% of 1% acetic acid buffer). The profiles of THQ decomposition obtained confirm the results of Kincaid [22], indicating that the new mobile phase did not negatively interfere with the decomposition profile of THQ. It was also determined that the total decomposition of THQ occurred in less than three days in the presence of metals (copper or iron) but that the decomposition only went to completion after 14 days in the absence of metals. Therefore, the presence of the metals greatly increased the decomposition of THQ. From this data, it was decided to use a concentration of metal in the cure systems, which would favour the decomposition of the reducing agents and, hence, the formation of decomposition products. At a ratio of 1:4 of reducing agent to cure system components the decomposition proceeded almost immediately. A ratio of 1:1 of reducing agent to cure system components was used to determine the decomposition profiles of THQ. However, in order to monitor the formation of the products over a period of time a balance of the concentration of reducing agent to all other components was required. Consequently, a concentration of 2% w/v metal in all cure component solutions was generally used for monitoring of products generated due to the decomposition of reducing agents.

2.6.2 Monitoring of Products Generated during the Decomposition of Reducing Agents

In the absence of metals the formation of products due to the decomposition of THQ and DHP was slow. This is due to the fact that the absence of metals slows down the decomposition of the reducing agents greatly. One major product was seen to form between saccharin and maleic acid in the THQ cure system. However, in the cure system containing saccharin as the only acid, this peak is not evident, indicating that this peak may be a product of a reaction between THQ and maleic acid in the presence of CHP.

In the DHP cure system, the decomposition of CHP, saccharin and maleic acid is slow in the absence of metals, with few products being formed even after 14 days. In contrast, a number of products were seen to form in the DHP cure system containing copper acetate. These peaks were predominantly formed between the maleic acid and saccharin. The unknown with a peak before the maleic acid peak is probably due to a reaction involving saccharin because it was not present in the chromatograms of cure systems containing maleic acid only.

For both reducing agents, the formation of products was faster in the presence of both acids and slowest in the presence of maleic acid only. This may support the hypothesis that maleic acid has a greater negative effect on the reducing agents than saccharin, thereby, causing a decrease in their reductive potential i.e. their ability to promote homolysis of the peroxide [30].

During the monitoring of the decomposition of the reducing agents in the presence of the copper acetate and iron nitrate, it was noted that in the copper solutions a blue precipitate was formed, while in the iron solutions a brown precipitate formed.

The blue precipitate may be copper saccharinate [33] (IV) George [33] discussed the ability of saccharin to oxidise metals because of its acidic character. The metallic ions generated decomposed the peroxy initiator and produced free radicals via free radical polymerisation mechanism. Possible future research into these compounds would involve extraction of the precipitate into a solvent for analysis by UV or thin layer chromatography. Presence of copper saccharinate in the solution would indicate that the saccharin has the ability to oxidise the metals. The metallic ions could then decompose CHP to produce free radicals to initiate the process of curing the adhesive.

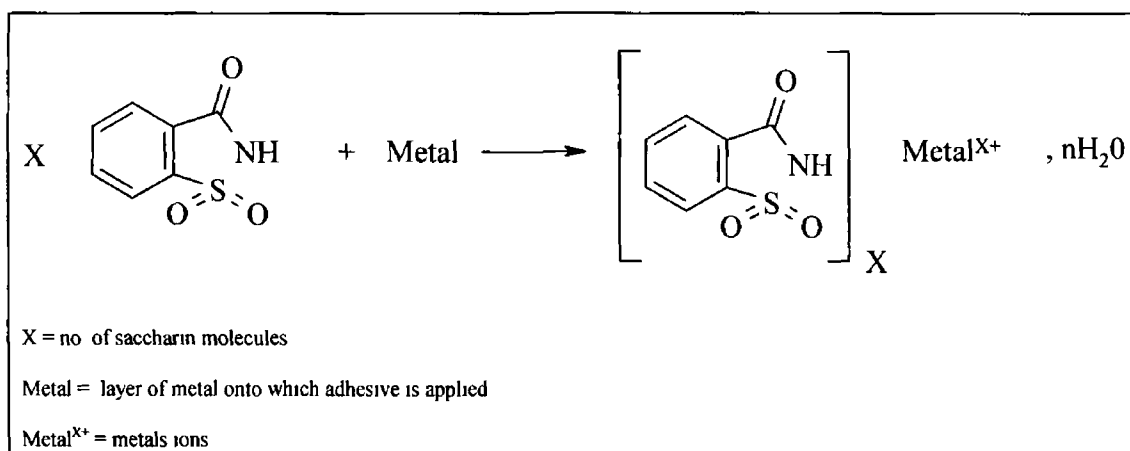


Fig 2 49 The oxidation of a metal by saccharin to produce a metallic ion capable of decomposing a peroxide to produce free radicals. Reproduced from [33]

A brown precipitate was also observed in THQ/Iron solutions containing saccharin, so it can be hypothesised that the iron was also oxidised by saccharin to generate iron ions.

2.6.3 LC/MS Analysis of Tetrahydroquinoline

Literature on tetrahydroquinolines is scarce, so their chemistry is generally predicted from their piperidine analogues. The piperidines are saturated amines and their reactions are in accord with those generally observed for this functional group. They are not very reactive toward nucleophilic species and form salts, nitroso, acyl, alkyl and aryl derivatives at the nitrogen.

The peak which was observed with a m/z value of 130.1 in some of the THQ cure systems corresponds to quinoline (mol. wt. 129g/mol). The purpose of THQ is as a reducing agent in the redox curing mechanism of the anaerobic adhesives. THQ is dehydrogenated to quinoline by many oxidants [36]. Shimizu and his colleagues [37] oxidised THQ and found that the main product formed was quinoline. This hypothesis agrees with the cure mechanism for AHP/copper – based systems which was proposed by Raftery [38] (chapter 1 section 3). In this mechanism, the AHP reduces Cu (II) to Cu (I) in the initiation step for the generation of peroxy free radicals. It is probable that the same reaction takes place with THQ.

In the absence of any NMR or IR information it is difficult to assign structures to a number of peaks in the mass spectra. However, from the mass spectrums of THQ/copper powder/saccharin (Fig. 2.45) and THQ/copper powder/maleic acid (Fig. 2.46), it was seen that the peak of m/z 261.2 is present in THQ solutions containing only saccharin and the peak at m/z 273.2 is present in THQ solutions containing maleic acid only. So it can be postulated that these compounds are the result of a reaction between the THQ and CHP in the presence of the acids.

The decomposition of the reducing agent was considerably slower in the THQ/Iron cure system (Fig 2 47) than the THQ/copper systems This can be seen in the presence of a peak with a m/z value of 134 1 at the retention time of THQ There is also a peak with a m/z ratio of 130 1, indicating that some oxidation of the THQ to form quinoline had occurred

2 6 4 LC/MS Analysis of Dihydropyridine

The chemistry of the dihydropyridines has been investigated in much more detail than the chemistry of the tetrahydroquinolines This is because the 1,6 dihydropyridine ring system exists in the reduced forms of the co-enzymes nicotinamide adenine di- and tri- phosphate (NADH and NADPH) [39] These redox couples are responsible for a number of biological oxidations and reductions

One of the principle reactions undergone by the dihydropyridines is their oxidation to the corresponding pyridines, which are much more stable compounds Two mechanisms have been proposed for the oxidation of dihydropyridines, by loss of a hydride ion or by the transfer of a single electron and one proton It is the former mechanism that is the most widely accepted of the two Studies carried out by Loctite [24] involved the reaction of DHP with iron (III) acetylacetonate in the presence of glacial acetic acid Identification of the products was accomplished using GC-MS The products were iron (II) acetylacetonate and pyridinium acetate, proving that the mechanism for oxidation was via hydrogen ion abstraction

Oxidation may also take place in the presence of air with the formation of a pyridinium salt between the DHP and glacial acetic acid via an autoxidation mechanism [24] It is possible that this may have happened to the DHP because the mobile phase contained 1 % acetic acid solution (Fig 2 50)

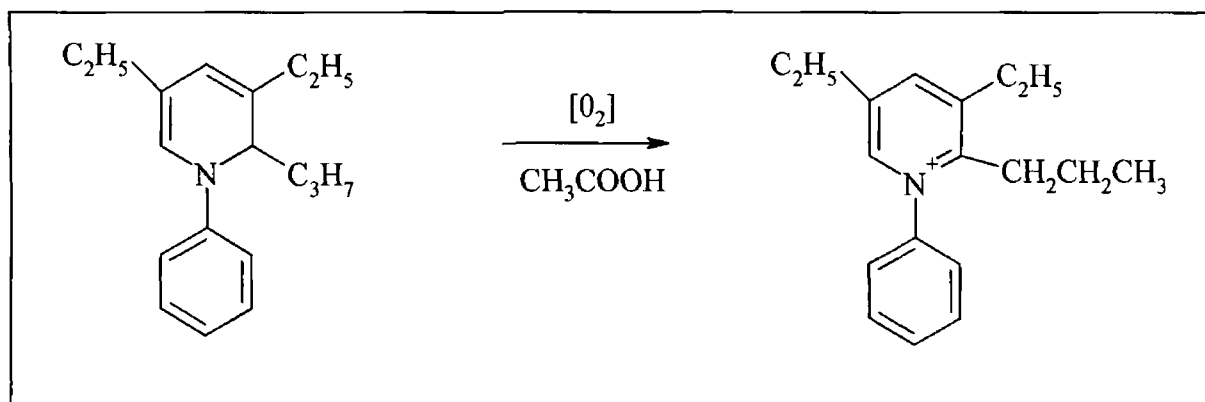
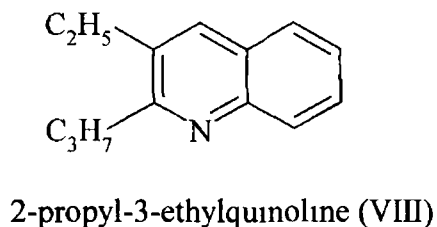


Fig 2 50 Air oxidation of DHP in the presence of acetic acid to form a pyridinium salt [24]

However, none of the peaks in Figure 2 48 have m/z values that correspond to the molecular weight of the salt so it is probable that this reaction did not occur because the concentration of acetic acid in the mobile phase was very low

Loctite [24] have identified an impurity in the starting material as 2-propyl-3-ethylquinoline (VIII) which has a molecular weight of 199 g/mole



However, no peak was detected which corresponded with this impurity. It is possible that it is present only in trace amounts and does not have any effect on the reactions undergone by DHP.

2.7 Conclusion

It has been seen from this study that THQ and DHP do undergo decomposition in both the absence and presence of metals and that a number of products are formed as a result. The separation of the cure components of THQ and DHP system was achieved using a mobile phase which was compatible with LC/MS detection. The LC/MS studies into determination of the products of decomposition show that numerous products are formed. However, further study using the LC/MS is required in order to properly identify the products formed due to the reactions between the reducing agents and saccharin or maleic acid. The identification of these products will allow for the determination of whether they affect the overall cure quality of the anaerobic adhesives. At present there is no spectral library on the LC/MS used for this study. The use of TLC may help to isolate some of these products so that they can be analysed using NMR and IR. The additional structural information can be used in conjunction with the spectra of the unknown products to enable full elucidation of the decomposition products. Another area for future work is using the LC/MS to monitor the decomposition of the products so that any intermediates formed can be determined as well. An extension of this study would be to use a photo-diode array detector to monitor the speed and extent to which DHP decomposes.

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Chapter 3

Analysis of Basic Compounds in Cyanoacrylate

Adhesives with Capillary Electrophoresis

3.1 Introduction

Adhesives based on the alkyl cyanoacrylate monomer differ significantly from other reactive adhesives because they are the only single component, instant bonding adhesives that cure via an anionic polymerisation mechanism at room temperature in the absence of any external energy sources. They have the ability to bind a diverse and dissimilar variety of substrates and, generally, only a single drop of the adhesive is required for most applications.

The general structure of the cyanoacrylic monomer (I) is as shown in Fig 3.1

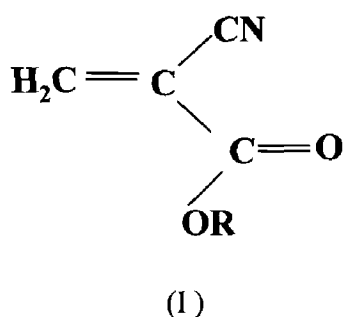


Fig 3.1 The general structure of the alkyl cyanoacrylate monomer, where R is an alkyl group

Nowadays, the most commonly produced cyanoacrylate adhesives are based on the ethyl and methyl monomers. As a rule, increase in the alkyl chain length brings about a decrease in the cure speed and strength of the adhesive.

Cyanoacrylate adhesives can be produced in two synthetic routes, both of which were developed by Ardis [1,2]. The first route involves the pyrolysis of an alkyl-3-acyloxy-2-cyanopropionate to form an alkyl-2-cyanoacrylate and a carboxylic acid [1]. The second, and more commercially popular route, involves a Knoevenagel condensation reaction [2]. This is essentially a three-step procedure (Fig. 3.2).

1 Condensation

The condensation reaction occurs between an alkyl cyanoacetate and formaldehyde in the presence of a base catalyst to yield a poly (alkyl-2-cyanoacrylate) and water. The prepolymer has a molecular weight of 1000-2000 Da. Piperidine is generally employed as the base catalyst. However, sodium hydroxide can also be used.

The reaction is carried out in a non-aqueous solvent (e.g. toluene), which allows for the dissipation of evolved heat. In addition, the solvent allows for the continual and efficient removal of water from the reaction mixture.

2 Depolymerisation

The crude alkyl-cyanoacrylate polymer is heated to 150°C in the vessel. This cracking process takes place under high vacuum in the presence of acid. The acid prevents any repolymerisation of the newly formed cyanoacrylate monomer. Normally, phosphoric acid, methanesulphonic acid or sulphuric acid is used to stabilise the monomer. The acid also neutralises any base catalyst, which may remain after condensation. Free radical inhibitors, e.g. hydroquinone, are also added at this stage to prevent repolymerisation caused by the generation of free radicals at the elevated temperatures. Typically, a yield in excess of 80% monomer can be achieved.

3 Purification

The crude monomer is purified in a fractionation process. A glutarate is produced as a side product of the reaction. It is then removed and recycled. The monomer is distilled onto radical and anionic polymerisation inhibitors and chilled to prevent any spontaneous polymerisation.

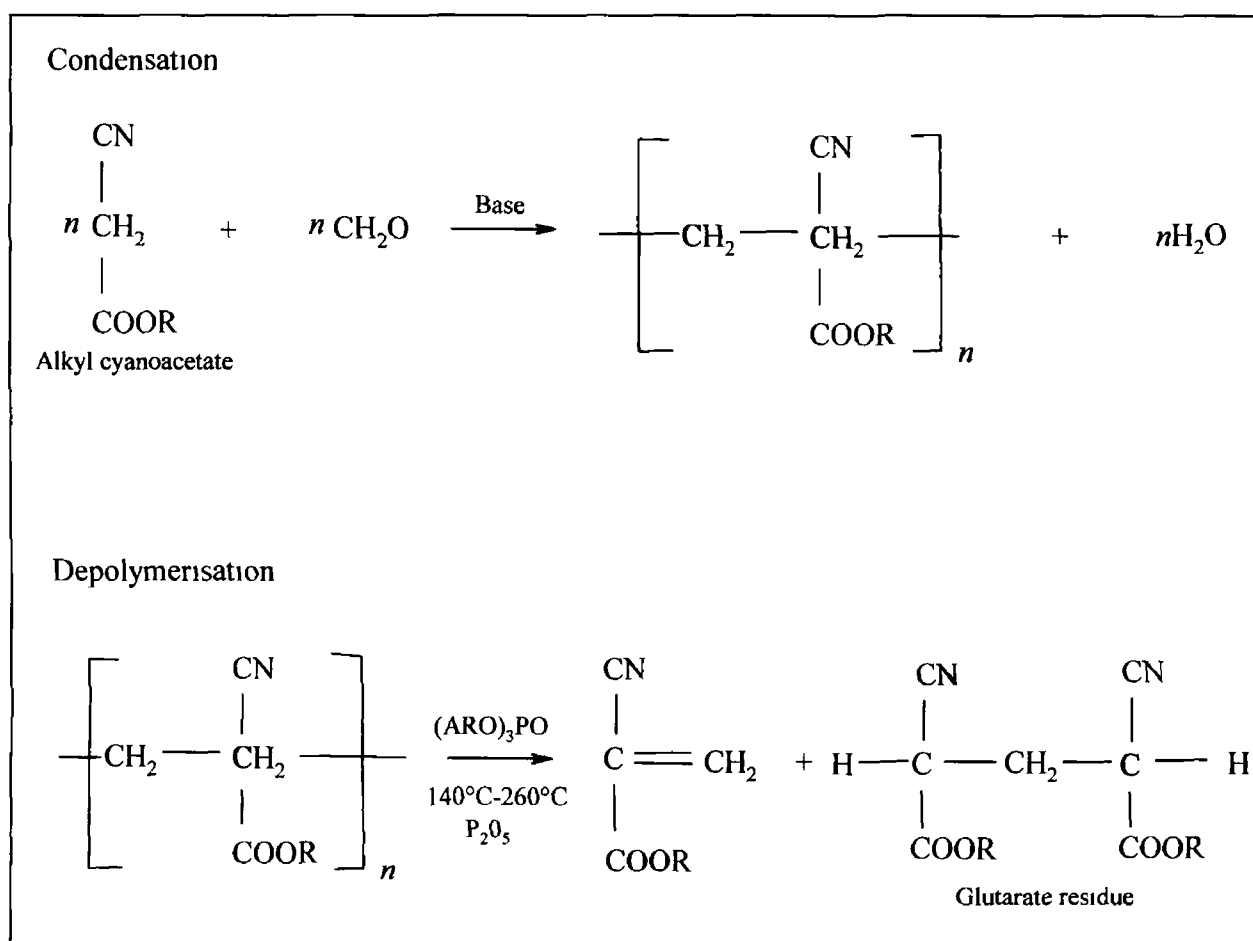


Fig 3.2 The Knoevenagel condensation alkyl cyanoacrylate monomer synthesis [3]

3.2 By- Products of Cyanoacrylate Monomer Synthesis

The synthesis of cyanoacrylate is theoretically simple. However, in reality it is difficult to control on an industrial scale. It has been observed that products are being formed during the cyanoacrylate synthesis, which may diminish the overall yield of monomer and, moreover, have a detrimental effect on the overall performance of the adhesive formulation.

As mentioned previously, a glutaric residue remains in the reaction vessel after depolymerisation. Reacting it with cyanoacetate to produce prepolymer recycles this residue. If the ratio of formaldehyde to cyanoacetate is not correct, the formaldehyde may also react with the glutaric, resulting in the eventual formation of a vinyl nitrile (Fig 3.3)

[4]

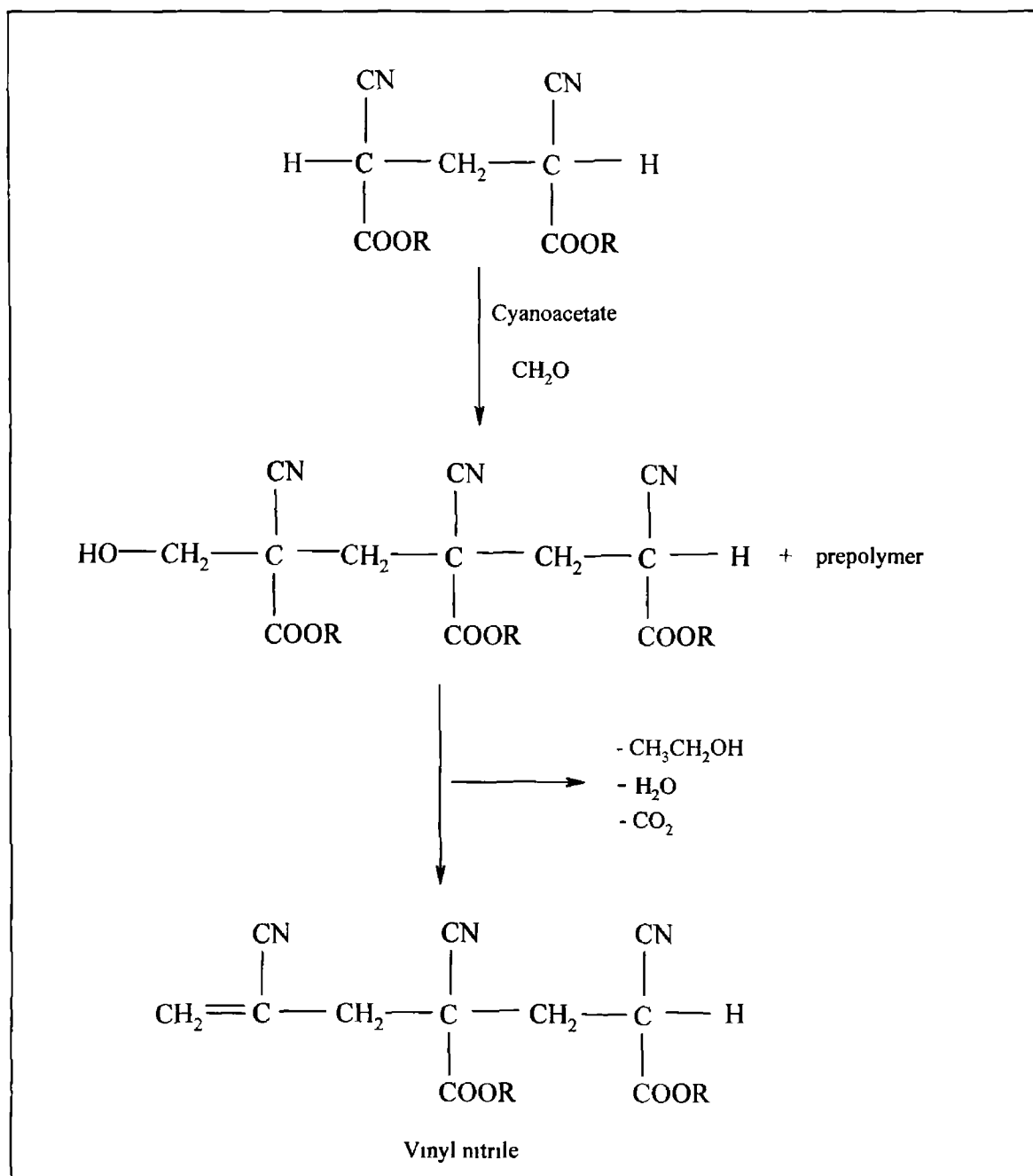
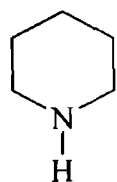


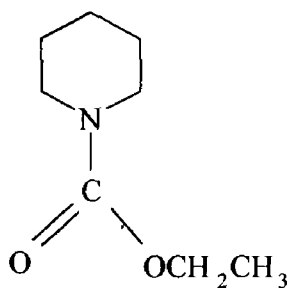
Fig 3 3 Recycling the glutarate to produce prepolymer If formaldehyde is present in excess it will react with the glutarate to produce a vinyl nitrile [4]

The presence of the vinyl nitrile is determined using Fourier transform infra-red spectroscopy (FTIR) [4] This vinyl nitrile can be hydrolysed to produce ammonia in the form of a salt of methansulphonic acid

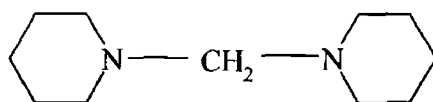
The base catalyst most commonly employed in the condensation stage of the synthesis of cyanoacrylate monomer is piperidine (II) It is thought that the piperidine may also participate in a side reaction with cyanoacetate to produce ethyl piperidine carboxylate (III) Yet another basic compound formed is dipiperidinomethane (IV) This compound is formed in a reaction between piperidine and formaldehyde



Piperidine (II)



Ethyl piperidine carboxylate (III)



Dipiperidinomethane (IV)

Fig 3 4 Basic compounds in cyanoacrylate adhesives

After condensation, the presence of a base in any other stage of the synthesis will cause repolymerisation of the monomer. Consequently, it is imperative that the amount of piperidine and the other basic impurities in the reaction vessel is monitored during all stages of production so that the correct amount of acid is added to neutralise them.

3.3 Purpose and Aims of Research

The aim of this project was to develop a method by which basic compounds found in the cyanoacrylate adhesives could be separated using CE. The basic compounds to be separated were piperidine, ethylpiperidine carboxylate (EPC), and dipiperidinomethane (DPM).

Piperidine is used as the catalyst for the production of the cyanoacrylate prepolymer. EPC and DPM are known by-products of the production of cyanoacrylate monomer. After formation of the pure cyanoacrylate monomer, any base present in the reaction vessel will cause the repolymerisation of the monomer. The reason for developing a CE separation of piperidine, EPC and DPM is to allow for a fast, quantitative determination of any basic compounds left in the reaction vessel. Presently, an excess amount of acid is added to the reaction vessel to neutralise the basic components. It is hoped that a quantitative CE method would allow for the actual amount of base present in the vessel to be determined. Consequently, the correct quantity of acid required for neutralisation of the base would be added. This would increase the amount of pure monomer produced and reduce the amount of acid wasted, thereby making the process more cost efficient.

CE, when coupled with UV detection, is a fast technique which is particularly attractive for the analysis of basic compounds, especially when one considers that their analysis by HPLC is difficult due to interactions with the polar HPLC column. The review of the literature has revealed that the application of CE to adhesives is sparse. The separation of the acidic components [5] has already been successful but a CE separation of the basic adhesive components would be a novel analysis. It would also increase the number of applications of CE for the analysis of adhesives.

3.4 Capillary Electrophoresis

Capillary electrophoresis (CE) is an automated technique that separates species by applying voltage across buffer-filled capillaries. Generally, the technique is used for the separation of ions, which move at different speeds when the voltage is applied depending on their size and charge [6]. The solutes are seen as peaks as they pass through the detector and the area of each peak is proportional to their concentration. In comparison with conventional liquid chromatographic (LC) techniques, CE offers the potential of more efficient separations in a shorter time, smaller sample and buffer volumes and a less complicated sampling system (Fig. 3.5).

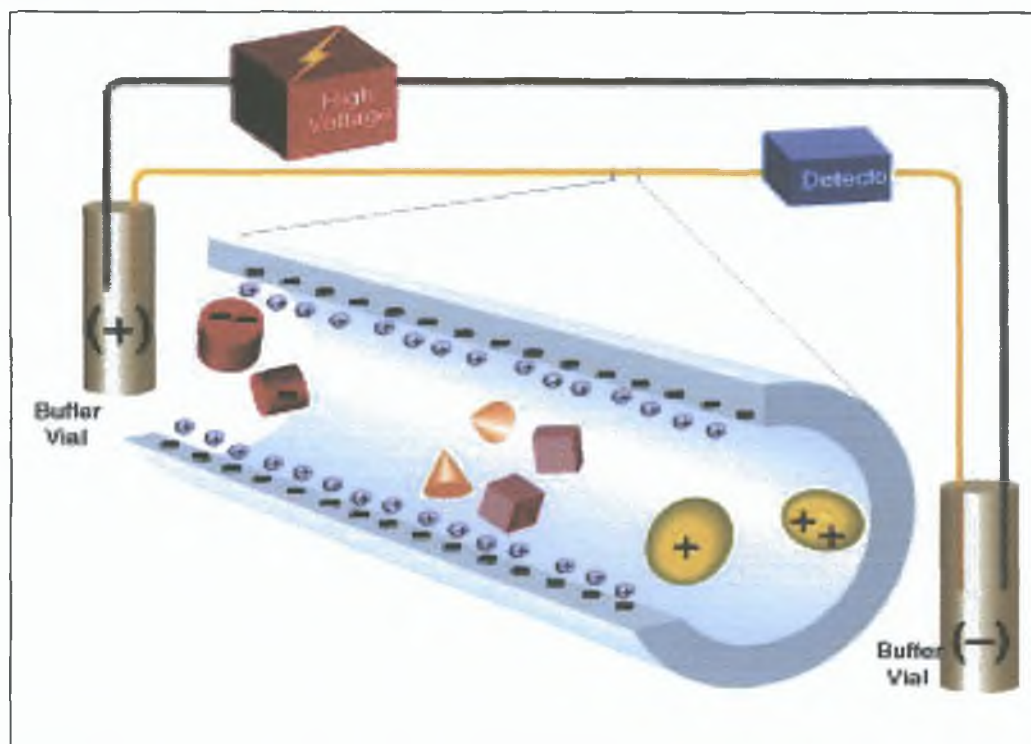


Fig. 3.5 Schematic showing the main components of a capillary electrophoresis instrument [7]. The magnified view depicts the capillary interior with molecules of different size and charge separating.

3.4.1 Theory

One of the fundamental processes that drive CE is the electroosmotic flow (EOF). Simply put, the EOF is the movement of the separation buffer as a result of the existence of a zeta potential (surface charge) at the interface between the buffer and the capillary wall.

Generally, fused silica capillaries are used in CE separations. These silica capillaries have ionizable silanol groups, which, when in contact with a buffer above pH 2, become deprotonated to form a negatively charged wall.

Cations in the buffer are attracted to the negatively charged wall forming an electrical double layer. The inner layer of cations are tightly bound to the wall, however, the outer layer of cations are more diffuse (Fig. 3.6).

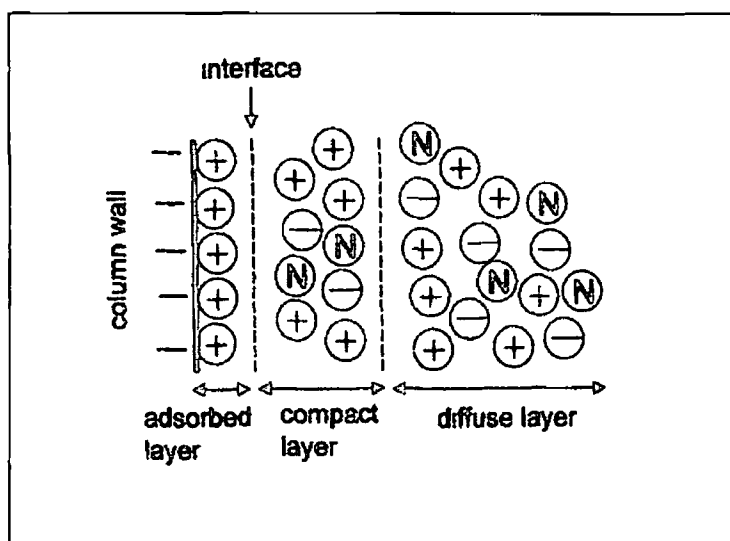


Fig. 3.6 Formation of the electrical double layer at the surface of a fused capillary. Reproduced from [8].

Upon application of an electrical field, the cations in the outer layer flow toward the cathode. These cations are solvated by water so the fluid in the separation buffer is mobilised as well and dragged along by the migrating charge (Fig 3.7). Separation is based on the size and charge of ions. Therefore, the smallest cations elute first and the largest anions last.

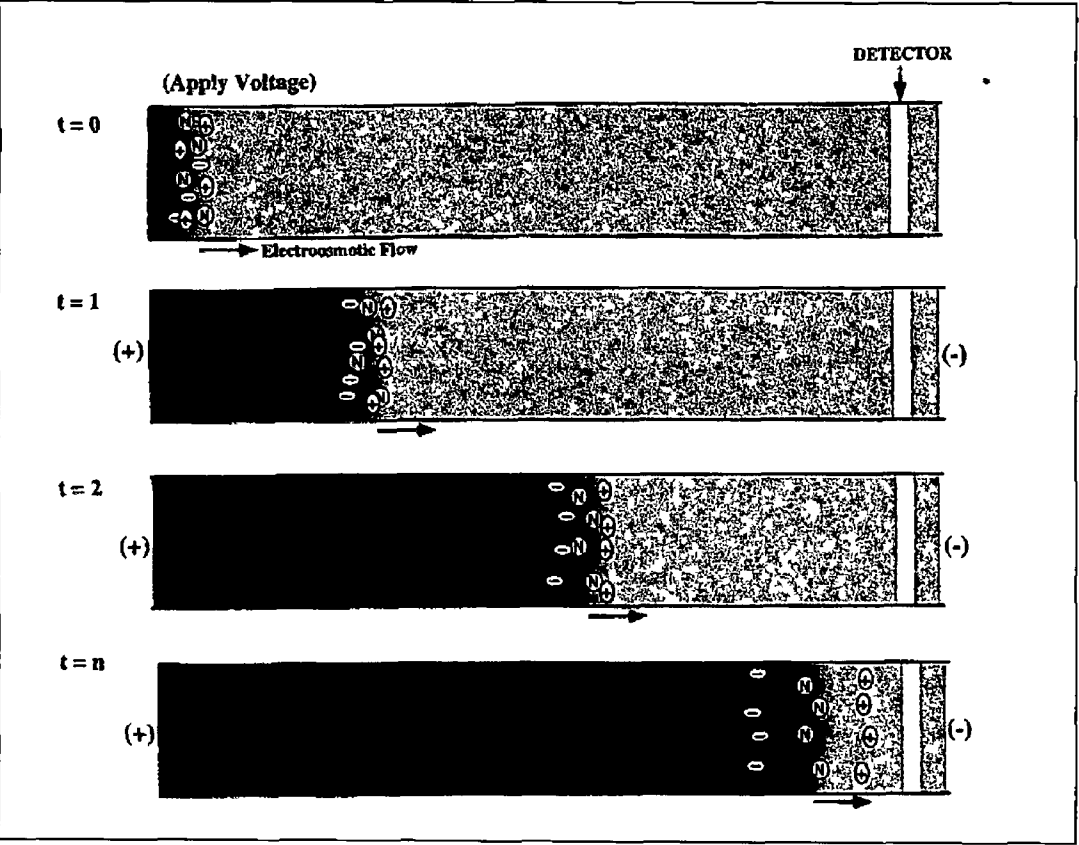


Fig 3.7 The mobility of charged and uncharged analytes in an electrical field
Reproduced from [9]

Mathematically, the velocity of the EOF is defined as

$$v_{eo} = \left(\frac{\epsilon \zeta}{4\pi\eta} \right) E \quad \text{Equation 1}$$

where ϵ refers to the dielectric constant, η is the viscosity of buffer, ζ refers to the zeta potential and E is the applied electric field. Manipulation of the EOF velocity by changing any of these factors is critical in achieving optimum separation in capillary electrophoresis [9].

Resolution between two solutes in CE is defined as

$$R_s = \frac{1}{4} \frac{\Delta\mu_{ep}\sqrt{N}}{\Delta\mu_{ep} + \mu_o} \quad \text{Equation 2}$$

where $\Delta\mu_{ep}$ is the difference in average mobility of the two solutes, and N is the number of theoretical plates [9]. From this equation it can be seen that an increase in voltage does not bring about an increase in resolution.

Instead, the selectivity of the separation must be controlled by choosing the correct mode of CE and by adjusting the pH, concentration and additives of the separation buffer. Resolution may also be improved by decreasing the EOF, thus increasing the effective length of the capillary. Resolution is then increased at the expense of run time.

The number of theoretical plates (N) in a CE separation is given by

$$N = \frac{\mu_{ep}V}{2D} \quad \text{Equation 3}$$

where V is the voltage applied across the capillary, μ_{ep} is the mobility of the solute and D is the diffusion coefficient of the solute. Band broadening is caused by longitudinal diffusion of the analyte in the capillary.

From this equation it can be seen that the efficiency is influenced by the applied voltage and not by the capillary length. An efficient separation will be obtained if a high voltage and a short column are used. This guarantees low band broadening, provided that there is efficient heat dissipation in the capillary.

Efficiency is expressed as a dimensionless quantity called the effective plate number. This reflects the number of times an analyte partitions between two phases during its passage through the column or capillary. The higher the effective plate number of a separation, the greater the efficiency and, hence, the better the quality of the separation. A capillary electrophoretic separation is much more efficient than a HPLC separation. This increase in efficiency in CE is related to the zeta potential of the capillary wall, which produces a flat EOF velocity distribution across the diameter of the capillary. In HPLC, where the separation is pressure-driven, the frictional forces of the mobile phase interacting with the walls of the column result in a radial velocity gradient throughout the column. This results in a parabolic flow profile. This results in bandbroadening and an overall decrease in separation efficiency (Fig 3.8).

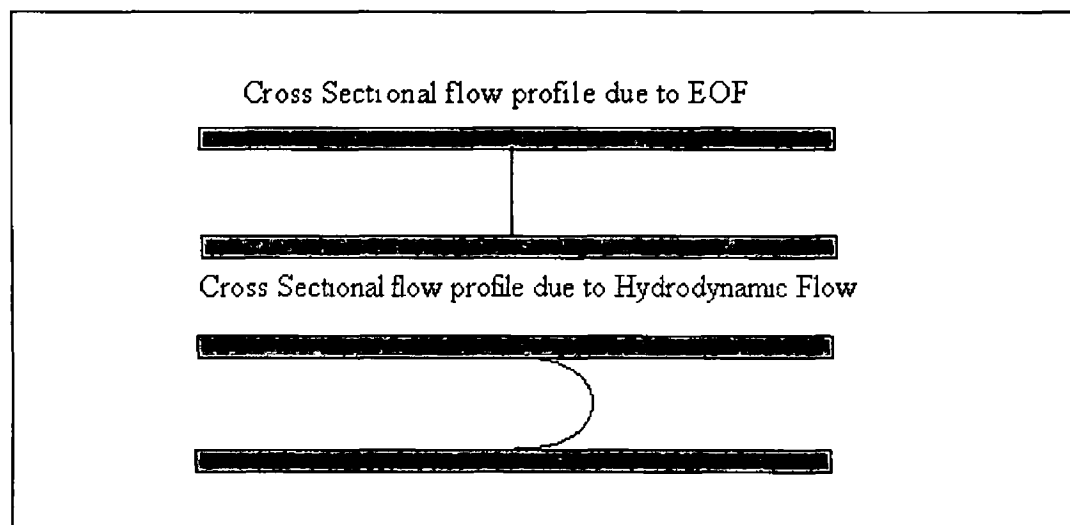


Fig 3 8 Diagram of the flow profile produced by the EOF in capillary electrophoresis and the flow profile due to laminar flow in HPLC

3 4 2 Analysis of Basic Compounds with Capillary Electrophoresis

3 4 2 1 Introduction

CE is particularly useful for the analysis of basic compounds. Traditionally, separations of basic compounds with HPLC have resulted in poor chromatograms. The basic compounds interact strongly with the polar HPLC column to produce peak tailing and low separation efficiencies [10].

In CE, basic compounds can be separated in their cationic form using low pH electrolytes. The main problem encountered when analysing basic compounds with CE is the cationic interactions, which occur between the analytes and the capillary wall. This problem can be overcome in two ways, first, one can alter the surface of the capillary to reduce cationic interaction with the wall and, second, one can lower the pH so that the capillary surface is neutral and the basic analytes are fully ionised [11].

Much of the work into the analysis of basic compounds with CE has been carried out on basic drugs (Table 3 1), however, many researchers have concentrated on heterocyclic, aromatic and biogenic amines (Table 3 2).

Analytes	Mode	Electrolyte	Detection Mode	Ref No
Narcotics, Diuretics	NACE	20 mM ammonium acetate in Methanol/Acetonitrile (50 50)	UV @ 214 nm	38
Amphetamines (1), tropane alkaloids (2), local anaesthetics (3)	NACE	(1) 25 mM ammonium formate, 1 M formic acid, in acetonitrile (2) 25 mM ammonium acetate, 1 M acetic acid in Methanol/Acetonitrile (25 75) (3) 25 mM ammonium acetate, 1 M acetic acid (40 60)	UV @ 200 nm	39
Ibuprofen and its major metabolites	NACE	50 mM ammonium acetate, 100 mM sodium acetate in MeOH/ACN (50 50) & 10% v/v glycerol	UV @ 254 nm	36
Procainamide, quinidine, chloramphenicol	CZE	30mM ethanesulphonic acid, pH 2.4, 20% ACN & 30mM Brj – S	UV @ 214 nm	26
Antihistamine and Morphine analogues	NACE	20mM ammonium acetate in MeOH/ACN/acetic acid (49 50 1)	UV @ 214 nm	40
Procainamide (PA) and acetylprocainamide (APA)	CZE	0.05 mM Na ₂ HPO ₄ @ pH 1.7–9.7	UV@ 200 nm for PA & 265 nm for APA	25
Anti-malarial drugs	CZE (1) MEKC (2)	(1) Na ₂ HPO ₄ @ pH 2.0 (2) 25 mM Na ₂ HPO ₄ pH 10 with 10% acetonitrile & 10 mM sodium dodecyl sulphate	UV @ 254 nm	30
Amitriptyline & Imipramine	MEKC	0.05 M 6-amino caproic acid @ pH 4.0 with 25 mM MAPS & 25 mM Tween 20	UV@ 214 nm	27
Adrenaline and precursors	MEKC	10 mM Na ₂ HPO ₄ @ pH 7.8 with 25 mM sodium dodecyl sulphate & 12.5 mM Tween 20	UV@ 214 nm	29

Key NACE Non Aqueous Capillary electrophoresis

CZE Capillary Zone Electrophoresis

MEKC Micellar Electrokinetic Chromatography

Table 3.1 Selected CE studies on the analysis of basic drugs

Analytes	Mode	Electrolyte	Detection Mode	Ref No
Heterocyclic Amines	CZE	50 mM Na ₂ HPO ₄ @ pH 2.1, with 20 mM citric acid & 26% methanol	UV @ 200 nm – 400 nm	12
Heterocyclic Amines	CZE	10 mM solution KCL/HCL@ pH 2.2	UV@ 214 nm	22
Heterocyclic Amines	MEKC	100 mM boric acid, 50 mM sodium dodecyl sulphate & 10% acetonitrile, pH 8.9	Fluorescence (derivatised with 6-aminoquinolyl-n-hydroxy succinimidyl carbamate)	32
Heterocyclic Amines	CZE	20 mM ammonium acetate @ pH 3.0 with 20% methanol	Mass Spectroscopy	14
21 Biogenic Amines (including histamine)	CZE	4 mM copper (II) sulphate, 4 mM formic acid, 4mM 18-crown 6 ether @ pH 3.0	UV@ 210 nm	15
Biogenic Amines	CZE	100 mM ammonium acetate @ pH 7.5 with 30% acetonitrile	UV@ 200 nm – 300 nm	23
Biogenic Amines	MEKC	100 mM boric acid with 20 mM sodium dodecyl sulphate @ pH 9.3	Fluorescence (derivatised with fluorescamine isothiocyanate)	31
21 Aromatic Amines (inc pyridine, aniline)	CZE	0.05 M Na ₂ HPO ₄ @ pH 2.35, with 7 mM 1-3 diaminopropane	UV@ 280 nm	18
Alkyl and aryl pyridine derivatives	CZE	0.05 M Na ₂ HPO ₄ @ pH 2.5 with 37.5 % polyethylene glycol (PEG)	UV @ 265 nm	19
Aminopyridines	CZE	0.05 M Na ₂ HPO ₄ @ pH 2.5	UV @ 210 nm	24
Pyrimidine derivatives	MEKC	25 mM sodium tetraborate, 50 mM sodium dodecyl sulphate with 20% propanol @ pH 9.4	UV @ 200 nm and 240 nm	33
Methyl quinolines	CZE	0.0176 M acetate-Tris @ pH 5.5 with 10% PEG	UV@ 225 nm	20

Table 3.2 Examples of CE studies on heterocyclic, aromatic and biogenic amines

3.4.2.2 Capillary Zone Electrophoresis (CZE)

The term capillary electrophoresis actually applies to a family of related techniques, including capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC) and non-aqueous capillary electrophoresis (NACE). Researchers have exploited all these techniques in their aim to separate basic compounds.

CZE involves the separation of species in free solution and is the simplest form of CE. Often, additives such as chiral selectors and organic modifiers, are added to improve the separation. The earliest studies on the use of CE to analyse basic compounds used CZE. Wu et al. [12] used a 50 mM phosphate solution, buffered with 20 mM citric acid and 30 mM NaCl to a pH of 2.1 to effect the separation of the 13 heterocyclic amines in rainwater. By employing an uncoated fused silica capillary of separation length 46.4 cm, an applied voltage of +18 kV and hydrodynamic injection, the separation of the amines was accomplished.

A group led by Mardones [13] used CZE to determine the heterocyclic content in meat and fish extracts. They performed the analyses with a fused silica capillary of separation length 49.4 cm. The electrolyte used was 30 mM NaH_2PO_4 buffered to a pH of 2.0 with 30 mM NaCl. 30 % (v/v) methanol was added as an EOF modifier. A constant separation potential of +19 kV, with the capillary maintained at ambient temperature, provided the optimum separation conditions.

A brief study was carried out on the optimum injection mode. In electrostatic injection, the sample ions are forced to migrate into the capillary by applying a potential across the capillary for a few seconds. Hydrodynamic injection involves drawing the sample ions into the capillary by application of a pressure difference at both ends of the capillary.

They found that hydrodynamic injection was less sensitive than electrokinetic injection but gave greater reproducibility at lower concentrations of amine

An organic modifier was added to an electrolyte to increase solubility of the analytes, change the EOF and reduce interaction of the analytes with the capillary wall. The study found that above 40% (v/v) methanol content the migration times of the amines became too long and no appreciable change in resolution was observed. The methanol had caused a decrease in the EOF leading to the longer migration times.

The velocity of the electroosmotic flow is greatly influenced by the pH of the buffer. At low pH the silanol groups of the capillary are undissociated. As a result, the magnitude of the electroosmotic flow is greatly decreased and separation at this pH is due to size/charge effects. However, as the pH increases the silanol groups dissociate to a greater extent. Separation is now due to the presence of an electroosmotic flow, the velocity of which increases with increased pH. To investigate this phenomenon and its effect on the separation of the amines, Mardones and colleagues adjusted the pH from 2.0 to 3.2. Above pH 2.8, the amine peaks were distorted. This is because separation was no longer based on the differences in their size/shape but on their electrophoretic mobilities, which are very similar [13]. With the optimum conditions determined during the study the heterocyclic amines were separated in 20 minutes.

The use of a mass spectrometer (MS) as a detector greatly increases the power of CE. Zhao et al. [14] analysed a mixture of six heterocyclic amines with CE/MS and evaluated their results against results obtained from a previously validated LC/MS method. An electrospray interface was used to connect the CE with the MS. Non-volatile buffers cannot be used in the mass spectrometer as they form salt deposits in the mass analyser. A method was developed that employed an electrolyte compatible with the MS. Originally, the researchers attempted to use electrolyte containing no micelles or non-volatile constituents. However, the heterocyclic amines adsorbed onto the capillary wall. To overcome this, the capillary was deactivated with polyvinylacetate (PVA), which is a water insoluble coating. Zhao and coworkers were then able to use a 20 mM ammonium acetate solution at pH 3.0 containing 30 % (v/v) methanol to obtain a separation of comparable quality to the LC/MS analysis.

Biogenic amines are organic bases present in animals and plants and can contribute to the spoilage of food. Common biogenic amines include histamine and tyramine. The biogenic amines have very similar mobilities so a number of parameters must be optimised before an adequate separation is achieved. In addition, many of these amines have little or no absorbance in the UV spectrum, so fluorescence or indirect UV detection is usually employed in their separation.

Arce and coworkers [15, 16] achieved the separation of 21 amines using indirect UV detection. In this detection technique a background electrolyte (BGE) generates a high UV absorbance at a wavelength of minimal absorbance of the analyte ions.

The presence of the ions is then detected as a decrease in the absorbance of the BGE because the analyte molecules have displaced its chromophore molecules. Consequently, a negative peak corresponds to the analyte.

The BGE must have a mobility equal or approximate to that of the analytes and an ionisation constant (pK_a) identical to that of the buffer [17]. Arce employed copper (II) sulphate as the BGE adjusted to pH 3.0 with formic acid. The amines exist in their protonated forms at this pH. The acid also prevents the hydrolysis of the copper in the electrolyte during CE separation. The modifier added to the electrolyte was 18-crown 6 ether.

The influence of instrumental variables on the separation was investigated. The velocity of the EOF increases with increase in applied separation voltage. However, at higher applied potentials, frictional collisions between mobile ions and buffer molecules generate heat in the electrolytic solution. This phenomenon is termed Joule heating. If the heat is not dissipated at an equal rate to its production, the temperature inside the capillary will rise, causing the buffer to boil and form bubbles. Thermal gradients also develop inside the capillary, leading to substantial bandbroadening and poor resolution. The study carried out by Arce and coworkers confirmed that, while they were separated in five minutes, the resolution of the amines was very poor at a separation potential of +30 kV. The optimum potential was determined to be +10 kV.

The influence of injector mode, buffer concentration, ionic strength and modifier concentration were also investigated and, ultimately, the 21 amines were separated in 16 minutes under optimum conditions.

Cavallero and colleagues [18] applied the optimised CE separation of 21 standard aromatic amines to their analysis of rainwater samples. Separation of the amines, including pyridine, aniline and their derivatives, was performed with an uncoated fused silica capillary (50 μm i.d.) of separation length 65 cm, UV detection and an applied potential of +30kV. A 7 mM solution of 1,3-diaminopropane was added to the 50 mM phosphate buffer (pH 3.5) to reduce retention time, drift and wall interactions (Fig 3.9).

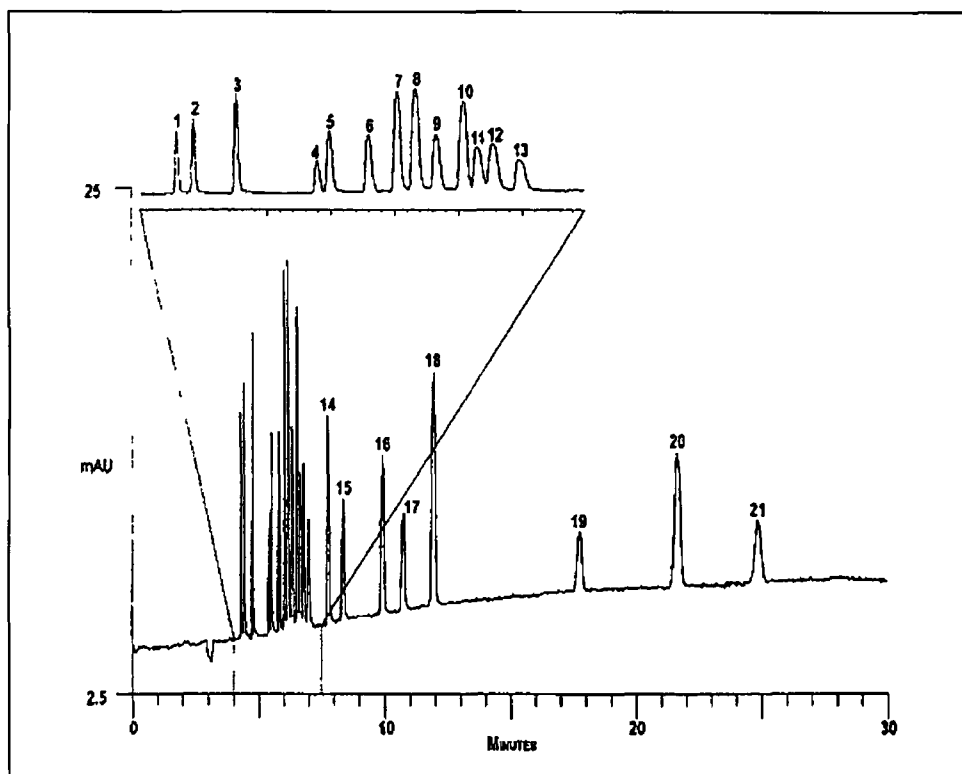


Fig 3 9 CZE separation for a standard solution of aromatic amines Fused silica capillary 65cms x 50 μ m i d , buffer 50mM NaH₂PO₄ with 7mM 1,3- diaminopropane (pH 2.35), applied potential +30kV, detection UV at 280nm Analytes 1 pyridine, 2 *p*-phenylenediamine, 3 benzidine, 4 *o*-tolidine, 5 aniline, 6 N,N-dimethylaniline, 7 *p*-anisidine, 8 *p*-chloroaniline, 9 *m*-chloroaniline, 10 ethylaniline, 11 α -naphthylamine, 12 diethylaniline, 13 N-(1-naphthyl) ethylenediamine, 14 4-aminophenazone, 15 ortho-chloroaniline, 16, 3,4-dichloroaniline, 17 3,3'-dichlorobenzidine, 18 2-methyl-3-nitroaniline, 19 2,4,dichloroaniline, 20 2,3,-dichloroaniline, 21 2,5 dichloroaniline [18]

It was found that, for the analysis of real samples, the CE method had a limit of detection (LOD) of $0.6 - 1.8 \mu\text{gml}^{-1}$, which was adequate for the analysis of water samples. However, it was determined that the method was not sensitive enough for the analysis of soil samples and could only be used on soil that had been contaminated.

The influence of the modifier, polyethylene glycol (PEG), on the separation of alkylpyridines was examined by Bednár et al. [19]. They found that interaction between the PEG chains and protonized pyridine bases caused an increase in their resolution. Additions of 12.5 %, 25 % and 37.5 % (w/v) PEG were made to 50 mM phosphate buffer, pH 2.5, and the effect on the separation investigated. Bednár et al. concluded that optimum separation was achieved with a PEG concentration of 37.5% (w/v). The applied potential was +30kV, with hydrostatic injection for 1 second and detection at 260 nm. An industrial sample containing pyridine derivatives was also analysed. Recently, Bednár and his colleagues [20] used PEG as a modifier for the separation of methylquinolines in industrial samples.

The separation of basic drugs on bare fused silica at low pH was demonstrated in a study carried out by Hudson and co-workers [21] who reported its use to screen complex samples from a library of more than 500 basic pharmaceuticals of toxicological interest. A capillary of separation length 50 cm was used with a 100 mM phosphate buffer of pH 2.38. The technique uses electrokinetic injection for 8 seconds at +10kV and an applied potential of $+350\text{Vcm}^{-1}$ (Fig. 3.10).

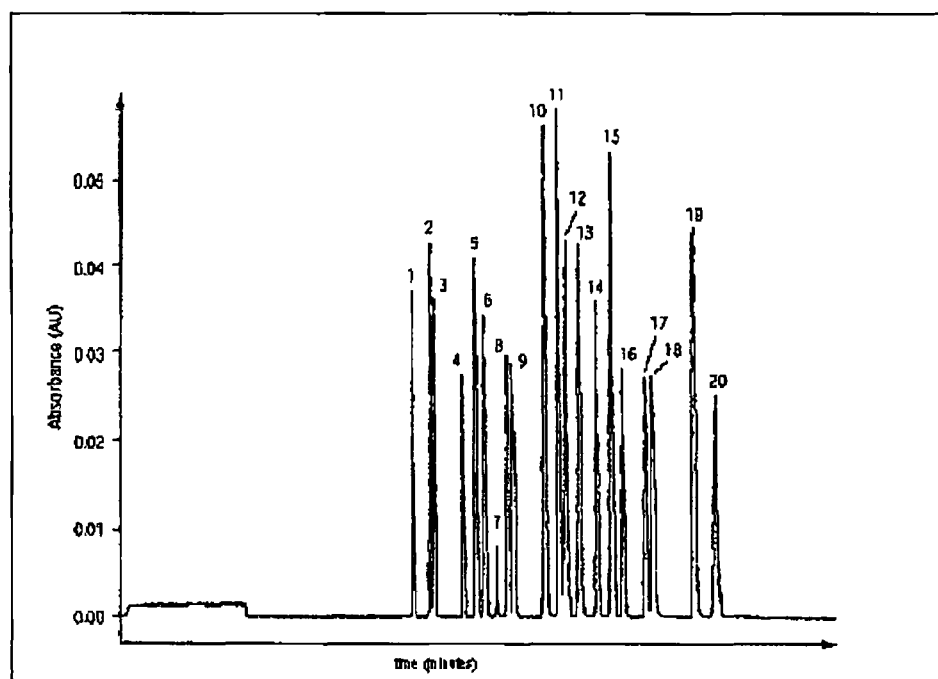


Fig 3 10 CZE separation of basic drug quality control sample using fused silica capillary
 Capillary 50cms separation length x 50 μ m Buffer 100mM phosphate (pH 2.38), applied
 potential 350V/cm, detection UV absorbance at 214nm, injection 8s at 10kV Analytes
 1 pheniramine, 2 chlorpheniramine, 3 brompheniramine, 4 anileridine, 5
 amphetamine, 6 methamphetamine, 7 trifluoroperazine, 8 pseudoephedrine, 9 ephedrine,
 10 methoxamine, 11 diphenhydramine, 12 dextromethorphan, 13 codeine, 14
 hydroxyzine, 15 salbuterol, 16 metoprolol, 17 trazodone, 18 haloperidol, 19
 verapamil, 20 Loperamide [21]

3 4 2 3 Micellar Electrokinetic Chromatography (MEKC)

MEKC allows for the simultaneous separation of neutral and charged solutes using CE instrumentation. One or more surfactants are added to the electrolyte solution above the critical micelle concentration (CMC) to form micelles. This micellar medium provides a pseudostationary phase into which analytes can partition. Consequently, separation in MEKC is based on the electrophoretic mobilities of the analytes in the micellar phase.

Surfactants are compounds that have a hydrophobic tail region and a hydrophilic head group. A commonly used surfactant in MEKC is sodium dodecyl sulphate (SDS), which is negatively charged, with the result that its electrophoretic velocity is towards the anode. Under normal conditions (positive polarity), with a fused silica capillary, the electrophoretic velocity moves from the anode to the cathode, so the SDS micelles oppose the EOF in the capillary. However, the anionic micelles will still progress towards the cathode, albeit at a slower rate, because the micelles' electrophoretic velocity is not great enough to overcome the overall electrophoretic velocity.

Separation of charged analytes in MEKC is accomplished in three forms: first, the analyte travels through the aqueous phase without any association with the micelles, second, the analyte forms an ion pair with the surfactant molecules and, third, the analyte is partitioned into the micellar phase.

When using anionic surfactants in the MEKC analysis of basic compounds, it must be remembered that, at the low pH required for the protonation of the basic analytes, the electrophoretic velocity of the micelle is toward the anode, so most researchers reverse the normal applied polarity of the instrument.

Ding and Fritz [26] separated a mixture of basic and neutral, hydrophobic drugs, including imipramine and lidocaine, in a 30 mM ethanesulphonic acid solution at pH 2.4 with 20% acetonitrile and 30 mM lauryl poly (oxyethylene) sulphate (Brij – S). They had previously carried out the separation of the basic compounds with great success in the absence of the surfactant. However, the addition of Brij - S enabled them to separate both the basic and neutral drugs.

MEKC separations of hydrophobic compounds with SDS are generally of poor quality, with long migration times, reduced sensitivity and poor resolution, because the hydrophobic compounds become incorporated in the micelle and, thus, cannot be separated. Ding and Fritz used Brij-S, an anionic surfactant. In the acidic medium of the electrolyte, the compounds are not incorporated in the Brij-S micelles as much as in the SDS micelles, so the separation was performed with success. The largest analyte compounds form the most stable association complexes with the sulphonated Brij- 30 (Brij – S) and, therefore, have the shortest migration times.

Hansen and his colleagues [27] studied the separation of basic drugs with similar structures with MEKC. Both zwitterionic and nonionic surfactants were employed to obtain separations of imipramine, amitriptyline and their analogues. The researchers chose to use a zwitterionic substance, 3 N-N dimethylmyristylammonium propanesulphonate (MAPS) and Tween 20, a non-ionic surfactant because these surfactants do not change the ionic strength of the separation buffer. In a previous study [28] they had discovered that addition of SDS to an electrolyte brought about an increase in the ionic strength of the buffer which limited the use of higher separation voltages and, hence, increased the separation time.

By exploiting small differences in the affinity of the analytes for the micellar phase, Hansen et al obtained a separation of all compounds

In a similar study, Esaka et al [29] used Tween 20 to improve the MEKC separation of hydrophobic cations. They had reached the same conclusion as Hansen [27], that the hydrophobic cations became totally dissolved in the SDS micelles, and proposed the use of Tween 20 to weaken the attractive ionic interactions between the cationic analytes and the SDS micelles. By varying the ratio of Tween 20 to SDS the selectivity of the separation was controlled. Esaka and co-workers obtained a separation of adrenaline and its six precursors using the mixed micelle system of 12.5 mM Tween 20 and 25 mM SDS in 10 mM phosphate at pH 2.5. A reverse polarity of -18kV was used, with the capillary, of separation length 20 cm, maintained at ambient temperatures.

In addition, it was also found that the polyether chain of Tween 20 served as a hydrogen acceptor, forming hydrogen-bonding interactions with hydrogen donating analytes, further increasing the selectivity of the separation buffer.

Taylor and Reid [30] evaluated CZE and MEKC as methods for the analysis of anti-malarial drugs and compared them with a previously validated HPLC method. The CZE method employed a 25 mM phosphate solution at pH 2.0 with 10% (v/v) acetonitrile and 10 mM SDS. They opted to use a buffer at pH 10 for the MEKC separation because it was assumed that the basic analytes would be neutral at this pH. Subsequently, the separation would be as a result of the preferential solubilization of the analytes in the SDS micelles. A forward (normal) polarity was used for both separations.

The researchers discovered that the resolution under MEKC conditions was inferior to that under the CZE conditions. In fact some of the analytes were not resolved at all with MEKC. Stacking techniques were used to improve the limit of detection (LOD) of the CZE method and a separation of comparable quality to the established HPLC method was eventually achieved.

Buchberger et al. [33] developed a MEKC method for the separation of a mixture of pyrimidines. Phosphate and borate buffers in the pH range 7.5 to 11.5 were prepared. They contained concentrations of SDS varying from 15 mM to 35 mM. The method used forward polarity with hydrostatic injection and UV detection. Buchberger et al. were unable to achieve a separation, so volumes of organic modifiers were added to the electrolytes to improve the separation. Organic solvents can have a significant impact on the structure of the micelles. They can also alter the distribution of the analytes between the micelles and the aqueous phase [34]. Eventually, optimum separation selectivity was obtained when the carrier electrolyte (25 mM sodium tetraborate and 50 mM SDS) was modified with methanol or propanol and adjusted to pH 9.4.

3.4.2.4 Non-Aqueous Capillary Electrophoresis (NACE)

Non aqueous capillary electrophoresis (NACE) is a relatively new technique in CE that offers great promise, especially for the easy manipulation of separation selectivity in the analysis of hydrophobic compounds. Organic solvents have vastly different physicochemical properties that allow for the control of the electroosmotic flow and analyte migration order simply by changing the solvent.

An organic solvent affects the acid/base properties of an analyte permitting the separation of analytes difficult to resolve in aqueous buffer

A theoretical study carried out by Karbaum and Jira [35] tested the suitability of eleven organic solvents for the separation of three basic compounds, propanolol, carteolol and imipramine

The solvents were assessed on the basis of solubility, selectivity, their chemical equilibria and Joule heating. The demands of the solvents on the CE system were also outlined. NACE requires a stable current between the electrodes and high purity solvents that do not have the same UV absorbance as the analytes.

A major benefit of NACE is that higher applied separation voltages can be utilised because Joule heating of the separation buffer is minimised. Organic solvents have a lower dielectric constant than water. Therefore, less solvent-separated free ions, which facilitate charge transfer, exist in the capillary. Consequently, lower electric currents are achieved at the same voltage in organic solvents as in aqueous buffers. This leads to a decrease in the level of Joule heating which occurs during the NACE separation. Accordingly, higher applied voltages can be used which lead to shorter analyte migration times.

Karbaum and Jira found that seven solvents were suitable for the separation. However, the solvent separation selectivities were widely different because of their contrasting acid/base conditions, viscosities and ionising properties. Mixed solvents were found to be better because they allowed the analysts to tailor the system to the separation required.

Two other studies [36, 37] also investigated the effect of various solvents on the selectivity of imipramine and its derivatives. The developed method was then applied to the analysis of ibuprofen and its metabolites [36]. Using an ammonium acetate buffer (because it is soluble in all solvents) and acetic acid or methanesulphonic acid to alter the apparent pH of the solutions, the effects of the different solvents on the separation of the basic drugs was investigated.

The apparent pH (pH^*) is used as a measure of the acid and base properties of non-aqueous solutions. As pH is only defined in aqueous solutions it has no meaning in non-aqueous solvents. However, acidic or basic conditions will still have a major influence on the selectivity of a NACE separation, so the pH of non-aqueous solutions is measured as pH^* .

A study carried out by Porras and co-workers [38] evaluated different electrolytes in methanol/ acetonitrile solvent mixtures for the separation of narcotics and other basic pharmaceuticals. Various alkali metal acetates at a concentration of 20 mM were assessed and it was found that the change in apparent migration times of the analytes was due to the change in the pH^* of the electrolyte solution. Addition of acetonitrile to ammonium acetate in methanol solutions was found to alter the electrophoretic mobilities of the basic pharmaceuticals studied. Changing the electrolyte anion or cation altered the resolution and migration order of the analytes, probably because of ion interactions in the solutions.

Cherkaoui and his colleagues [39] used NACE to obtain very rapid separations of amphetamines and local anaesthetics. A 25 mM ammonium formate solution with 1M formic acid in acetonitrile was used to separate amphetamines in less than 1 minute.

The non-aqueous solution allowed a higher applied voltage of 30kV to be used without a large current being generated. This greatly decreased the chances of Joule heating happening. The amphetamines were injected under pressure for 1 second and a 32.5 cm capillary of internal diameter 50µm was used.

Anaesthetic drugs were also rapidly separated under the same experimental conditions except that an ammonium acetate in methanol/acetonitrile (40/60) electrolyte was utilised.

3.4.2.5 Coated Capillaries

Nearly all CE separations have been performed on fused silica capillaries. These capillaries have many advantages, namely, flexibility, good thermal and optical properties in the UV range and their availability in very small internal diameters. However, their major drawback is that charged molecules interact with the silica surface. Also, capillary surfaces must be completely equilibrated, by using a complex series of rinsing steps, prior to every separation.

One answer to these problems is to coat the capillary to deactivate or “shield” the silanol groups. Consequently, the EOF is virtually eliminated. Generally, any neutral polymer that is soluble or swells in water can be used. Examples include methylcellulose and non-cross linked polyacrylamine. These polymers can also be dissolved in the electrolyte to suppress the EOF in CZE [41].

Polyacrylamide is commonly used in coating capillaries because it has been found to form a well-defined monomolecular layer of the polymer coating on the capillary wall. Wan and his co-workers [42] used a polydimethacrylamide - coated capillary to separate basic and acidic proteins. A reverse polarity (negative potential) was applied for all the separations on coated capillary. The buffer used for the analysis of the basic proteins was a 50 mM acetic acid- Tris solution of pH 2.4. Wan et al. discovered that they obtained very high efficiencies with polydimethacrylamide - coated capillary when analysing the basic proteins because the absorption of the analytes onto the capillary was negligible.

Liu and Pietrzyk [43] used a sulphonated polymer - coated capillary for the CE separation of pyridines and pyrimidines. A 20 mM Tris-trichloroacetic acid buffer at pH 2.0 with a forward polarity of +20 kV was utilised to obtain the separation of the organic bases.

It was discovered that the EOF for the sulphonated polymer-modified silica capillary was nearly constant over a pH range from pH 2 to pH 9. Therefore, altering the buffer pH influences weak base and acid analyte migration times according to analyte pK_a value. Also, at low pH the EOF in the polymer-modified capillary is much greater than that in the bare fused silica, resulting in shorter migration times.

Liu and Pietrzyk also investigated the effect of the addition of an inorganic cation to the electrolyte. By altering the concentration of Mg^{2+} in the buffer at pH 2.0 they were able to influence the analyte migration time and resolution because increasing the inorganic cation concentration caused a decrease in the electrophoretic velocity.

In capillary electrochromatography (CEC) the fused silica capillary is packed with a HPLC stationary phase and a voltage is applied across the capillary, which generates an EOF. Separation is based on both differential partitioning and electrophoretic migration of the analytes in the capillary. It is therefore possible to obtain unique separation selectivity using CEC compared to both HPLC and CE. There are many advantages of CEC that combine the best aspects of both HPLC and CE. The plug-like flow profile of EOF reduces flow - related band broadening and separation efficiencies of several hundred thousand plates per metre are often obtained in CEC. There is no back pressure when EOF occurs, so small particle sizes such as 1-3 micron can be used to improve separation efficiencies. CEC is useful for hydrophobic compounds that can be difficult to analyse by CE because carrier electrolytes containing high levels (40-80%) of organic solvents such as methanol or acetonitrile are employed.

An early study on the separation of basic proteins with coated capillaries was carried out by Xu and his colleagues [44]. A silica capillary was modified with copolymers of vinylpyrrolidone and vinylimidazole. This coating greatly decreased the EOF. In addition, the positive layer on the capillary surface, formed due to the cationic character of the polymers below pH 7, repelled attractions between the wall and the basic proteins. The authors claimed to have obtained a good separation of the proteins in ten minutes below pH 5. Above this pH the stability of the coating was poor and the separation was not reproducible.

Smith [45] investigated the use of various HPLC stationary phase-packed capillaries with aqueous and non-aqueous buffers for the separation of basic compounds including tricyclic anti-depressants and pyridine.

Smith found that the best separations were obtained when a non-aqueous buffer with electrolyte was employed, specifically, a Waters symmetry shield RP 8 packed capillary of length 48.5 cm and an acetomtrile-100mM Tris solution (70/30) at a pH of 9.0. This resulted in baseline resolution of six basic compounds. A high capillary temperature (40°C) and a forward polarity of +30kV were employed.

A mixture of basic compounds was analysed by Wei et al. [46] using a capillary column packed with 3µm silica particles and carrier electrolyte in the pH range 7.5-10.5. They found that the separation mechanism was a multifunctional one, including ion-exchange and reversed-phase interactions as well as electrophoretic migration of the analytes (Fig. 3.11).

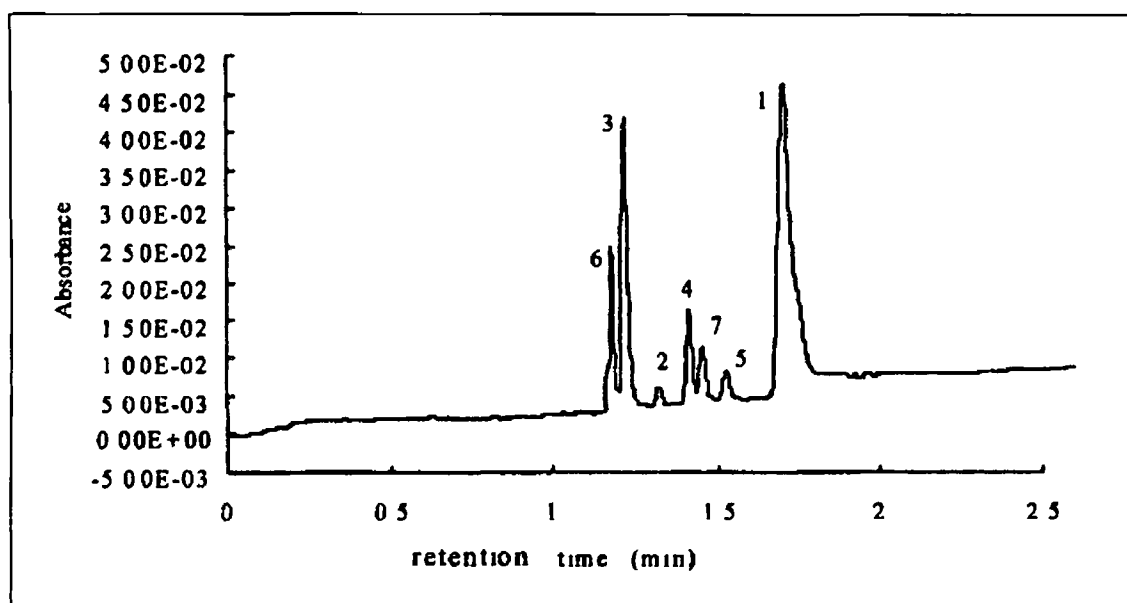


Fig. 3.11 Electrochromatogram of the separation of seven basic drugs by CZE. Experimental conditions: open tubular column 27 cm (20cm separation length) x 75µm i.d., mobile phase 100 mM buffer (pH 8.29). Analytes: 1 aniline, 2 cocaine hydrochloride, 3 berberine hydrochloride, 4 thebaine, 5 jatrorrhizine hydrochloride, 6 ephridine hydrochloride, 7 codeine phosphate [46].

Klampfl and Haddad [47] separated basic compounds with a capillary packed with cation exchange resin in low pH electrolytes. It was discovered that the ion-exchange interactions occurring between the cation-exchange resin and the analytes could be manipulated by changing the composition of the carrier electrolyte in type (e.g. Li^+ , Na^+), and in concentration. Klampfl and Haddad were then able to manipulate the separation times and selectivity for most of the basic analytes (Fig. 3.12).

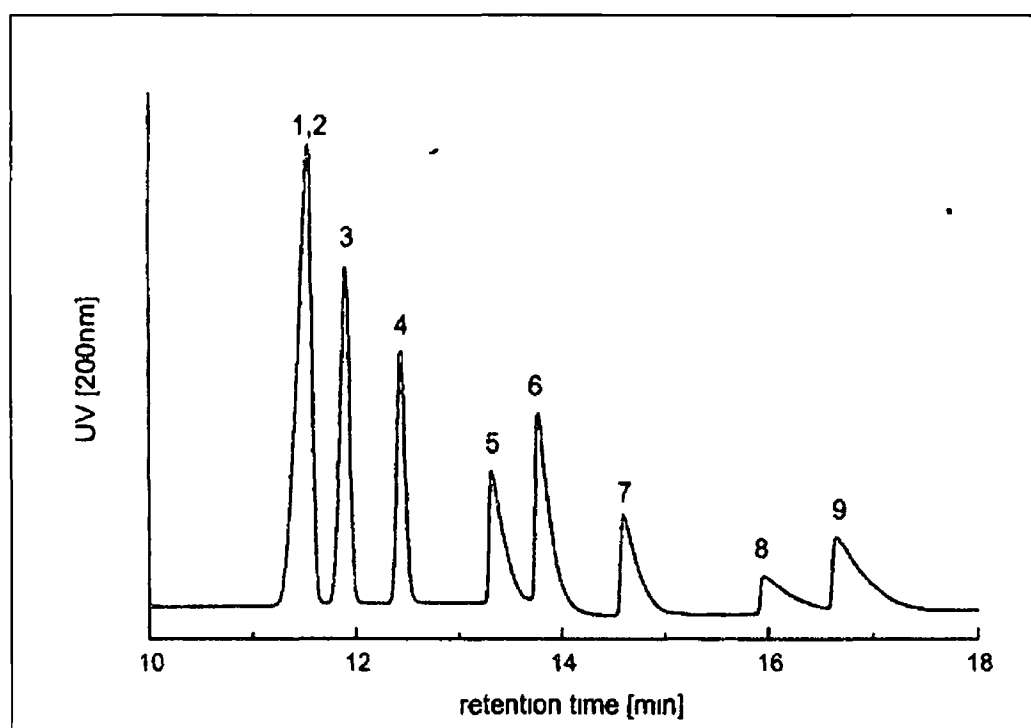


Fig. 3.12 Separation of a standard mixture of basic compounds. Experimental conditions: Capillary: 35cm (26.5 packed bed) \times 75 μm i.d., packed with 3 μm SCX, mobile phase: 20mM NaH_2PO_4 -acetonitrile (30:70) pH adjusted to 3.0 with H_3PO_4 , voltage: +15kV. Analytes: 1 *o*-toluidine, 2 aniline, 3 *m*-toluidine, 4 *p*-toluidine, 5 benzylamine, 6 4-aminopyridine, 7 3-aminopyridine, 8 pyridine, 9 2-aminopyridine [47].

3.5 Experimental Details

3.5.1 Reagents and Apparatus

The sources of chemicals were as follows

Chemical Name	Supplier	Purity
Piperidine	Sigma-Aldrich	99%
Ethyl piperidine carboxylate	Sigma-Aldrich	97%
Dipiperidinomethane	Sigma-Aldrich	98%
Acetonitrile	Labscan	HPLC Grade
Methanol	Labscan	HPLC Grade
Sodium dodecyl sulphate	AnaLar (BDH)	99%
Polyvinylsulphonic acid (sodium salt)	Sigma-Aldrich	25% weight in water
Tween 20 (polyoxyethylenesorbitan monolaurate)	Sigma-Aldrich	n/a

Two CE instruments were used a P\ACE 2000 equipped with a high power voltage supply and connected to the mains nitrogen and a P\ACE MDQ, both of which are Beckman instruments The P\ACE MDQ was used following the breakdown of the original system Both systems were equipped with a Hg lamp equipped with UV filters The detector window was 100 μm x 200 μm in size

Polyimide-coated fused silica capillaries with an internal diameter of 50 μm were supplied by Composite Metal Services They had a separation length of 50 cm with a total length of 57 cm The samples were injected hydrostatically for 10 seconds All sample vials were glass with silicon seals and they were loaded onto an autosampler All electropherograms were recorded and analysed using the Beckman P\ACE software on a Gateway P5-133 (P\ACE 2000) and IBM 300GL (P\ACE MDQ 5000) personal computers

3.6 Method

A variety of parameters, both instrumental and chemical, must be investigated to ensure a successful separation in capillary electrophoresis. Method development involves choosing the correct type of buffer, its concentration and pH, optimisation of temperature, voltage and capillary length, type of detection and injection mode required and whether a modifier is required to alter the electroosmotic flow.

All solutions were prepared with 18 M Ω deionised water (from a Millipore Waters System). 500 ppm stock standards of piperidine, dipiperidinomethane and ethyl piperidine carboxylate were prepared in each buffer studied. These stock standards were kept for up to one week. All buffer electrolyte solutions and standard solutions were filtered through a 0.45 μ m filter prior to use.

3.6.1 Capillary Preparation

The polyimide coating must be removed from a small section of the capillary to create a detector window. This was accomplished by burning the appropriate area with a butane lighter and then cleaning the window by wiping it carefully with acetone.

The capillary was changed every two weeks because a variety of different buffers was used during the project. Conditioning of the capillary was as follows. Initially, it was washed for 2 minutes with 18 M Ω deionised water followed by a rinse with 0.1 M HCl for 2 minutes. The capillary was then washed for a further 2 minutes with deionised water, then with 0.1 M NaOH for 10 minutes. After conditioning, the capillary was equilibrated with buffer for 15 minutes.

Prior to every sample run, the capillary was rinsed for two minutes with methanol, two minutes with NaOH and two minutes with buffer. Rinsing the capillary with methanol ensures that any compounds or impurities attached to the capillary are washed off.

CE involves the constant movement of ions through the capillary from the injection side to the detector side. Consequently, there is a build-up of ions in the vial at the detector end of the capillary. The buffer was replaced after every 6 runs because this difference in the composition of the two vials can lead to variations between each sample run.

3.6.2 Determination of UV Absorbance of the Basic Compounds

The instrument used was a Perkin-Elmer Lambda 900 equipped with UV WinLab L800 software on a Dell personal computer. An acetonitrile blank was used. The samples were prepared in acetonitrile in the concentrations given in section 3.6.1.

3.6.3 Determination of Acid Dissociation constants (pK_a) of Basic Compounds

A 100 ml solution of Britton-Robinson (BR) buffer was prepared to which one or two drops of the analyte were added. The buffer was a solution containing 0.04 M boric acid, 0.04 M acetic acid and 0.04 M phosphoric acid. The pH of the solution (buffer with analyte) was determined and a UV spectrum recorded around the wavelength of maximum absorbance of the analyte. 0.05 M NaOH was added to the solution to change the pH by 0.05 units. A UV spectrum was recorded again and the maximum absorbance was also noted. The experiment was continued in this manner until no further change in absorbance was observed.

The pK_a of the compound was then ascertained by plotting the change in pH vs the absorbance of the compound. The midpoint of the curve was taken to be the pK_a of the compound. In order to ensure that the pK_a s determined from the midpoint of each curve were accurate, a first derivative plot of each was carried out.

A Shimadzu UV-Vis spectrometer was employed to monitor the change in UV absorbance of the solution while the pH was observed using an EDT Instruments RE 357 Microprocessor pH meter. Measurements were at room temperature (22°C).

3.7 Results

3.7.1 Determination of UV Absorbance of the Basic Compounds

The CE instruments were equipped with UV detectors with four filters 200 nm, 214 nm, 254 nm and 280 nm. To determine the UV absorbance of the compounds a solution of each was prepared in acetonitrile (UV cutoff 190 nm). Using 100 ppm solutions initially, it was determined that the compounds gave rise to absorbance between 200 nm and 220 nm, so 10 ppm and 1 ppm solutions of each were prepared.

All the compounds had slightly different absorbance maxima (Figures 3.13-3.15). However, it was decided to use the 214 nm filter. Dipiperidinomethane (DPM) had a maximum absorbance at 210 nm and it is the least UV absorbing of all the compounds having almost no absorbance at 200 nm (Fig. 3.13). Ethylpiperidine carboxylate (EPC) exhibited strong absorbances from approximately 190 nm- 220 nm (Fig. 3.16), whilst piperidine was strongly absorbing at 200nm (Fig. 3.14). However, piperidine was fairly absorbent over the whole range from 190nm – 220 nm. Since most of the standards to be analysed on the CE were prepared at concentrations of 50 and 100 ppm it was decided to use the 214 nm filter. All compounds absorbed at that wavelength and it was the closest to the maximum absorbance of the least strongly absorbing compound, DPM.

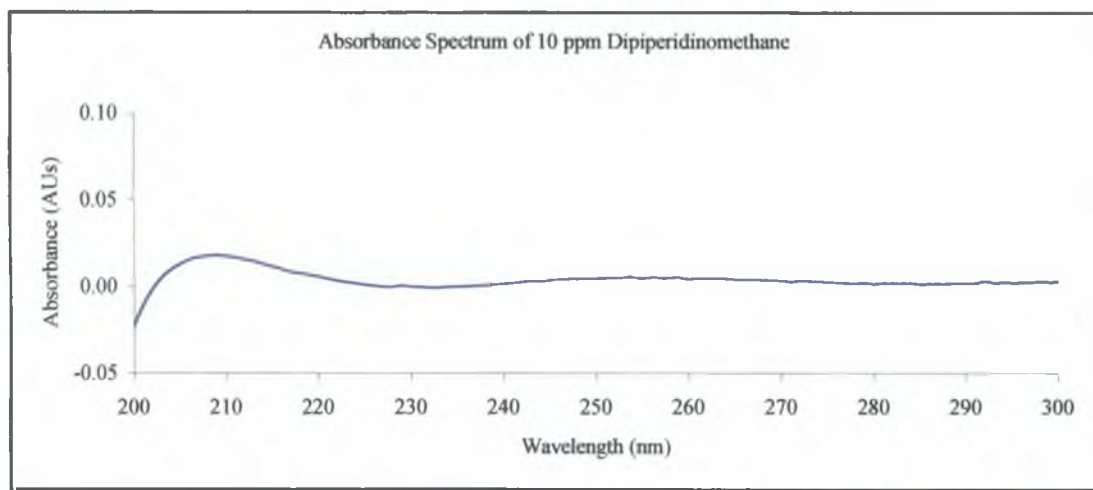


Fig. 3.13 UV absorbance spectrum of dipiperidinomethane with its maximum absorbance at 210nm.

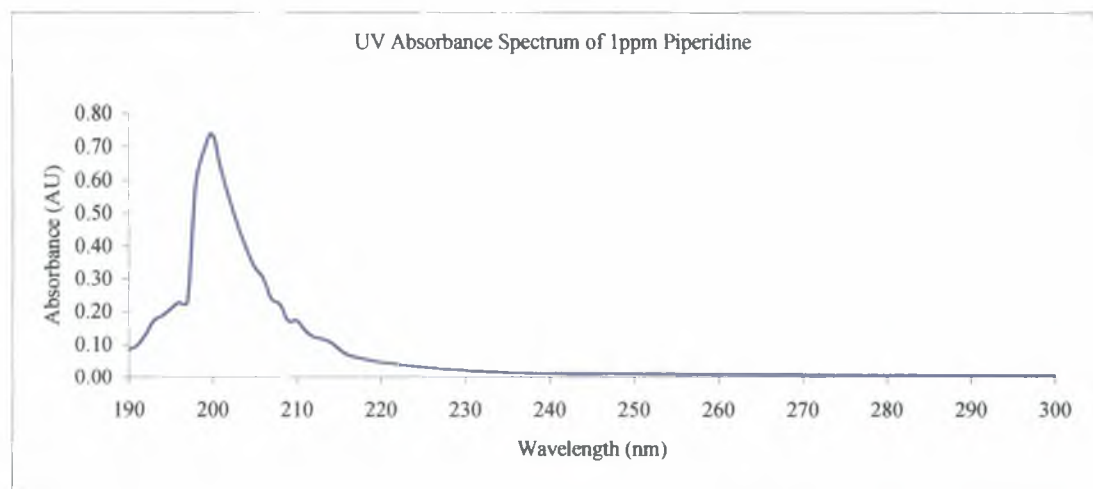


Fig. 3.14 UV absorbance spectrum of piperidine with a maximum absorbance at 200nm.

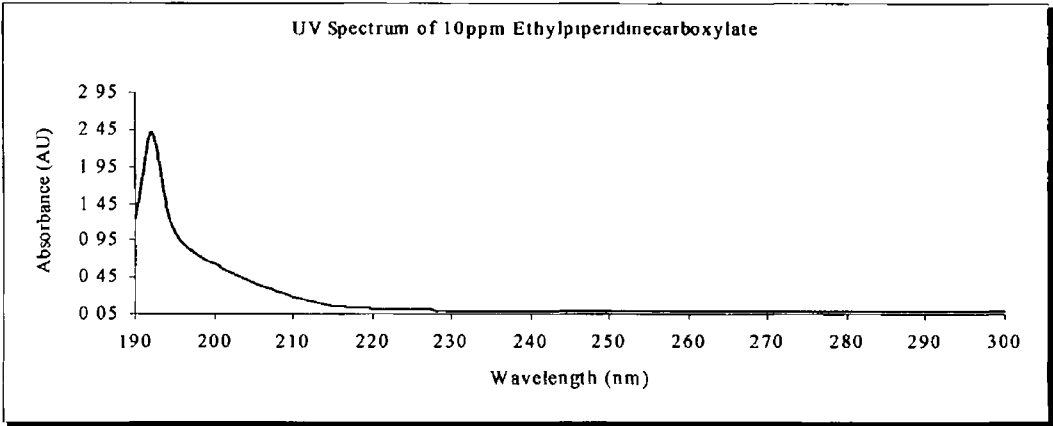
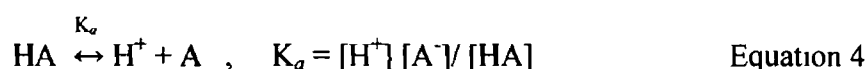


Fig 3 15 UV absorbance spectrum of ethyl piperidine carboxylate with a maximum absorbance at ~192nm

3 7 2 Determination of Acid Dissociation Constant (pK_a) of Basic Compounds

It is important to determine the pK_a values of the compounds because this can allow one to determine migration order of analytes in CE. The acid dissociation constant, K_a , is a measure of the extent to which an acid is ionized in an aqueous solution. A weak acid (HA) is one that is not completely dissociated and is defined by the reaction



The pK_a is the negative logarithm of the equilibrium constant, i.e.

$$pK_a = -\log K_a = -\log [H^+][A^-]/[HA] \quad \text{Equation 5}$$

Therefore, as the value of K_a increases, the value of the pK_a decreases, and vice versa, i.e. the stronger the acid, the lower the pK_a value. The dissociation constants can be determined using CE, as demonstrated by Medonsa and Hurtubise, who determined the pK_a 's of eight heterocyclic aromatic amines [48]. However, approximate pK_a values can also be determined by monitoring the change in UV absorbance of a solution in BR buffer at a certain wavelength as its pH is altered (section 3 6 3). From the literature, the dissociation constant of piperidine is 11 123 [49, 50], taken at a temperature of 25°C. Consequently, the pK_a value determined for piperidine from this method would give a good indication of how accurate a procedure it was for calculating the pK_a 's of DPM and EPC.

Figures 3 16, 3 19 and 3 21 depict plots of the pH versus absorbance for each compound, while the change in absorbance of each compound as a function of pH is depicted in Figures 3 17, 3 20 and 3 22. The pK_a values were determined from these plots as shown in Fig 3 18. The pK_a of piperidine was determined from Figures 3 16 and 3 19 to be 11 8.

As stated earlier, the literature value for piperidine of 11 123 was determined at 25°C. The dissociation constants of the basic compounds were taken at 22°C so this would have had a slight effect on the determined values. However, the determined dissociation constants were only approximate values and were, therefore, used only as a general guideline.

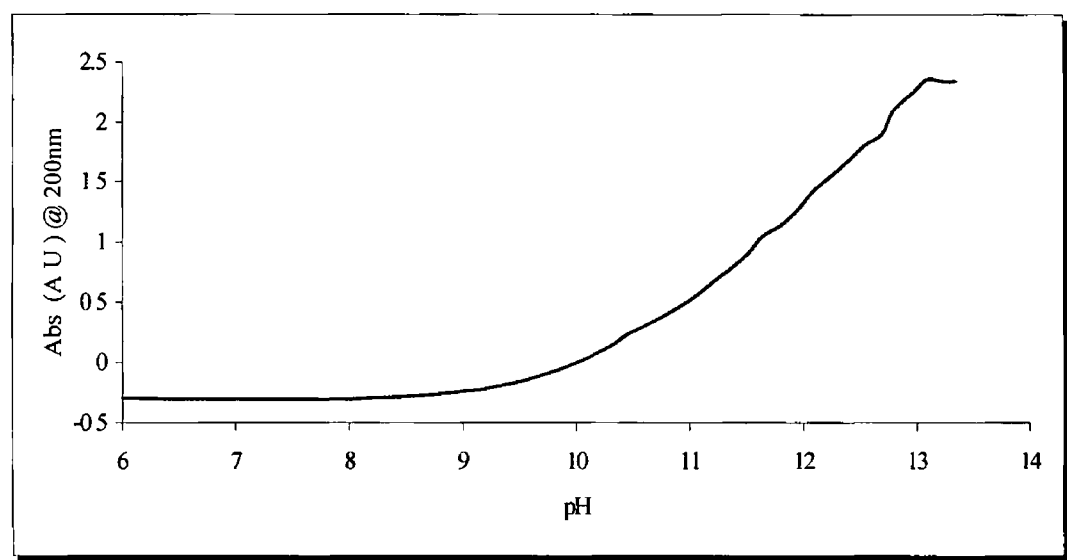


Fig 3 16 Plot of pH vs absorbance of piperidine in Britton-Robinson buffer

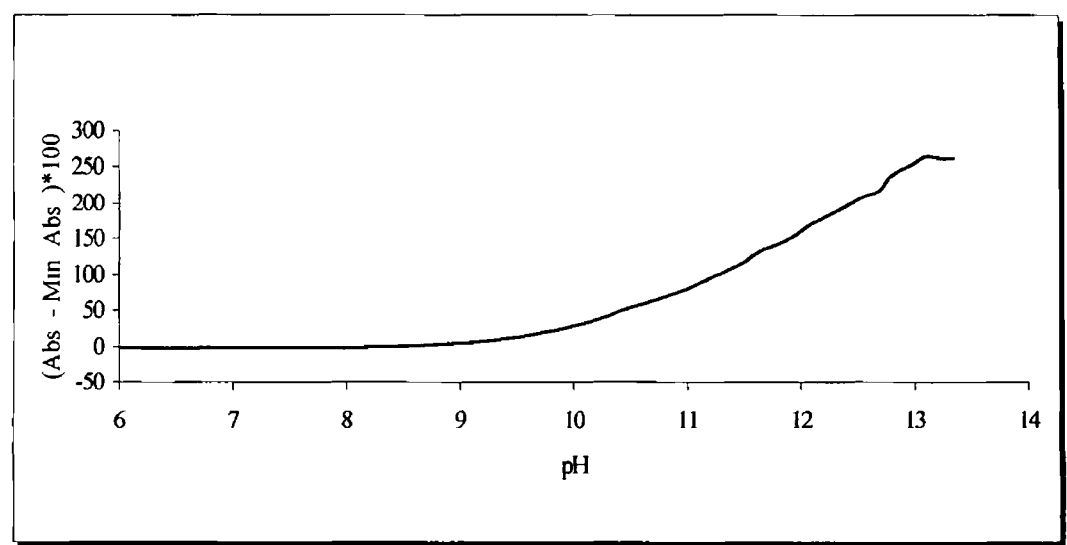


Fig 3 17 Plot of pH vs change in absorbance of piperidine in BR buffer

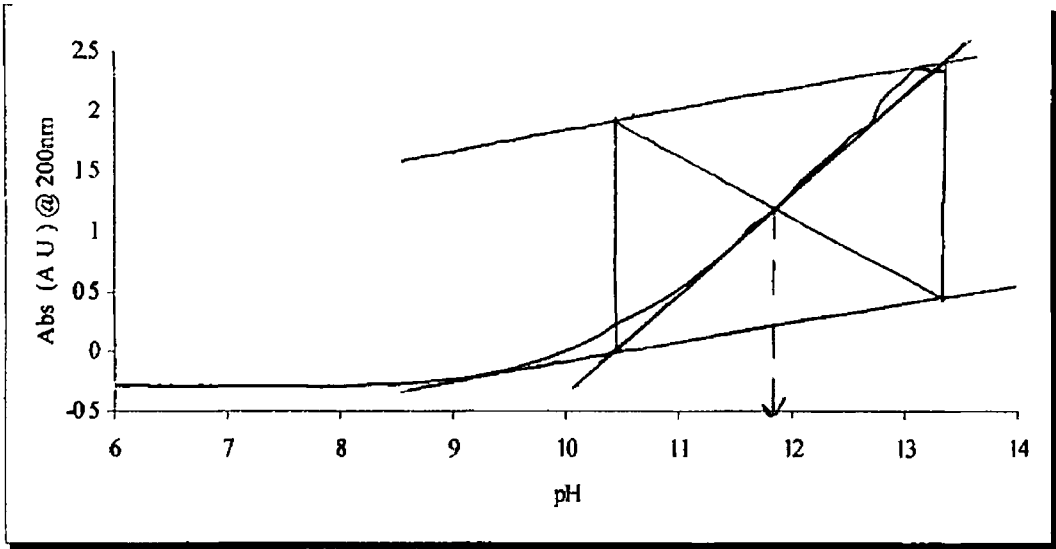


Fig 3 18 Plot of pH vs absorbance of piperidine in BR buffer, showing the method for determination of the dissociation constant of piperidine

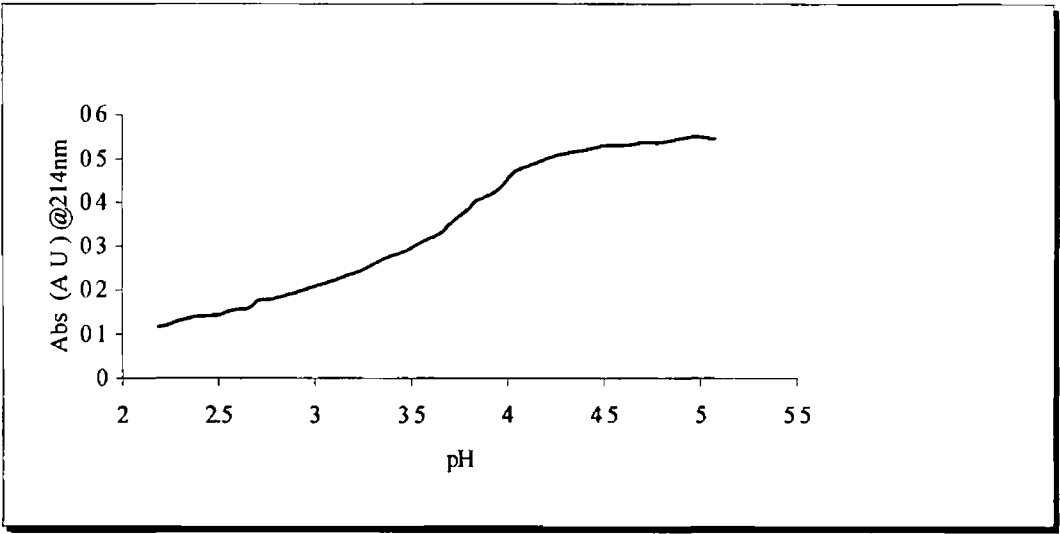


Fig 3 19 Plot of pH vs absorbance of dipiperidinomethane in BR buffer

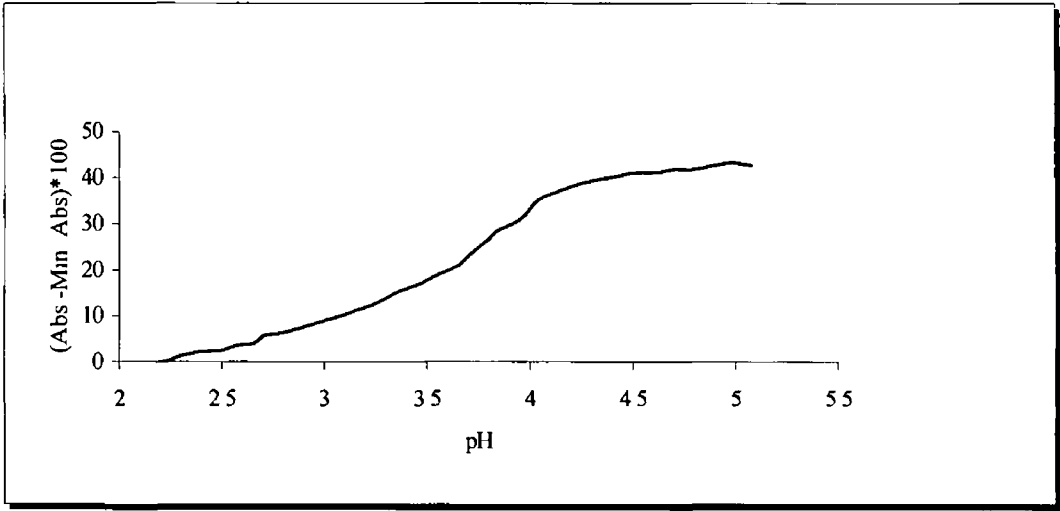


Fig 3 20 Plot of pH vs change in absorbance of dipiperidinomethane in BR buffer

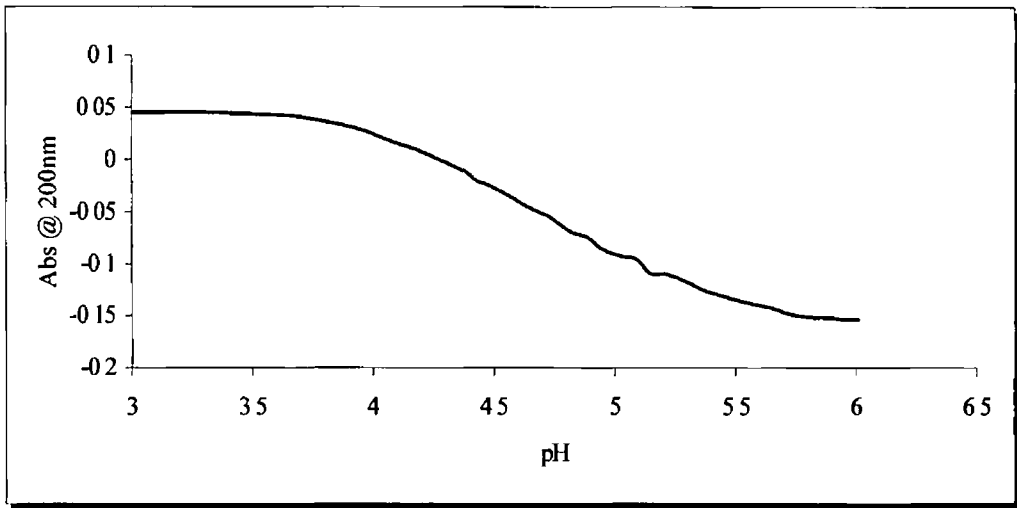


Fig 3 21 Plot of pH vs absorbance of ethylpiperidine carboxylate in BR buffer

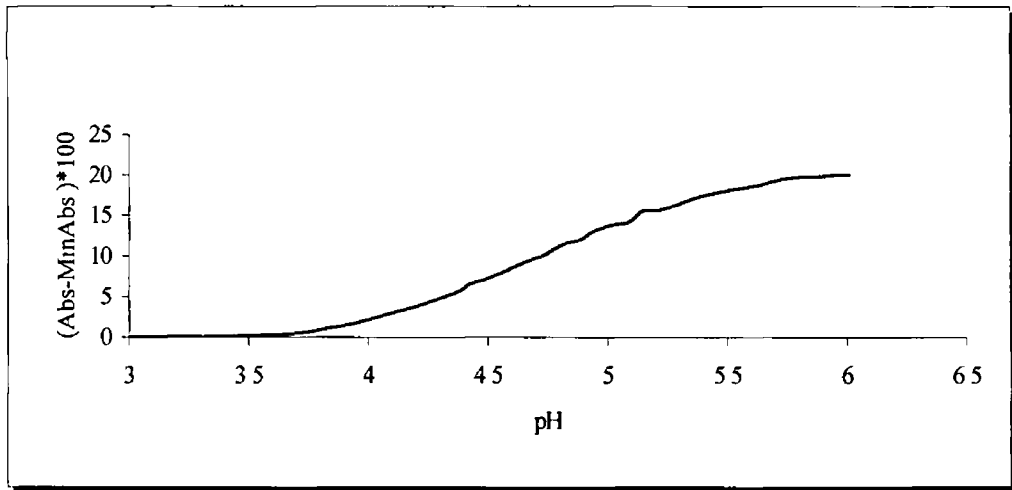


Fig 3 22 Plot of pH vs change in absorbance of ethylpiperidine carboxylate in BR buffer

In order to establish whether the pK_a values determined for EPC and DPM from the plots of pH versus absorbance and change in absorbance were accurate, graphs of the first derivative plots of the pH versus absorbance for each of the compounds were plotted (Figures 3 24 and 3 25) From Figures 3 24-3 25 it was determined that the pK_a of DPM was 3.5 and the pK_a of EPC was 4.6. These values compare well to those calculated from Figures 3 18 to 3 22.

From the determination of the dissociation constants of the three compounds, it was clear that the pK_a of piperidine was significantly different from the pK_a of EPC and DPM, indicating that piperidine was the most basic of the three compounds. This implied that the conditions required to analyse piperidine by CE may be different from those required by EPC and DPM because the pH at which piperidine is fully ionised is very different to the pH at which EPC and DPM are ionised. Separation in CE is based on the migration velocities of analytes under the influence of an electrical field. At the pH at which piperidine is fully ionised, DPM and EPC are only partially ionised and, therefore, the optimisation of their resolution would be difficult.

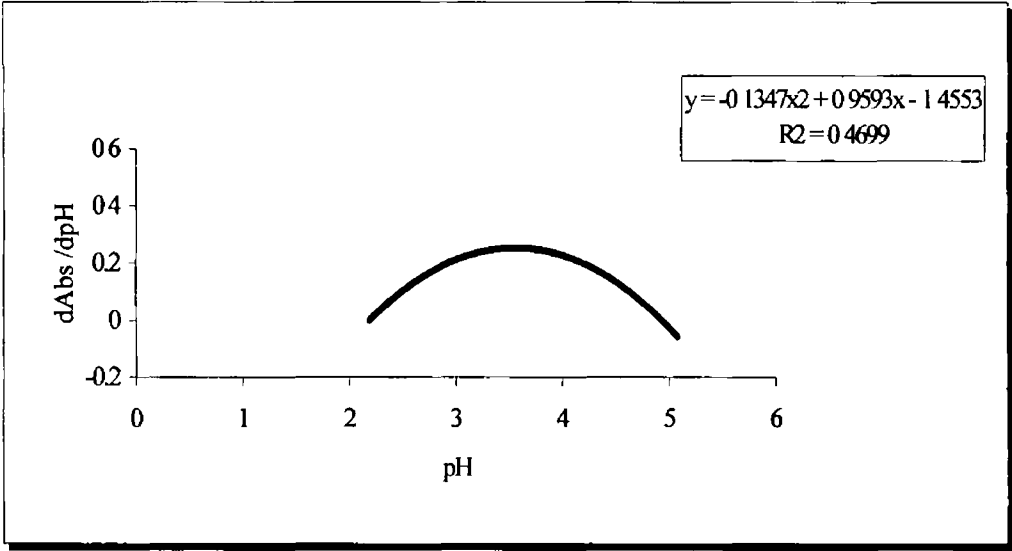


Fig 3 23 First derivative plot for dipiperidinomethane

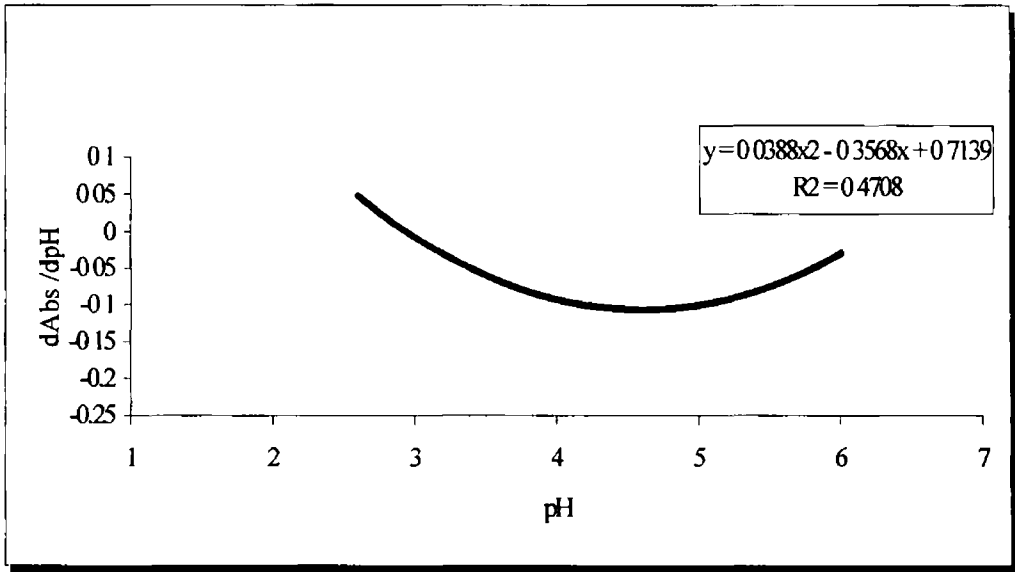


Fig 3 24 First derivative plot for ethylpiperidine carboxylate

3 7 3 Temperature and Voltage

Temperature control is important because both the analyte mobility and the magnitude of the EOF are temperature related. The capillary was thermostatted to 25°C for all separations performed. The operating voltage of a separation affects the migration time of the analytes; a high voltage ensures a fast separation. However, the resolution will be decreased. An Ohm's law plot of voltage vs. current was carried out for every new buffer used so that the optimum voltage for the separation could be determined. An example is given for a 50 mM phosphate solution of pH 3.0 (Fig. 3.25). Any voltage in the area where the graph is linear can be employed for the separation.

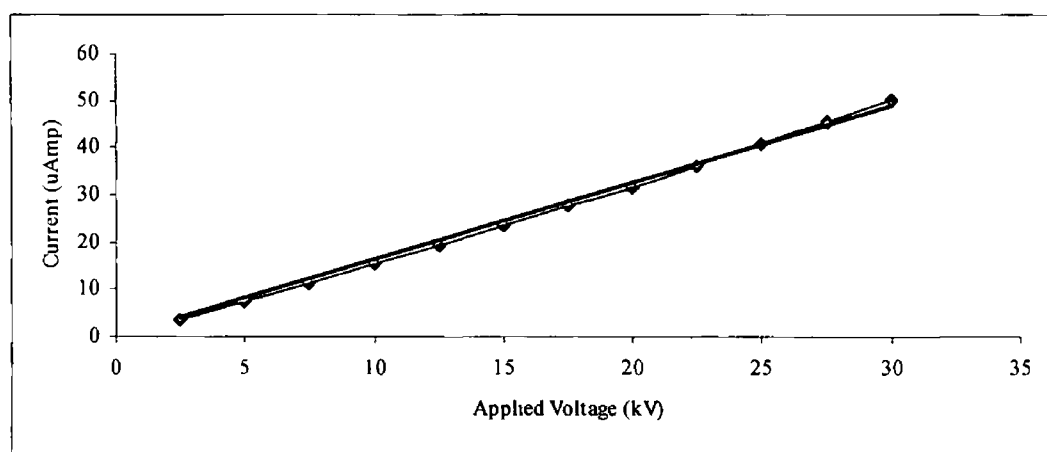


Fig. 3.25 Ohm's law plot for 50 mM phosphate buffer, pH 3.0

3.7.4 Buffer

The main purpose of the buffer is to provide precise pH control of the carrier electrolyte. This is particularly relevant to the separation of basic compounds because the buffer must be at an acidic pH in order to maximise the protonation of the analytes. As explained in section 3.3.1, separation is based on size/charge effects at this pH, rather than on the mobility of the analytes. In addition, the silica capillary is essentially neutral at low pH, so, the interactions between the analytes and the capillary are negligible. A buffer must be operated between ± 1 pH unit of its pK_a otherwise the buffer component becomes fully ionised and causes excessive conductivity. Also, the buffer may change pH, requiring frequent replacement. A number of buffers were suitable for separations at a low pH, including citrate (pK_a 3.12), formate (pK_a 3.75) and phosphate (pK_a 2.14). Phosphate was chosen for this method because it has a very low pK_a and it is commonly used in the separation of basic compounds (section 3.3.2).

An electropherogram of piperidine separated in borate buffer is shown in Fig. 3.26. The pK_a of borate is 9.236 [47], at which piperidine is ionised. However, in this buffer no peak is observed for either DPM or EPC because their dissociation constants are much lower than piperidine. It is also evident in Fig. 3.26 that the response of piperidine is quite low. This is because it is not at its wavelength of maximum absorbance, as seen in Fig. 3.14. This issue indicated that the separation of all three compounds by CE using UV detection may prove difficult.

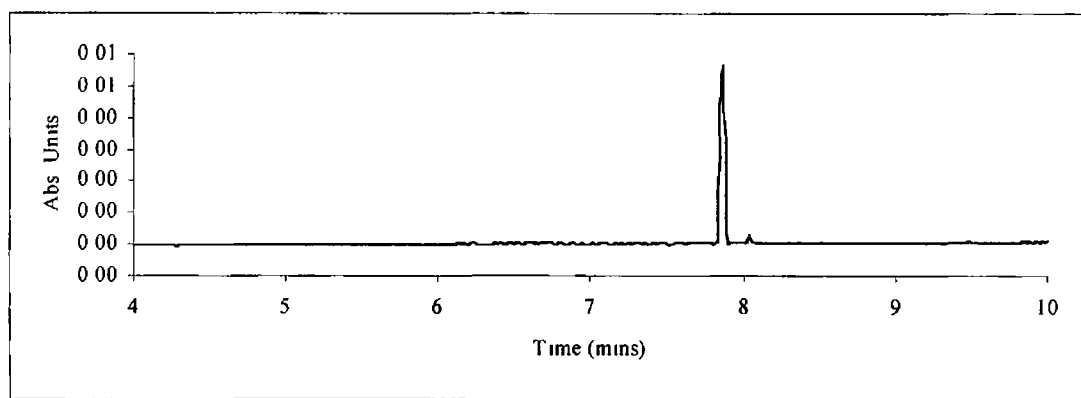


Fig 3 25 100 ppm Piperidine separated in borate buffer of pH 9.27. The borate buffer is at its pK_a thus providing optimum separation conditions. Buffer: 80 mM sodium tetraborate at pH of 9.27. Capillary: 50 μ m i.d., 50 cm separation length. App. potential: 15kV. Hydrodynamic injection for 10 seconds. Detection at 214 nm.

The concentration of the buffer is important in providing the ionic strength necessary for electrical continuity during the separation. The fastest separations are obtained with the most dilute buffers. The reason for this is that as the ionic strength of the buffer increases the zeta potential of the capillary wall and, as a result, the EOF, decreases in proportion to the square root of the buffer concentration [48].

The solubility of the analytes in a buffer is of paramount importance. This is of particular concern when using non-polar compounds. Piperidine was found to dissolve in water. However, dipiperidinomethane and ethylpiperidine carboxylate were only soluble in acetonitrile and, even then, only in small concentrations (< 100 ppm).

Also, DPM and EPC only dissolved in NaH_2PO_4 solutions when they were in concentrations below 50 ppm. On addition of acetonitrile to the phosphate solution, DPM and EPC were soluble. So, it was decided that the separation buffer would have to contain acetonitrile in order to aid dissolution of the analytes. Consequently, all standards were also prepared in the same buffer used for the separation.

3.7.5 Modifier

Organic solvents are used to increase solubilisation, reduce the EOF and alter selectivity. As discussed in section 3.3.5, acetonitrile was added to the phosphate buffer to solubilise DPM and EPC, however, acetonitrile has a very small effect on the EOF in comparison with other solvents, such as methanol. Other modifiers investigated were SDS and an ion-pairing reagent polyvinylsulphonic acid (sodium salt).

3 7 6 Development of Optimum Separation of the Basic Compounds

3 7 6 1 Introduction

A number of factors had to be taken into account when considering the separation of piperidine, EPC and DPM by CE using UV detection

1 The difference in the dissociation constants of piperidine, EPC and DPM

The pK_a of piperidine is 11.1, while the pK_a s of EPC and DPM are much lower (determined to be approximately 4.6 and 3.5 respectively). This meant that selection of a buffer which could be used to provide optimum separation all three compounds, particularly a good separation between DPM and EPC, would be difficult. In addition, basic compounds have a tendency to interact with the capillary wall. An answer to this problem is to use a buffer of low pH. At this pH the capillary is essentially neutral and so, cationic interactions between it and the basic compounds are reduced.

2 The wavelengths of maximum absorbance of the three compounds are quite different

Selection of a wavelength to use for the separation was limited by the fact that only four filters were available on the CE instrument, of which the 214nm filter was the one at which all three compounds would absorb. Even at this wavelength the absorbance of DPM was poor. An alternative was to consider derivatisation of the compounds to make them more absorbent in UV or fluorescent light. However, derivatisation is cumbersome and the goal was to achieve a fast, straightforward technique to analyse these compounds. So, it was decided to use UV detection and tackle the improvement of detector response, possibly through a stacking technique, after a separation was achieved.

3 The solubility of the three compounds differs enormously

Piperidine is soluble in water and, therefore, is soluble in most buffers. However, as explained in section 3.7.6, EPC and DPM were only soluble in acetonitrile. This also meant that these compounds were slightly hydrophobic in character, which may cause an interaction at low pH between them and the epoxide moiety of the fused silica capillary wall, leading to bandbroadening, tailing and irreproducibility of separation.

Bearing these factors in mind, the feasibility of a CE separation of the three compounds was addressed. In Fig. 3.25 it can be seen that piperidine was successfully analysed in borate buffer. However, the conditions were not suitable for the separation of DPM and EPC. Taking into account that, at a pH of 2.4, all three compounds should be ionised and the capillary wall will be neutral, a 50 mM phosphate solution of pH 2.4 with 20% ACN was prepared. A higher concentration of phosphate could not be used as DPM and EPC would not dissolve in higher concentrations of the buffer. Electropherograms of both DPM and EPC (Figs 3.26, 3.27) were obtained. In these figures, it can be seen that each of the compounds had very similar migration times, with DPM and EPC splitting into two peaks. It is possible that the peak splitting was due to weak interactions between the compounds and the capillary wall. However, at 2.4 the silica capillary wall should be almost neutral, so there should be virtually no cationic interactions between it and the compounds. No peak was observed for piperidine in 50mM phosphate buffer at pH 2.4, possibly due to the very low EOF, which exists at this pH. However, in 100mM phosphate buffer at a pH of 2.4, a peak for piperidine was obtained (Fig. 3.28) this was probably due to the increase in the ionic strength of the buffer.

It should be noted that only one peak was observed for piperidine, indicating that the peak splitting of EPC and DPM at pH 2.4 may have been caused by hydrophobic, rather than cationic, interactions with the capillary wall.

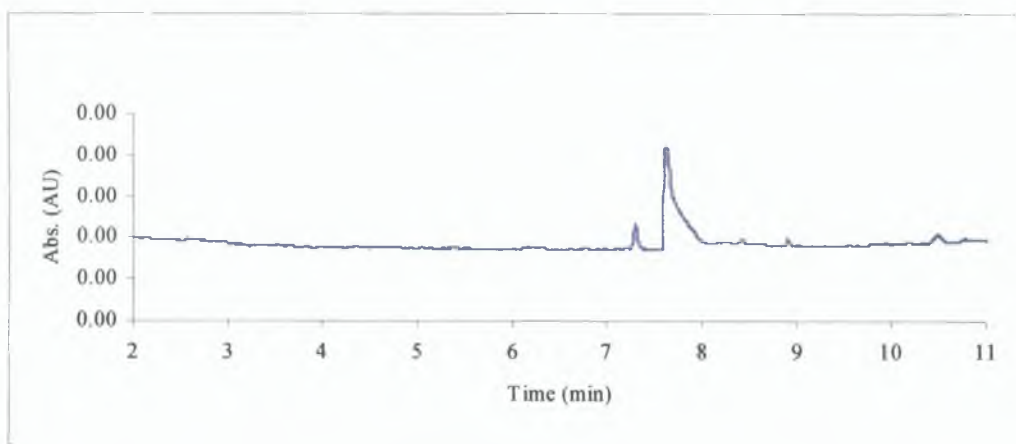


Fig. 3.26. 50 ppm DPM in 50 mM phosphate buffer at pH 2.4 with 20% ACN. Capillary: 50 μ m i.d. of separation length 50 cm. Applied potential: 15kV. Injection in buffer for 10 secs. Temperature: 25°C.

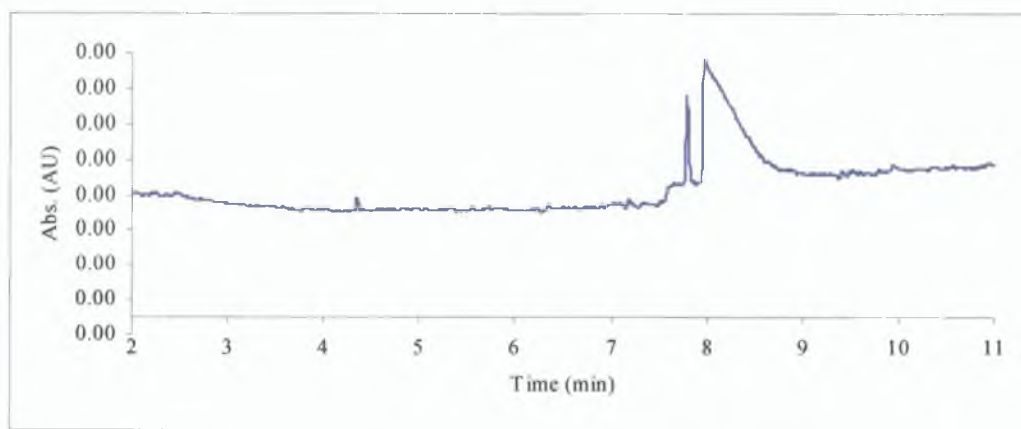


Fig. 3.27. 100 ppm EPC in 50 mM phosphate buffer, pH 2.4 with 20% ACN. Capillary: 50 μ m i.d. of separation length 50 cm. Applied potential: 15kV. Injection in buffer for 10 secs. Temperature: 25°C.

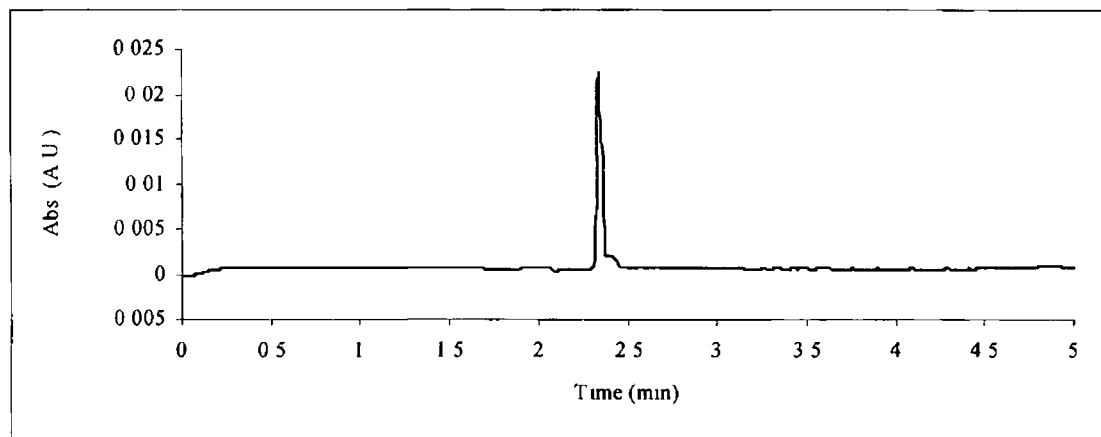


Fig 3 28 100 ppm piperidine in 100 mM phosphate buffer, pH 2.4 with 20% ACN. Capillary 50 μ m i.d. of separation length 50 cm. Applied potential 15kV. Injection in buffer for 10 secs. Temperature 25°C.

In Fig 3 29 the electropherograms of DPM and EPC at pH 5.0 are presented. Phosphate buffer of a higher pH was prepared to determine whether the peak splitting, which was observed in Figures 3 26 and 3 27, was due to a cationic interaction between the analytes and the capillary wall. At pH 5.0 the silica capillary wall is deprotonated, so some interaction between it and the basic compounds would be expected. Two peaks were observed in the electropherograms of each compound, indicating that some splitting due to the compounds interacting with the capillary wall occurred, even though the high ionic strength of the buffer should have prevented some of the interaction between the ionised silanol groups and the charged analyte ions.

The pKa of the silanol groups is approximately 4.0. However, it has been demonstrated that a slight EOF can still exist at pH 2.0 [49], so it is possible that the peak splitting observed at pH 2.4 (Figures 3.26 and 3.27) was due to a small amount of interaction between the analytes and the capillary. It is also possible that hydrophobic interactions at pH 2.4 between the capillary wall and EPC and DPM may have caused two peaks. However, the fact that the electropherogram of piperidine did not display two peaks at pH 2.4 indicated that, if the peak splitting was caused by an interaction between EPC and DPM and the capillary wall, it was due to the analytes hydrophobic nature.

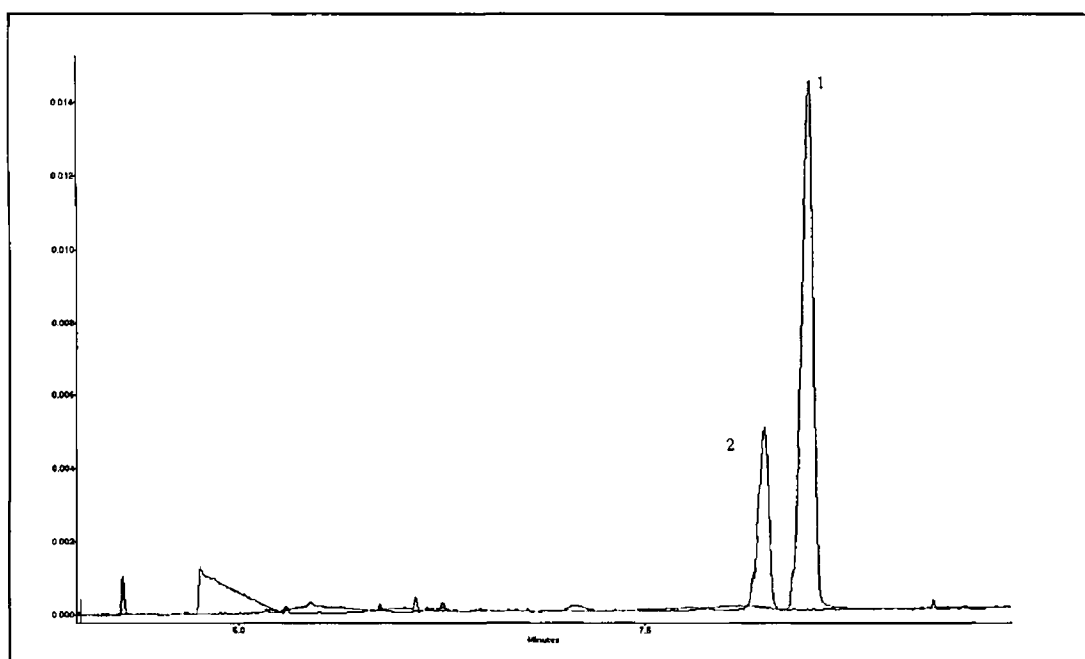


Fig. 3.29 1. dipiperidinomethane, 2. ethylpiperidine carboxylate, analysed in 50 mM phosphate buffer, pH 5.0 with 20% ACN. Capillary: 50 μ m i.d., 50 cm separation length. Applied voltage: +15 kV. Hydrodynamic injection in buffer for 10 secs. Temp: 25°C.

Fig. 3.30 shows the analysis of piperidine in 100 mM phosphate buffer of pH 5.0 with 20% ACN. Only one peak is observed for piperidine. However, its response is significantly lower than that of DPM and EPC (Fig. 3.29) and it eluted at the same time as EPC. In addition, the elution time of piperidine was greatly increased from Fig. 3.28, probably due to the change in pH and concentration of the buffer. A better response was obtained for EPC and DPM in the pH 5.0 buffer. Therefore, the peak splitting in figures 3.26 and 3.27 cannot be due to ionic interactions between the capillary wall and the analyte ions. This is because the silanol groups are significantly ionised at pH 5.0 and, consequently, an interaction between the capillary wall and the analytes would be more pronounced at pH 5.0.

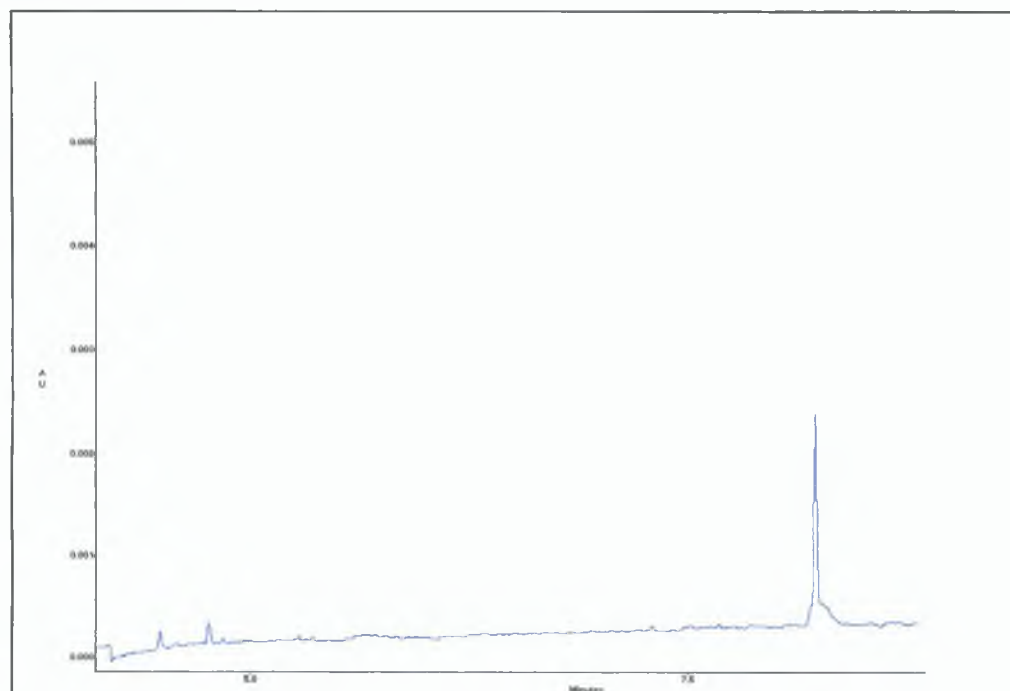


Fig. 3.30. 100ppm Piperidine analysed in 50 mM phosphate buffer, pH 5.0 with 20% ACN. Capillary: 50 μ m i.d., 50 cm separation length. Applied voltage: +15kV. Hydrodynamic injection in buffer for 10 secs. Temp.: 25°C.

Observing the electropherograms in figures 3 29 and 3 30, it can be seen that all three analytes can be analysed in the 50mM phosphate buffer with 20% ACN. However, piperidine and EPC elute at the same time. Manipulation of the resolution between EPC, APM and piperidine by altering the organic modifier concentration was not possible, because at concentrations of ACN less than 20% the EPC could not dissolve in the buffer. EPC was soluble in higher concentrations of phosphate buffer. However, this caused Joule heating in the capillary leading to poor quality peaks and very poor resolution on the electropherogram. Therefore, it was decided to investigate the use of other additives to effect the separation of the three compounds. Focussing, initially, on achieving a separation between DPM and EPC, an investigation into the use of additives to prevent interactions with the capillary wall was carried out. It was hoped that this would improve their resolution and prevent peak splitting. A successful separation of DPM and EPC could then be applied to the analysis of piperidine as well.

3 7 6 2 Investigation into the Separation of EPC and DPM using Buffer Additives

A previous study [51] on the separation of protonated amino acids used ethane sulphonic acid (ESA) to improve the separation between analytes by increasing selectivity and resolution of the peaks. It was indicated in the report that the ESA coated the capillary, preventing any analyte-capillary interaction. It was also thought that the ESA modified the migration of the cations by weak ion-pair formation. By formation of these weak ion-pairs the PVSA may have increased the migration times of the basic compounds.

The use of polymers to coat capillary walls has been well documented in the research in recent years (section 3.3.2.5). However, polymers can also be added to the separation buffer to improve separation. Schutzner [52] et al. added polyvinylpyrrolidone to a separation buffer to improve the separation of diastereomers of warfarin and phenprocoumon, while Breadmore and his colleagues [53] used a cationic polymer solution in the separation of anions. However, reports on the use of soluble polymers for the separation of basic compounds are very scarce.

Fig. 3.31 is an electropherogram of the EPC when polyvinylsulphonic acid (sodium salt) (PVSA) was added to the separation buffer. One peak, with a very poor detector response, is present, indicating that EPC was not splitting due to interaction with the capillary wall. The PVSA appeared to prevent ethyl piperidine carboxylate from interacting with the capillary. The splitting of the dipiperidinomethane peak seemed to have been reduced by the presence of the PVSA (Fig. 3.32). However, the detector response is very poor, which was caused by the presence of the PVSA. In addition, the migration times of both EPC and DPM have greatly increased.

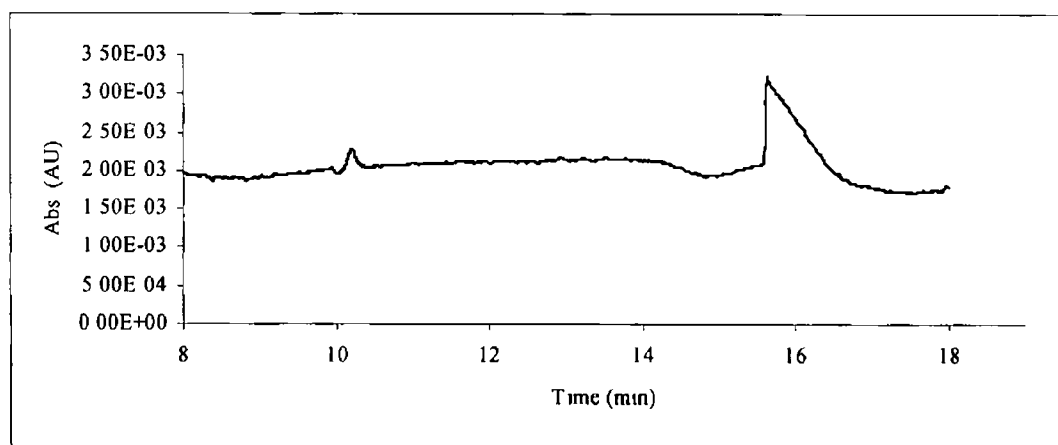


Fig 3 31 50 ppm EPC in a phosphate buffer, pH 2.4 with 20% ACN and 5% v/v polyvinylsulphonic acid (sodium salt) Capillary 50 μ m i.d., 50 cm separation length Applied voltage +20kV Hydrodynamic injection in buffer for 10 secs Temp 25°C

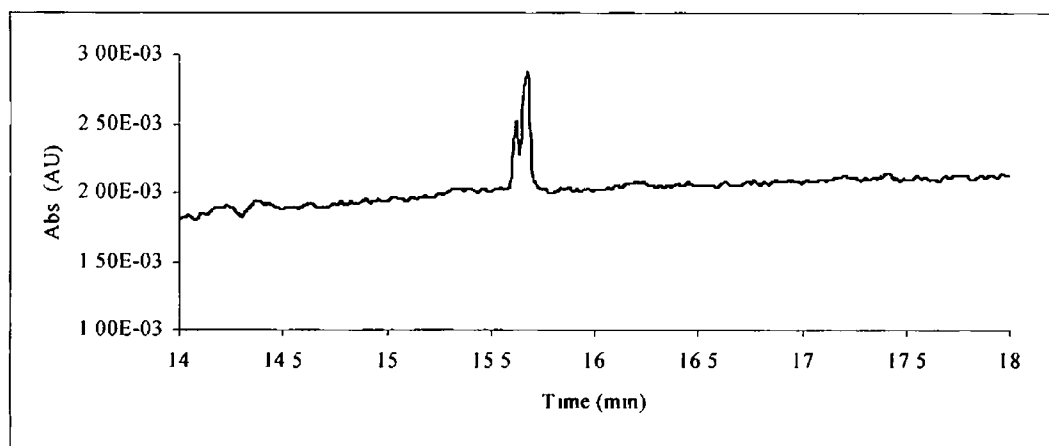


Fig 3 32 50 ppm DPM in a phosphate buffer, pH 2.4 with 20% ACN and 5% v/v polyvinylsulphonic acid (sodium salt) Capillary 50 μ m i.d., 50 cm separation length Applied voltage +20 kV Hydrodynamic injection in buffer for 10 secs Temp 25°C

At other concentrations of PVSA, no peaks were observed at all for either EPC or DPM. Above the concentration of 5% PVSA the analysis of the analytes was not improved. So it was decided to investigate the use of micellar electrokinetic chromatography (MEKC) for the analysis of the basic compounds. If an anionic surfactant is employed at a low pH, the micelles can form ion-pairs with the cationic analytes and separation is based on the affinity of the analyte for the micelle and its mobility in the EOF. In section 3.3.2.3, the mechanism of the overall movement of the anionic surfactant towards the cathode is outlined. However, at low pH there is a negligible EOF so the anionic surfactant will be attracted to the anode. Consequently, the polarity of the instrument was reversed before any MEKC separations were carried out. Initial studies on the surfactant, sodium dodecyl sulphate (SDS), were performed.

The effect of the addition of ACN to buffers containing SDS was investigated. Acetonitrile was added to all buffers because the analytes were non-polar and, therefore, might have been incorporated into the micelles. Addition of an organic modifier to the buffer system would increase the affinity of the analytes for the mobile phase.

Fig. 3.33 shows the electropherogram of DPM analysed in phosphate buffer at a pH of 2.4 with 35mM SDS. There are a number of peaks before the main peak indicating that the addition of SDS to DPM did not improve the quality of the separation. It is possible that the peaks before the main peak were caused by air bubbles in the capillary. It was also noted that DPM and EPC were soluble in the phosphate buffer at a concentration of 100mM upon addition of SDS. The separation of DPM at concentrations of 25mM and 35mM SDS was also investigated. However, the DPM peak was very poorly resolved in both buffers.

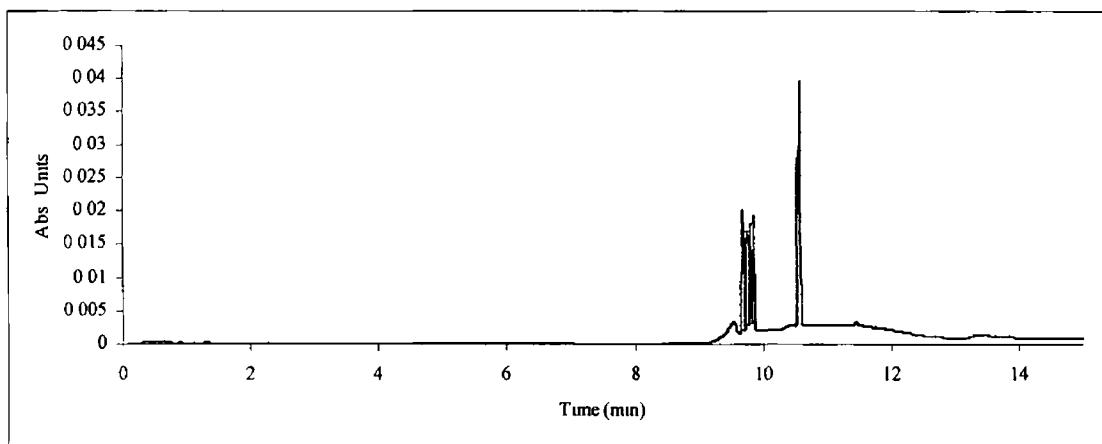


Fig 3 33 100 ppm DPM in 100 mM phosphate buffer at a pH of 2.4 with 7% ACN and 45 mM SDS. Capillary: 50 μ m i.d., 50 cm separation length, Applied Voltage: -10 kV, hydrodynamic injection in buffer for 10 secs, Temp: 25°

In Fig 3 34, the electropherogram of EPC analysed in 100mM phosphate buffer at a pH of 2.4 with 45mM SDS can be seen. The peak shape is poor with a weak detector response in comparison to DPM in the same buffer.

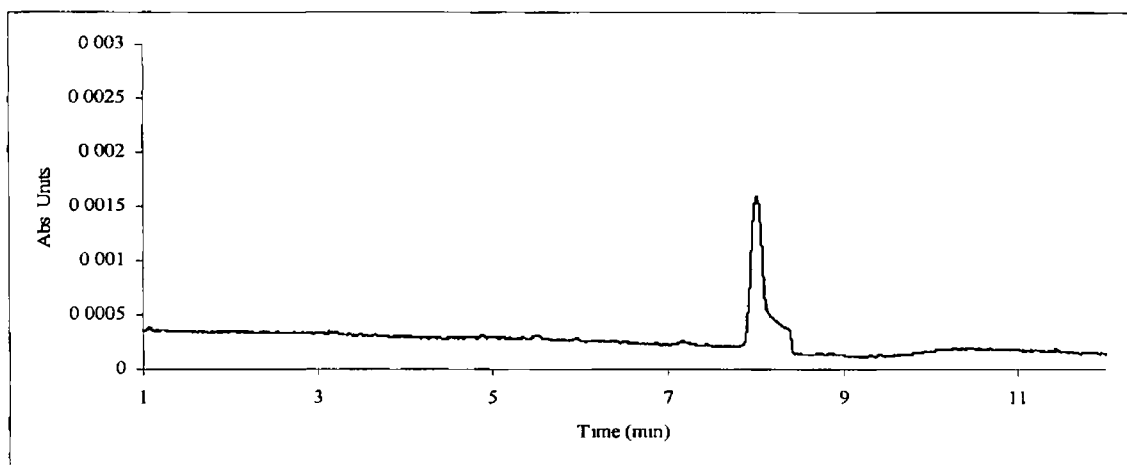


Fig 3 34 50 ppm EPC in 100 mM phosphate buffer at a pH of 2.4 with 7% ACN and 45 mM SDS. Capillary 50 μ m i.d., 50 cm separation length, Applied Voltage -10 kV, hydrodynamic injection in buffer for 10 secs, Temp 25°

The analysis of DPM and EPC in this buffer was difficult because the presence of SDS in the buffer caused the inside of the vial caps to become wet. This, in turn, caused an abrupt increase in the separation current, which caused the instrument to abort the separation. Care had to be taken to ensure that the vial caps were dried before each analysis. Fig 3 35 shows a separation of EPC and DPM in the phosphate buffer with 45mM SDS. In addition, the separation could not be achieved in lower concentrations of SDS.

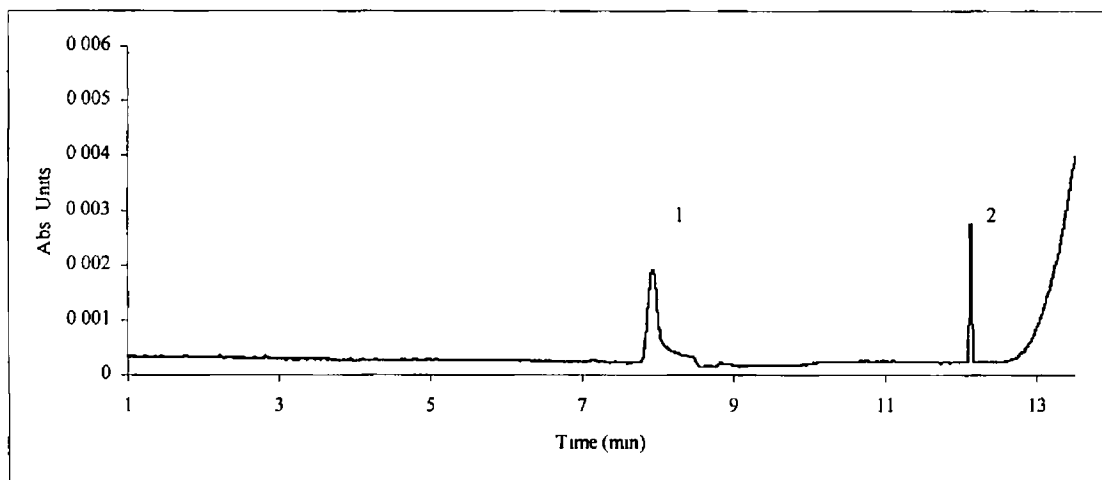


Fig 3 35 50 ppm EPC (1) and 50 ppm DPM (2) separated in 100 mM phosphate buffer at a pH of 2.4 with 7% ACN and 45 mM SDS. Capillary 50 μ m i.d., 50 cm separation length, Applied Voltage -10 kV, hydrodynamic injection in buffer for 10 secs, Temp 25°

From the analysis of EPC and DPM in 50mM phosphate buffer and 7% ACN which is shown in Fig 3 29, it can be seen that the detector response of the analytes is superior to that in Fig 3 35. In addition, in Fig 3 29 it can be seen that both analytes eluted in under 9 minutes.

Piperidine was analysed in a 100mM phosphate buffer at pH 2.4 containing 35mM SDS and a peak eluting at 9 minutes (Fig 3 36 (1)) was present on the electropherogram (Fig 3 36). However, at a concentration of 45mM SDS, no peak was obtained for piperidine. Consequently, no separation of the three compounds could be achieved.

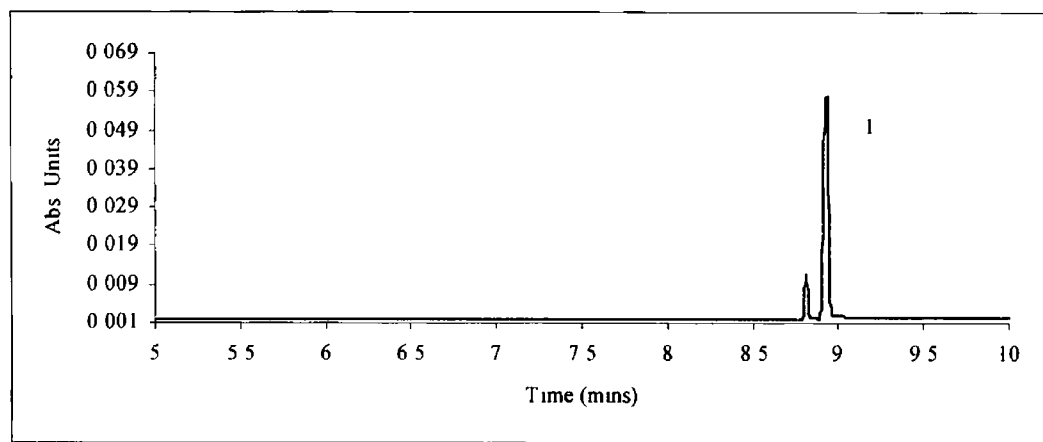


Fig 3 36 100 ppm piperidine in 100 mM phosphate buffer at a pH of 2.4 with 7% ACN and 35 mM SDS. Capillary: 50 μ m i.d., 50 cm separation length, Applied Voltage: -10 kV, hydrodynamic injection in buffer for 10 secs, Temp: 25°

3.8 Discussion

The development of a capillary electrophoretic separation method for the analysis of the three basic compounds in cyanoacrylate adhesives was unsuccessful. However, a separation of DPM and EPC was obtained and the analysis of piperidine was also achieved.

On initial examination of the three compounds, a separation was thought to be an unrealistic goal due to the differences in their solubility, detector response and dissociation constants. The separation of piperidine was obtained by using a phosphate buffer containing no additives. The analysis of EPC and DPM could not be achieved in phosphate buffer alone. The addition of ACN to buffers for DMP and EPC was necessary because the analytes were not soluble in less than a 20% concentration of ACN. Analysis of separate mixtures of the three compounds in phosphate buffer at pH 5.0 with 20 % ACN was achieved. However, a separation of a solution of the three compounds in the same pH 5.0 buffer was not obtained. It was decided to investigate the use of additives that could be added to the solution to resolve the analytes. Firstly, the separation of DPM and EPC was investigated because they had similar characteristics.

Initially, the use of a phosphate buffer at low pH containing an organic modifier to solubilise the analytes was investigated. An analysis of both EPC and DPM was obtained. However, an extra peak was observed in the electropherograms of both compounds, which may have been caused by an interaction between the analytes and the capillary wall, so it was decided to explore other additives to improve the resolution of the basic compounds.

REFERENCE

The use of a soluble polymer gave poor results. Only one peak was observed for each analyte. However, the migration times of the analytes were extremely long and the response of the compounds was extremely poor. This is probably due to the fact that the EOF was greatly reduced at the low pH and, also, because the capillary was coated by the PVSA, resulting in a further decrease in the magnitude of the EOF. It also seems that the EOF was unstable at low pHs when the PVSA was added because there were significant variations in the migration times of the analytes from run to run.

A 100 mM phosphate buffer of pH 2.4 with 45 mM SDS and 7% ACN gave the best result for the MEKC analysis of DPM and EPC. At higher concentrations of ACN, a number of peaks were observed. This could be caused by a breakdown in the micellar structure of the SDS by the solvent. At higher concentrations of the surfactant, no peaks were observed for either EPC or DPM. They slightly hydrophobic analytes may have become solubilised in the micelles, which may explain why no peak was observed at higher concentrations of SDS. A peak for piperidine was obtained with phosphate buffer at low pH with 25mM SDS. However, the analysis was not as good as that in the absence of SDS, as evidenced on the electropherogram by a peak present near the main peak.

In all buffers the response of the analytes was very poor, which probably reduced the quality of the separations even further. Because the limit of detection was poor, higher concentrations of the analyte had to be analysed. In particular, when attempting to separate EPC and DPM, the use of higher concentration of analytes presented the problem of their solubility in the buffers. This limited the range of buffers available for investigation. One way to overcome this may be stacking [9], a technique which has the effect of amplifying the response of the analytes at the detector.

Another answer to the problem of low limit of detection would be to derivatise the analytes with a fluorescent tag and employ a fluorescence detector. However, this technique is time-consuming and cumbersome.

In conclusion, CE with UV detection can be used to detect piperidine. It can also be used to obtain a separation of EPC and DPM. However, separation of the three compounds proved difficult due to a combination of factors including differences in their solubility, detector response and dissociation constants.

3.9 Conclusion

CE has many advantages over other analytical techniques. It is a fast, reproducible method that produces highly efficient separations with a low use of consumables. However, for the analysis of some systems, the method development can be complex, requiring a variety of parameters to be optimised.

A separation of EPC and DPM was achieved and a solution of piperidine was analysed using the same buffer. However, resolution between the three compounds was difficult to achieve due to the fact that the organic modifier concentration could not be changed. The use of MEKC allowed for the use of less organic modifier but the concentration of SDS required to separate the three compounds was different.

However, much work is being carried out into the separation of basic compounds with coated capillaries and soluble polymers. From the results obtained with polyvinylsulphonic acid (sodium salt), separation of these compounds may be possible by employing coated capillaries or soluble polymers.

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Chapter 4

Conclusions

4. Conclusion

This thesis comprises three chapters concerned with the separation of components found in anaerobic and cyanoacrylate adhesives. It was the aim of the study to garner greater insight into the chemistry of these adhesives through the use of novel separation techniques. The first chapter is a general introduction to the chemistry of anaerobic and cyanoacrylate adhesives and includes a literature survey on capillary electrophoresis.

4.1 Products of Decomposition of Reducing Agents in Anaerobic Adhesives

Interest in the cure chemistry of the anaerobic adhesives has been of considerable interest lately. A review of the literature has shown the large volume of work dedicated to this area of adhesives research. The role of the reducing agents in the cure chemistry of anaerobic is complex. Primarily, the reducing agents reduce the transition metals from their higher oxidation state to their lower state, allowing for initiation of the curing process.

However, reducing agents have been shown to react with cumene hydroperoxide and other accelerators present in the cure system, e.g. saccharin and maleic acid, to form products which may, or may not, impede the curing performance of the adhesive over time. There are a number of products formed by the decomposition of tetrahydroquinoline (THQ) and dihydropyridine (DHP). They were too numerous and present only in small quantities, which made identification by conventional methods difficult. The coupling of liquid chromatography with mass spectrometry is ideally suited to this type of analysis.

A number of obstacles were overcome in order to identify the products with LC/MS. A mobile phase was developed, which allowed for the separation of the analytes and was compatible with LC/MS. The problem of salt formation was conquered by carrying out the reactions over iron filings or copper powder. A number of initial studies were carried out with the LC/MS which identified that the reducing agents could be detected using the mass spectrometer in electrospray ionisation mode. However, there were not enough samples analysed with the LC/MS, which would have allowed for identification of all products formed in the cure systems. Further study would involve carrying out more analysis with the LC/MS on samples containing differing amounts of the metals, the reducing agents and the other cure components. Separation of the solutions with TLC may allow for the retrieval of products, which could be analysed by NMR or IR to give structural information about the products.

4.2 Separation of Basic Components in Cyanoacrylate Adhesives

The separation of the acidic components in cyanoacrylate adhesives with capillary electrophoresis has already been achieved. It was hoped that the separation of basic components would also be possible by CE, potentially increasing the number of applications of this technique to adhesives and providing a simpler and more efficient method of identification and quantification than presently used methods.

The survey of literature presented in chapter one shows the great number of CE separations of basic compounds that have been achieved since the introduction of the technique approximately 20 years ago.

While presenting more of a challenge than anions, the separation of basic compounds using CE is theoretically simple, particularly when the novel techniques developed recently, such as non-aqueous CE (NACE) and polymer – coated capillaries, are exploited. Micellar electrokinetic chromatography (MEKC) and soluble polymers were both investigated as possible separation techniques for the basic components of cyanoacrylate adhesives. However, no successful separation of the three components was achieved. This was probable due to the attraction of the analytes for the capillary wall. Also, they are quite non-polar so the choice of electrolyte was limited to those in which the analytes were soluble.

It is possible that the analytes could be separated with another technique, e.g. ion-chromatography or gas chromatography. A separation with CE could be possible with a coated capillary and the limit of detection could be improved by exploiting stacking techniques.