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Analysis of photographic developer solutions

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ANALYSIS OF PHOTOGRAPHIC DEVELOPER SOLUTIONS

By

Timothy Paul Desmond

A Doctoral Thesis

submitted in partial fulfilment of the requirements

for the award of Doctor of Philosophy of

Loughborough University of Technology.

November 1994

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Department of Chemistry

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Abstract

Analysis of photographic developer solutions

Timothy Paul Desmond

November 1994

The project has investigated the degradation products of colour developing agents CD-3 and CD-4 that are likely to be present as significant constituents in used colour developer solutions. Some of the major components have been identified and confirmed earlier proposals made on model systems of solutions of developing agents. Liquid chromatographic and capillary electrophoretic methods have been established for the determination of the individual components of the developer solutions and both Kodak and competitor products have been examined.

The liquid chromatography methods developed for RA4/RA100 colour developer solutions, using ion-pair reagent and diode array detection, enabled the separation of CD-3 developing agent, Phorwite stain reducing agent and the developing agent degradation products. A liquid chromatography method was also developed for the analysis of CD-4, CD-4 sulphonates and CD-4 non-polar degradation products in C-41 colour developer solutions. A capillary electrophoretic method was developed for the analysis of CD-4 developing agent and CD-4 sulphonates. However this method did not allow for the separation of non-polar degradation products. Electrophoretic methods, using high pH buffers and micellar forming buffers, were investigated for the analysis of RA4 colour developers solutions. The liquid chromatographic methods and electrophoretic methods were complementary to each other. Liquid chromatography was found to be the best method for the separation of RA4 developer solutions and capillary electrophoresis was found to be the best method for the separation of C-41 developer solutions. The methods developed could be used to monitor the degradation of photographic developer solutions during use and before disposal.

Over a period of time much of the colour developing agent in RA4 and RA100 developer solutions was converted into a highly coloured tar which precipitated from solution. Most of the tar was soluble in organic solvents and on chromatography gave a complex mixture of components which appear to be primarily dimerisation and polymerisation products of colour developing agents. A number of significant non-polar degradation products were isolated and identified from solutions containing CD-3 developing agent alone and from the corresponding developer solution, RA4. These included a yellow azo dimer, a blue quinoneenamine and a purple compound, postulated to be a phenazine.

Similar yellow azo and blue quinoneenamine compounds were isolated from degraded solutions containing CD-4 but no corresponding purple compound was found. As C-41 developer solutions contain a high concentration of sulphite, the main degradation products are the CD-4 sulphonates.

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Chapter 1

Introduction

1.1 Introduction to colour photography

From the first Stone Age hunters who painted images on the walls of their caves to the first moon voyagers who turned their cameras back upon a swirling blue planet, mankind has sought to capture the colours of the world. Light was first shown to be the source of colour when Isaac Newton passed a beam of sunlight through a glass prism and produced the rainbow array of hues of the visible spectrum. The first practical colour photography was invented by J.C. Maxwell in 1861 when he attenuated light by means of black and white positives made from negatives which had been exposed through filters. The same filters were used for projection which produced a superimposed representation on the screen, giving rise to a colour image [1].

However, it was not until 1896, when it was realised that the brown image produced by pyrogallol developers was composed of silver and formed by the oxidation products of the developer, that the basis of modern colour photography was born. Routine, facile development of colour film was not possible until 1935 when Mannes and Godowsky, working at Eastman Kodak, Rochester, produced Kodachrome, a colour film for home movies [2]. This gave rise to numerous different types of colour development, one of the most important being the colour negative method of development, which is now sold under various names such as Kodacolor.

1.2 Aims of project

The present study undertook to look at the degradation of colour developer solutions. Although colour developing agents have been used for many years, few of the products of their degradation have been identified, although some degradation routes have been proposed. There is concern that it is necessary to gain a greater understanding of these compounds to aid in strategies for their monitoring and disposal.

1.3 The colour process explained

The colour negative process involves a two stage process in which a silver emulsion is exposed in a camera and a latent image is formed which has to be revealed by development [3]. This is carried out in a solution containing one or more reducing agents (developing agents) which convert silver halides to metallic silver. The latent image consists of silver halides that have received enough exposure to light for silver atoms to be released which collect at specks of silver sulphide on the crystals and at mechanical defects in the crystals. These silver atoms act as development centres at which the reducing process begins. Exposed crystals must be reduced more rapidly than unexposed crystals so an essential property of a photographic developing agent is the difference in the rate at which it reduces exposed and unexposed silver halides.

In colour or 'chromogenic' development a special developing agent is used, typically a p-phenylenediamine derivative, the oxidation products of which combine with colour couplers to produce coloured dyes. The amount of dye produced is directly proportional to the amount of silver deposited. Different colour couplers produce different coloured dyes with the same oxidation products of the developing agent. In colour photography the dyes are yellow, magenta and cyan and in most colour films the dyes are incorporated into the emulsion layers of the photographic film, such films are called 'substantive'. However there are some 'non-substantive' films such as Kodachrome where the couplers are in the colour developer solution. A separate colour developer containing the appropriate coupler is then needed for each emulsion layer of the photographic film [3].

When the couplers are incorporated into the photographic film emulsions, means have to be found to prevent them from migrating into other emulsion layers and into the developer solutions. This can be achieved by two different means, in both cases the couplers are located in gelatine layers.

(i) The couplers can be made of large molecular size compounds which although soluble, are prevented from moving around in the small spaces of the sponge-like network of the gelatine by their size. The sponge-like nature of the gelatine makes it possible for the smaller developing agent to diffuse into each layer to develop the latent image and for the

oxidation products to couple with the dyes and produce the desired image [4].

(ii) The couplers can be dispersed in the emulsion in tiny oil globules which do not move around in the gelatine. These couplers are insoluble in water and cannot diffuse out of the globules. Following reaction of the developing agent with the silver the oxidation products diffuse a short distance through the gelatine until it encounters a coupler droplet and reacts to form an image dye. The resulting aggregate of coupler droplets form a dye cloud around the silver grains that have been developed, eventually resulting in the desired image reproduction [5].

All colour negative films incorporate couplers in their emulsions. The blue-sensitive top layer incorporates a yellow dye producer, the green sensitive middle layer a magenta dye producer and the red sensitive bottom layer a cyan dye producer.

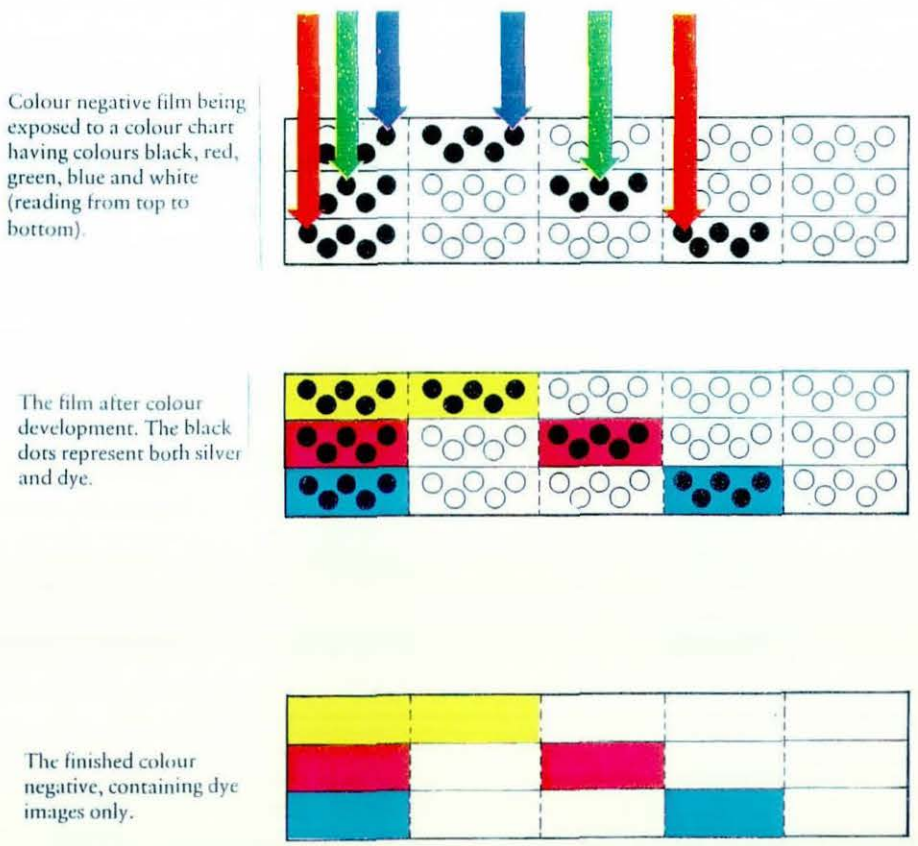


Fig. 1.1 Principles of colour negative development process [3].

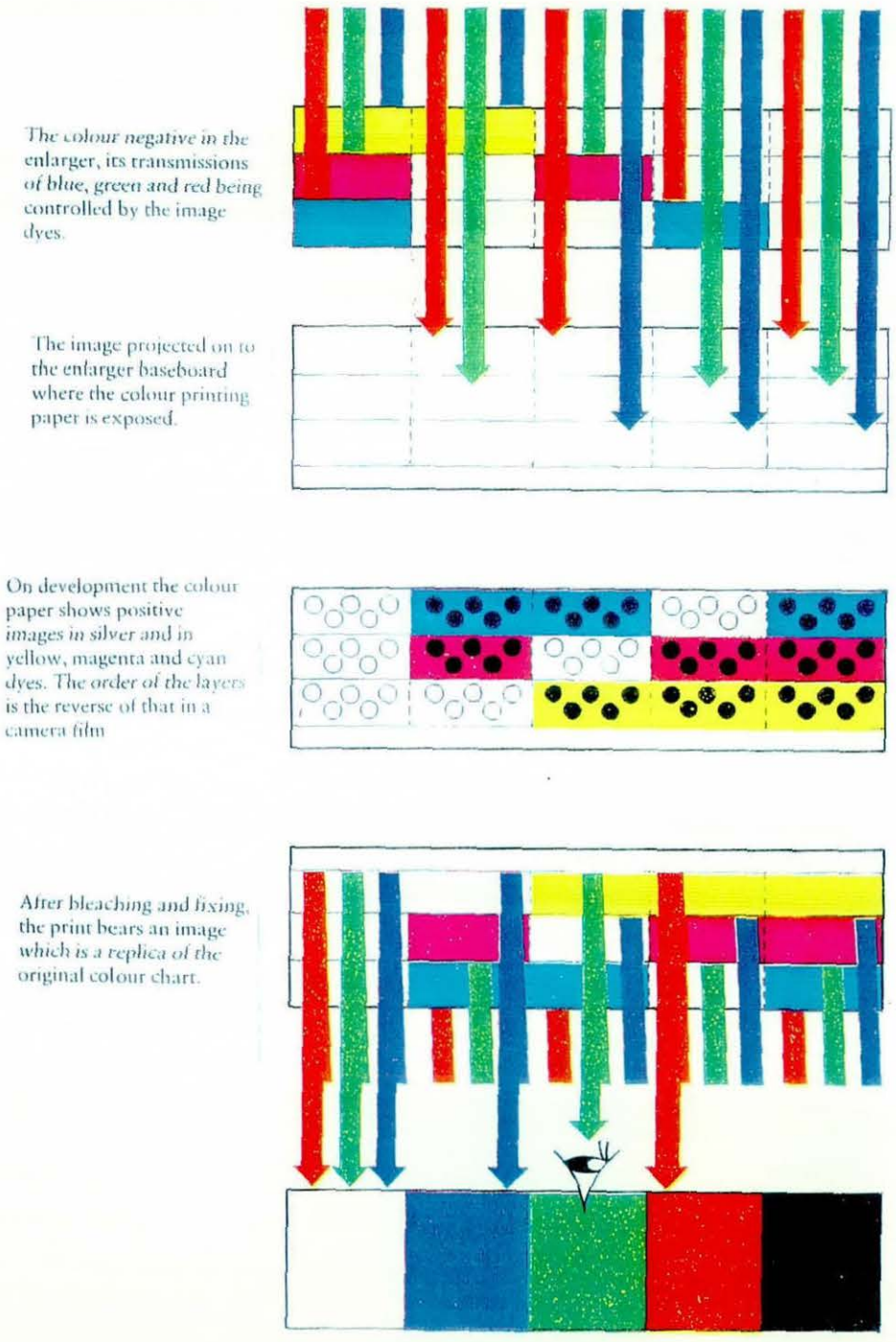


Fig. 1.1 Principles of colour negative development process [3].

When a colour negative film has been exposed, the first stage of processing involves a more or less straightforward black and white development during which uncoloured negative images in silver are produced in the three coupler layers. An example of this process is when a colour chart containing black, red, green, blue and white colours (read from top to

bottom) is exposed to colour negative film (*A*) and the latent image is formed. The film is developed using a colour developer solution. The developing agent reduces the silver bromide to silver and the resulting oxidation products of the developing agent react with the dye couplers to form coloured dye images. The black dots represent both silver and dye (*B*). The finished colour negative, contains dye images only, as the silver is bleached out by a bleach bath where the silver is oxidised back to silver bromide and then, after a wash cycle, a fixing bath converts the silver bromide in the emulsion layers to soluble compounds which are removed from the film (*C*). The colour negative is placed in an enlarger, its transmissions of blue, green and red being controlled by the image dyes (*D*). The image is projected onto an enlarger baseboard where the colour printing paper is exposed (*E*). On development the colour paper shows positive images in silver and in yellow, magenta and cyan dyes. The order of the layers is the reverse of that in a camera film. After removal of the silver by bleaching and fixing the image bears an image which is a replica of the original chart.

1.4 Chemistry of photographic development and developers

Developer solutions containing the developing agent produce the image from which photographs are produced. All modern developer solutions employ organic developing agents although interest has also been shown in inorganic developing agents such as ferrous oxalate. There are in existence many thousands of formulations for developer solutions [6]. Most compounds such as metol and phenidone are given historical names rather than their systematic names. Most of the descriptions which characterise developers are overlapping and confusing but they are indicative of the advances made in developing solutions over the last hundred years. The majority of modern developing solutions are provided in the form of liquid concentrates. The main components of developing solutions are:-

(a) the developing agent,
(b) alkali,
(c) preservatives and anti-oxidants,
(d) sesquestering agents,
(e) restrainer,
(f) development accelerator,
(g) surfactants,
(h) stain reducing agents

The function of each is explained below.

(a) ***The developing agent***

Some of the requirements for a developing agent are:-

(i) reducing agent for silver ions,
(ii) reduces exposed silver halide faster than unexposed silver,
(iii) water soluble,
(iv) relatively stable to aerial oxidation,
(v) non-toxic,
(vi) ecologically acceptable,

One of the most widely used organic developing agents, p-phenylenediamine, was first discovered by Andersen in 1888 and gave rise to a whole family of related compounds [1]. Various side groups have been added to increase specificity, lower the risk of dermatitis and increase the photographic effect. The developing agents are usually stored as the acid salts. The free bases, which are liberated in alkaline solution, readily oxidise to give quinonediimines. The chemical constitution, electrochemical, photographic and allergenic properties of p-phenylenediamines are well known [7]. The two developing agents studied in this project were Kodak CD-3 and Kodak CD-4, whose structures are shown in Fig. 1.2.

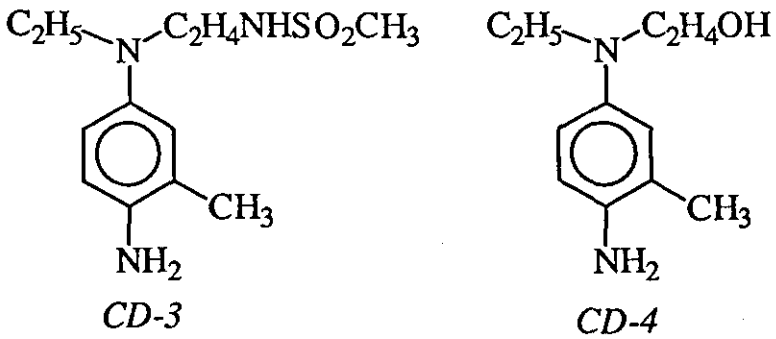


Fig. 1.2 Structures of Kodak developing agents CD-3 and CD-4.

CD-4 is used in the development of colour film, whereas CD-3 is used in the development of colour paper. As seen earlier, photographic development is a two stage process (Fig. 1.1) whereby a developing agent (CD-4) reduces the silver in the film to produce an image. This image is then exposed to light sensitive paper and produces an image, the image is then developed by a second developing agent (CD-3).

A general equation for colour development is given in Fig. 1.3. Colour development begins with the oxidation of p-phenylenediamine to quinonediimine and the reduction of one mole of silver ion to metallic silver. This may be followed by the reaction of the semiquinone with another mole of silver ion to form the quinonediimine and another mole of metallic silver. Or the semiquinone may desorb from the reactive site and dismutate to form quinonediimine and more p-phenylenediamine (see Fig. 1.3). The dismutation is favoured in alkaline solution as it produces hydrogen ions.

After development of the silver the oxidised developing agent adsorbs onto the photographic paper and reacts with couplers in the photographic paper to form coloured images, see Fig. 1.4. A typical dye forming reaction scheme is shown in Fig. 1.5.

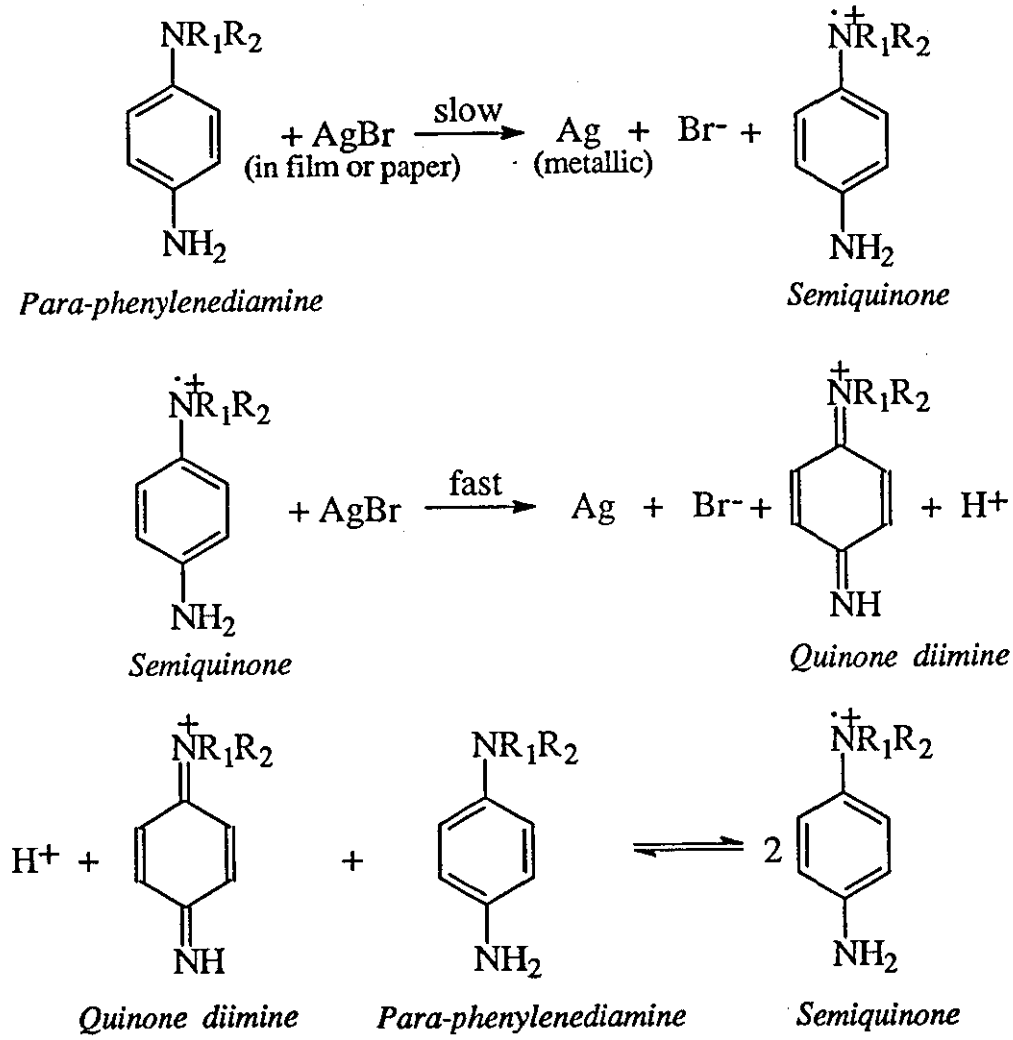


Fig. 1.3 General equation for colour development [8].

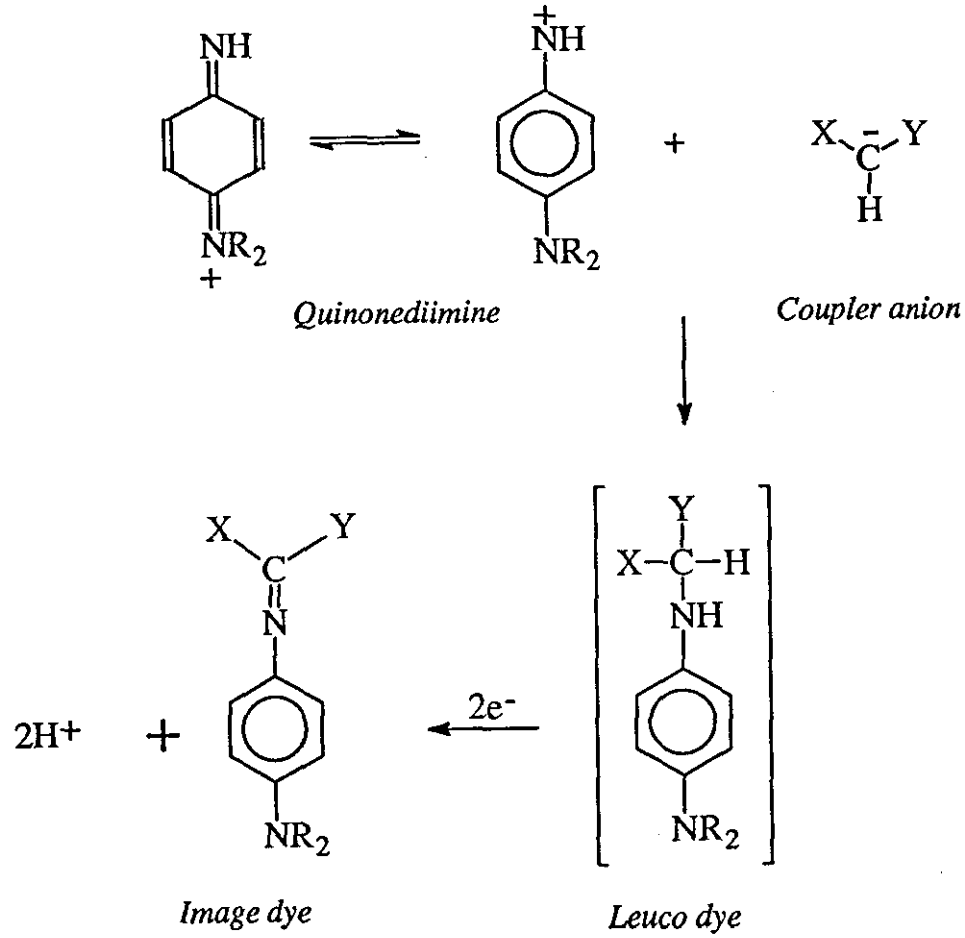


Fig. 1.4 Formation of image dye from quinonediimine and coupler [8].

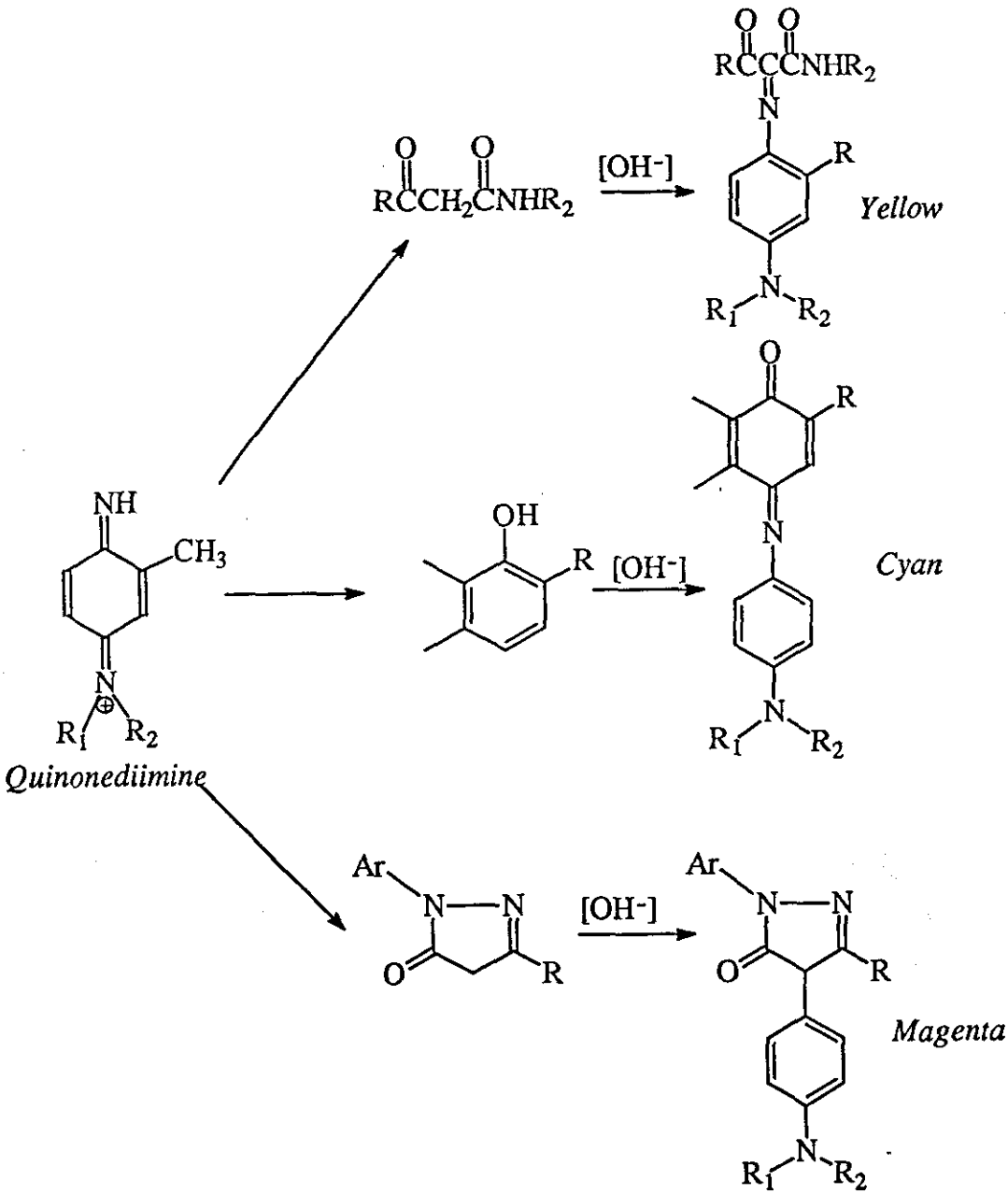


Fig. 1.5 Typical image dye forming reactions of quinonediimine [8].

(b) ***The alkali***

The alkali is the compound or mixture of compounds in the developer solution which determines and maintains the pH of the solution at the required value throughout the useful life of the developer [9]. Alkali is added in the form of hydroxide ions or carbonate ions. The redox potential of all organic developing agents are influenced by pH and as the pH rises the developing agent becomes a more powerful reducing agent with respect to silver. This effect is a direct consequence of the necessary involvement of

hydrogen ions in the oxidation/reduction equilibrium. This is obvious in Fig. 1.3 where the semiquinone reduces silver ions to metallic silver producing hydrogen ions and the quinonediimine in the process. With a lower concentration of hydrogen ions the reaction is driven more to the right, therefore favouring the reduction of silver ions. This in itself will result in a higher rate of photographic development but other factors can also influence the rate of development either way.

When development of silver halide takes place a hydrogen ion is produced for each atom of metallic silver formed, see Fig. 1.3. In order that the rate of development will not be slowed by a local drop in pH, these hydrogen ions must be rapidly neutralised by an abundance of hydroxyl ions:

$$H^+ + OH^- = H_2O$$

Hence the developer solution must have an alkaline pH. But more importantly, there must be a large reservoir of potential hydroxyl ions, whatever the pH of the solution might be. In other words the solution must be well buffered [9].

The storage properties of a developer are seriously affected by the pH. Because the reduction potential of all developing agents increases as the pH increases, the rate of reaction with molecular oxygen will also increase. Thus a high pH makes the developer more susceptible to aerial oxidation, which must be counteracted with more preservative, an excess of developing agent or protection from oxygen. When most developing agents are oxidised by air, hydroxyl ions are produced which tend to raise the pH still further. A good buffer capacity is therefore essential to offset this effect too.

(c) ***The preservative and anti-oxidant***

The original function of the preservative was to protect the developing agent against aerial oxidation. For organic developing agents sulphite ion is employed in varying concentrations and sodium or potassium sulphite are the preferred salts. Sometimes a metabisulphite is added, but this is converted to the sulphite in alkaline solutions. The aerial oxidation of developing agents is autocatalytic in that the quinonediimine formed reacts with more phenylenediamine to form more semiquinonediimine, see Fig. 1.3. The

removal of quinonediimine by sulphite breaks this chain of reactions, see Fig. 1.6. In the case of colour photography, the oxidation product (quinonediimine) of the reduction of silver ions by the developing agent is required to undergo further reaction with the couplers in the photographic emulsion to form image dyes, see Fig. 1.4. However reaction of quinonediimine with sulphite to form sulphonates could be a competing reaction and prevent colour image formation. So the concentration of sulphite must be kept low.

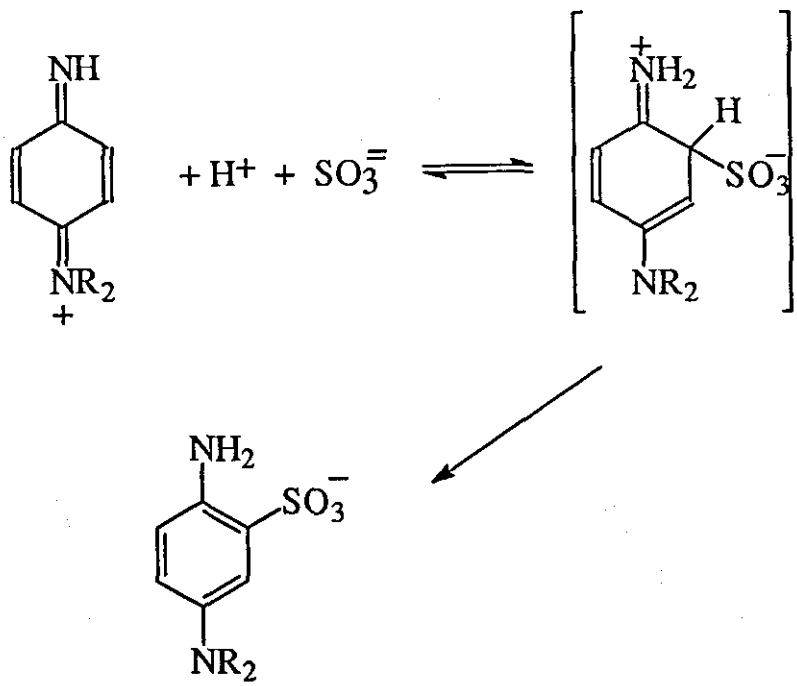
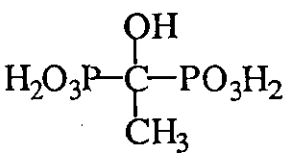


Fig. 1.6 Formation of developing agent sulphonates.

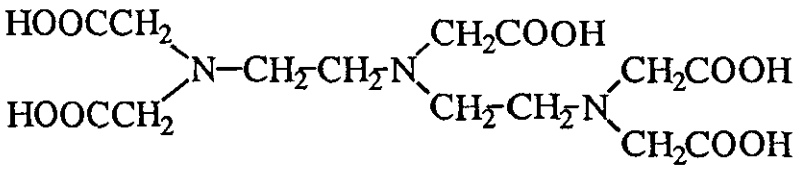
As a lower concentration of sulphite would make the developer solution more prone to atmospheric oxidation other antioxidants are added. Most of these compounds are organic reducing agents and hence possess some development properties themselves. Diethylhydroxylamine and hydroxylamine sulphate are extensively used. These anti-oxidants are preferentially oxidised by atmospheric oxygen, ensuring that the developing agent is unaffected.

(d) *Sesquestering agents*

The catalytic effect of metal ions on the aerial oxidation of developing agents, hydroxylamine and sulphite must also be counteracted and sequestering agents such as AntiCal No. 5 and No.8 (see Fig. 1.7) are added to combat this effect. However, AntiCal No.5 is being phased out as it also complexes sodium ions and is a poor complexing agent for calcium. If calcium is not complexed it can react with the carbonate of the developer solution to form calcium carbonate precipitates on the film.



AntiCal No.5



AntiCal No.8

Fig. 1.7 Structures of sequestering agents [8].

(e) *Restrainer*

Soluble bromide is always present as a restrainer and is sometimes supplemented by other compounds. Bromide results in retardation of development of silver ions but the retardation is greatest for unexposed silver ions and least for exposed silver ions. Each silver bromide crystal is surrounded by a double layer of Br^- ion and K^+ ions. The height of this barrier can be increased or decreased by increasing or reducing the concentrations of the restrainer or accelerator. Therefore by adding more bromide the double layer is increased making the silver bromide crystal less accessible to the developing agent.

(f) *Development accelerators*

These compounds increase the rate of development. The type of accelerators used in the developer solutions studied in the present project were organic amines, specifically triethanolamine. The mechanism involved is unclear but the compounds possess weak solvating properties for silver halide which promotes physical development. Physical development is the production of a visible image by depositing metallic silver on the development centres, from the solution containing a soluble silver salt and a reducing agent. The accelerator also works by dissolving in the coupler dispersion within the emulsion layers causing it to increase to a larger volume [5].

(g) *Surfactants*

Surfactants are included in the developer solution to ensure uniform wetting of the paper and so prevent the deposition of salts which can cause clouding of the developed paper [9].

(h) ***Stain reducing agents***

Sensitising dyes are necessary to extend the sensitivity of silver halide into the visible region of the spectrum. These large molecules have limited solubility and adsorb onto the photographic paper leading to a degradation in the whiteness of the product. Large organic molecules called stilbenes have been found to be effective at removing the sensitising dyes from the paper. These stain reducing agents are widely used in the textile industry as optical brighteners. In some examples, the stilbene is retained in the photographic paper and by its fluorescent effect, giving the paper a 'whiter white' [5].

1.5 Literature review.

Before investigations into the analysis and monitoring developer solutions and isolation/identification of degradation products could occur, it was necessary to review previous work on developer solutions, developing agents and other related components by other authors.

1.5.1 Analysis of black and white developing agents

Up to recently classical methods of analysis along with paper chromatography were the preferred methods of analysis for black and white developers. Paper chromatography had been extensively used for analysis of developing agents. The developer was spotted onto paper or extraction with an appropriate solvent was used. Infra-red examination of the residue after solvent extraction was also shown to be useful for qualitative analysis [10].

As developing agents are reducing agents it was possible to use titrimetric methods for their quantitative analysis. Hydroquinone can be titrated with an oxidant such as iodine and metol can be titrated as a base. N,N'-diethyl p-phenylenediamine was extracted from developer solution into ethyl acetate and titrated [10].

More recently liquid chromatography has been applied to the analysis of black and white developing agents. Hammers and Rhemrev-Boom [BF] separated a mixture of developing agents such as phenidone (1-phenyl-3-pyrazolidone) and metol (para-methylaminophenol). The samples were prepared in a synthetic matrix of sulphite, hydroxide and carbonate, typical components found in developer solutions. The developing agents were eluted using a water/methanol gradient on an octadecylsilica column using UV detection at 254 nm.. They applied this method to kinetic studies of the degradation of phenidone and concluded that it degraded according to a first order process until an equilibrium state was attained. However, Hammers did not apply the method to the separation of real developer solutions which can also contain polar and non-polar degradation products of developing agent , in addition to the active developing agents, and various salts and other organic components.

Hydroquinone and metol black and white developing agents were determined in photographic developers by Chen *et al.* [12] using a reversed-phase ion-pair chromatography system with amperometric detection. A potassium phosphate buffer with sodium 1-heptanesulphonate ion-pairing reagent was used for the mobile phase and the developing agents were separated on a phenylsilica column. Metol has an amino group (pK_a = 5.9) and is thus protonated in acidic media. However, hydroquinone (pK_a = 9.97) is neutral in acidic media. Therefore it is possible to separate the

hydroquinone molecule and metol/heptanesulphonate ion-pair on the weakly polar phenylsilica column.

Ambrose *et al.* [13] looked at the analysis of hydroquinone which was measured colorimetrically. The study also looked at another developing agent, *N*-methyl-p-aminophenol, which was analysed by gas chromatography on a 3% OV-17 column using a flame ionisation detector. The practicality of using macroreticular resins for the recovery and recycling of colour developing agents from used developer solutions was also investigated. Studies were also carried out on the reaction of CD-3 with sulphite to form sulphonates and the monosulphonate of CD-3 was analysed on an anion exchange microparticle packing. The authors also looked at sludges from photographic solutions and extracts of the sludges were examined by liquid chromatography. A number of yellow compounds were seen but none were characterised.

In the second paper of this series Ambrose *et al.* [14] examined the kinetics of the reaction of benzoquinone with sulphite. In a later paper Ambrose *et al.* [15] produced a HPLC method for the determination of sulphonates of 4-(methylamino)-phenol and hydroquinone.

1.5.2 Analysis of phenylenediamines

Although little has been documented on the chromatography of developing agents a multitude of papers have been published on the analysis of phenylenediamines, the most commonly used developing agents. These papers can afford an insight into the analysis of phenylenediamine developing agents, if not in the context of their developing properties.

1.5.2.1 HPLC analysis of phenylenediamines

Reddy *et al.* [16] developed a liquid chromatographic method for the determination of nitrobenzenes and their reduction products, aniline and phenylenediamines on a Zorbax C_8 column using a water: methanol gradient system. Tailing of the phenylenediamines led to poor peak shape and resolution. However on addition of 0.1% triethylamine in water the

resolution and sharpness dramatically improved, leading to a baseline separation of the ortho, meta and para phenylenediamines.

The usefulness of amine modifiers such as triethylamine to reduce tailing on reversed phase columns has been well demonstrated [17]. Kiel *et al.* addressed the mechanisms involved in the separation of charged analytes in the presence of alkylammonium modifiers. They suggested that hydrophobic interactions were of little importance but that interactions between the analytes and residual silanol groups on the modified silica were of key importance. It was postulated that the amine modifier 'masked' the silanol groups. Secondary and tertiary amines were shown to have stronger hydrogen-bonding capabilities than primary amines and would be expected to show stronger interactions with the silica surface.

The main actions of the triethylamine modifier were firstly to form hydrophobic interactions with the silica bonded organic components to create a charged surface and repel solute molecules. Secondly to form ion-exchange interactions with negatively charged silanols, thus blocking potential retention sites and thirdly, to form hydrogen bond interactions with uncharged silanol groups, which would block these retention sites.

Gennaro and Bertolo [18] presented an HPLC method for the analysis of nitrites, nitrates, aliphatic and aromatic amines contained in the same mixture. The amines were separated on an octadecylsilica column using an octylammonium salicylate mobile phase. The aliphatic and aromatic amines gave rise to ion-pairs with the salicylate of the mobile phase and so separation was possible. Detection was at 254 nm by way of indirect UV, as salicylate gave rise to a large absorbance at this wavelength. The use of octylammonium salicyclate facilitated the separation of cationic and anionic species through ion-pairing with both the octylammonium adduct and salicylate adduct. However resolution of the amines was poor. Subsequently Gennaro *et al.* [19][20] optimised the reversed phase ion-interaction of nitrite, nitrate and phenylenediamine isomers. Again octylammonium salicylate was used as the mobile phase, to ion-pair with the phenylenediamines.

1.5.2.2 CE analysis of phenylenediamines

Capillary electrophoresis (CE) involves the separation of charged species by way of their charge, ionic size and the nature of the separation buffer used. As colour developing agents, such as CD-3 and CD-4, are water soluble ionisable compounds they should be ideally suited to separation by CE. Previous work on the separation of phenylenediamine isomers has been reported by Nielen [21]. This method employed minor differences in the pK_a values of o-, m- and p-phenylenediamines to obtain a separation. A 40 mM solution of tris(hydroxymethyl)-aminomethane (Tris) buffer (pH 4.8) was used to separate the isomers on a 50 cm fused silica capillary, using UV detection and a voltage of +25 kV. The electroosmotic flow marker eluted at 7.8 mins and all phenylenediamine isomers eluted before this, indicating that the isomers are partially protonated and are attracted to the cathode (Fig. 1.7).

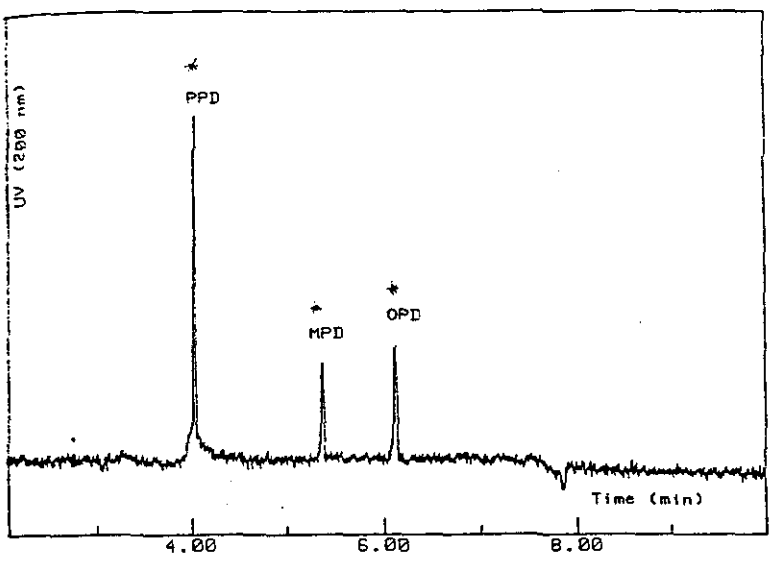


Fig. *1.7 CE separation of phenylenediamines [21].*

1.5.3 Analysis of colour developing agents

Some papers have been published on the analysis of colour developing agents but most of these have been internal Kodak technical papers.

In 1977 Harbison *et al.* [22] reported the use of an reversed phase system for the separation of a mixture of colour developers by coupling with 1-naphthol and chromatographic separation of the resulting dyes. Conversion of the developing agents to their corresponding dyes was necessitated as at low concentrations there can be losses due to oxidation. The dyes are much more stable and were analysed using octylsilica and octadecylsilica columns. However, peak shapes for the eluted compounds were poor. This may have been caused by residual silanol groups on the stationary phase giving rise to tailing peaks.

A method for the analysis of colour developing agents in fresh and seasoned developer solutions has been published internally by Kodak [23]. This method separated the colour developing agent CD-3 (see Fig. 1.2 for structure) on a base-deactivated octadecylsilica column.*

The developer solution was diluted with ascorbic acid to reduce degradation of the developing agent and separated using an ammonium acetate buffer (pH 4.8)/acetonitrile isocratic buffer. The acetate buffer contained sodium heptanesulphonate ion-pairing agent (0.05 % w/v) and triethylamine (0.05 % v/v) was added to the acetonitrile organic modifier to reduce peak tailing. The developing agent was protonated at the pH of the buffer (pH 4.8) and ion-paired with the heptanesulphonate to give a good separation.

Another solution to the problem of peak tailing of amines on reversed phase columns involves the use of ion-exchange columns for the separation of colour developers. Ohno *et al.* [24] used ion-exchange chromatography

*Base-deactivated octadecylsilica is modified silica which has been chemically treated to endcap residual silanol groups. These deactivated columns give better separation and resolution compared to non-deactivated columns, particularly for basic amines.

with conductometric detection and UV detection for the simultaneous determination of ionic species and UV active species in colour developers, such as Kodak C-41 and E-6. The column packing material was prepared by mixing cationic exchange resin, anionic exchange resin and a hydrophilic gel. This method gave a good separation of the ionic salts present in developer solution, such as sulphite, sulphate and bromide on the ion-exchange resin.

However, separation of the colour developing agents CD-3 and CD-4 was very poor. Separation of degradation products was unsatisfactory and no separation of the individual products was seen, as coelution occurred (see Fig. 1.8).

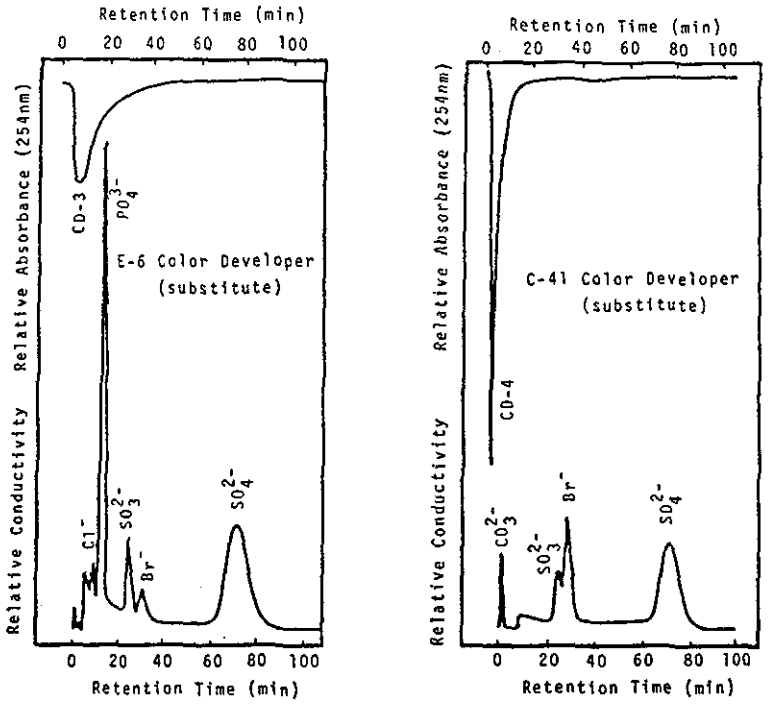


Fig. 1.8 Analyses of used photographic developer solution [24].

Studies on the kinetics and mechanism of sulphonation of CD-3 were undertaken [25]. It was deduced that the reaction was first order in sulphite and developing agent. Oxidation products of CD-3 were extracted from solutions of oxidised developing agents and examined by mass spectrometry. However, no components were isolated or identified.

The method for determination of colour developing agents and their sulphonates was modified [26]. As previously, the developing agents were separated as the 1-naphthol dyes. The stability of solutions of

agents were separated as the 1-naphthol dyes. The stability of solutions of CD-3 in various media was also examined. It was found that at pH near neutral, CD-3 was particularly unstable. However, at low pH it is quite stable, hence its storage as an acid salt. The sulphonates of CD-3 were also separated on an aminosilica column, but experience showed that the aminosilica column had a particularly short lifespan and degraded quickly with usage. Harbison *et al.* [27] reported a method for the separation of developing agent sulphonates on an octadecylsilica column using an acetate/acetonitrile gradient mobile phase with detection at 254 nm. This paper also looked at the possibility of reactions other than sulphonate formation occurring. The authors looked at the formation of sulphamate as a possible competing reaction.

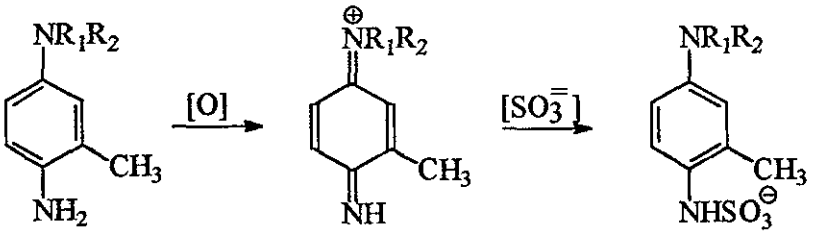


Fig. 1.9 Formation of sulphamate from CD-3/sulphite [27].

The CD-3 sulphamate was prepared by reaction of CD-3 with chlorosulphonic acid. This compound was then compared to a solution of CD-3, sulphite and ferricyanide and was found to present in a yield of less than 5%. The photolysis of CD-3 sulphonates was also examined.

1.5.4 Stability of colour developer solutions

As developing agents are reducing agents, they are prone to aerial oxidation and antioxidants are added to keep the level of concentration of developing agent at an acceptable level to facilitate development. Several specific studies have been carried out on the stability of Kodak processing solutions [28][29]. Huston and Buongiorne [28] studied the effects of oxidation on Kodak RA4 and Kodak C-41 developer. An antioxidant, *N,N'*-diethylhydroxylamine (BD-89) was added to RA4 colour developer solutions and acted as a preservative for the developing agent, CD-3. Air was bubbled

determined using gas chromatography. Little or no loss of CD-3 was seen until all the BD-89 had been oxidised, at which point the concentration of CD-3 started to drop off rapidly. A similar study was undertaken for the C-41 developer solution. Sodium sulphite and hydroxlamine sulphate were added to C-41 solutions to act as oxygen scavengers and antioxidants, respectively. Levels of CD-4, the developing agent present, were monitored, along with pH, sulphite and hydroxylamne sulphate. It was found that levels of developing agent, hydroxylamine sulphate and sodium sulphite started to drop immediately on aeration but the pH remained stable for 60 hours. The reasons for the different behaviour of the solutions was not explained.

Photographic developer solutions are relatively expensive to manufacture and in everyday use they are changed infrequently, sometimes the same basic solution is used for up to a year. As the solution is used to develop film and paper the concentrations of developing agent, antioxidants and stain reducers decrease with usage, therefore they need to be replenished. Replenisher solutions are used in the photographic industry to 'top up' working solutions, thereby ensuring a constant concentration of active developing agent. It was found that C-41 replenisher solution started to degrade immediately on exposure to air as in the previous example [28] but a sample subjected to aeration took about 100 hours for the developing agent to degrade whereas a sample allowed in air to stand took about 13 weeks to reach a similar level [29].

1.5.5 Analysis of photographic stain reducing agents

An important component of photographic developer solutions are the stain reducing agents. The build-up of oxidation products and salts in solution can cause staining and fogging of the photographic paper and so stain-reducing agents are needed to counteract this. Very little has been documented on the analysis of these agents in the context of their use in photographic materials. Photographic stain reducing agents tend to be commercially available stilbene fluorescent whitening agents and a multitude of papers have been published regarding their stability and analysis in this context.

1.5.5.1 Analysis of Phorwite

An article on the analysis of Kodak stain reducing agent (KSRA, also called Phorwite REU or Blankophor) has been published [30]. The stability of Phorwite REU in photographic solutions has also been studied [31].

Phorwite REU (Fig. 1.10) is a stain reducing agent used to reduce stains in photographic paper [31]. It is a member of the general class of 4,4'-bistriazinylaminostilbene-2,2'-disulphonic acids (Fig. 1.10).

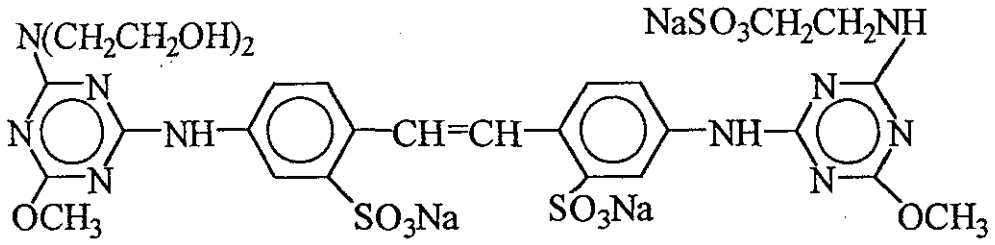


Figure 1.10 Structure of Phorwite REU stain reducing agent.

It is still unclear as to how these optical brighteners reduce staining but their fluorescent properties do not seem to play a role [31]. Vincent described an HPLC method for the analysis of Phorwite in developer solutions [30]. The method used a 2-amino-2-(hydroxymethyl)-1,3-propanediol/acetonitrile mobile phase (pH 7.6) and separated Phorwite on an octadecylsilica column followed by detection at 347 nm. At least nine peaks were separated using this method. However, the method did not seem to be reproducible as some of the peaks seemed to vary in detector response from run to run. This phenomena was reported but was not further investigated.

1.5.5.2 Stability of Phorwite

The method developed by Vincent was applied to the analysis of stain reducing agent in fresh and seasoned developer solutions and it was found that the concentration of stain reducing agent dropped with time, usage and exposure to light [31]. These findings were supported in another article by Vincent [30].

A study to investigate the decomposition of Phorwite stain-reducing agent with time and light was instigated by Vincent [30]. Solutions of Phorwite were stored in clear and actinic red glass reagent bottles. The solutions were analysed over a seven week period using the HPLC method previously described [30]. It was found that Phorwite decomposed at a rate of 6 % per week when stored in clear glass containers. No significant decomposition was observed for a solution kept in actinic red glass. The main photodegradation products of Phorwite were the aldehydes, acid and dihydro derivatives [32]. No conclusions were drawn for the decomposition of Phorwite in the dark environment of a developer tank where Phorwite is not exposed to light.

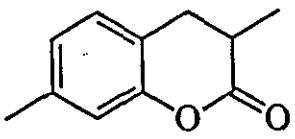
1.5.5.3 Analysis of optical brighteners (stain reducers)

Optical brighteners are also called whitening agents and are used extensively in the textile and printing industries. The methods of analysis given should be easily adapted for the analysis of optical brighteners (stain reducing agents) in photographic solutions and also to the analysis of optical brighteners in photographic wastes.

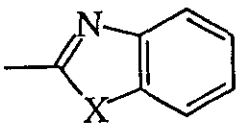
The use of HPLC for the determination of whitening agents in detergents has been reported [33]. Eleven stilbene sulphonate derivatives with structures similar to Phorwite, were separated on an octadecylsilica column, which was thermostated at 60°C, using a sodium acetate/methanol (43:57 v/v) mobile phase. Triethylammonium chloride was added as an ion-pairing agent. Detection was by fluorescence and the excitation and emission spectra of the eluted fractions were determined using a stopped- flow method. UV detection would have also been possible but would have been less sensitive.

Tsuji *et al.* [34] preconcentrated and isolated stilbene disulphonate fluorescent whitening agents in river waters on an octadecylsilica cartridge and separated them on an octadecylsilica analytical column using an acetonitrile:sodium phosphate buffer mobile phase with fluorescent and ultraviolet detection. The whitening agents separated tended to be less polar than the corresponding components found in developer solutions.

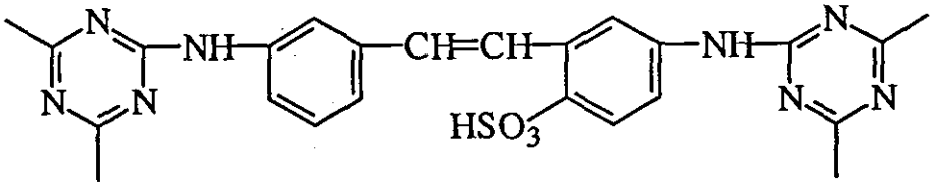
Thin-layer chromatographic (TLC) methods for the determination of whitening agents have been well documented. As all are fluorescent and UV active, detection is made easier. Lawrence and Ducherme [35] made use of plastic backed precoated silica gel plates for the determination of whitening agents in polymers. Coumarin, benzazole and stilbene derivatives were separated and detected. (Fig. 1.11). Chloroform was used as the eluent for the coumarin and benzazole derivatives, whereas a more polar pyridine-n-butanol-ethanol-ammonia (30:40:10:20) eluent was used for the stilbene derivatives. After elution and drying, the whitening agents were detected under UV light at 365 nm.



Coumarin



Benzazole



Stilbene

Fig. 1.11 Structures of main whitening agent groups [35].

Similarly TLC was used for the separation and identification of whitening agents in detergents by Lepri *et al.* [36]. Twenty whitening agents representing all major classes including stilbenes were studied . Reversed phase C_2, C_8 and C_{18} silica TLC plates were used. Compounds were eluted using acetic acid and potassium chloride (3%) in water/methanol (60:40 v/v) and acetic acid with hydrochloric acid in water: methanol (60:40). The spots were detected under UV light at 366 nm.

1.5.5.4 Stability of optical brighteners (stain reducers)

Many of the studies concerning the stability of optical brighteners can give a useful insight into the stability of these compounds in photographic solutions.

Milligan and Holt investigated the photodecomposition of a stilbene sulphonic acid, (bis-4,4'-(4"-methoxy-6"-phenoxy-5-triazin-2"-ylamino) stilbene-2,2"-disulphonic acid (Fig. 1.12), similar to Phorwite, in solution and on wool [32]. The compound was irradiated in solution and rapidly produced an equilibrium mixture of cis (Z) and trans (E) isomers. Further decomposition to the corresponding aldehyde, acid and dihydro derivatives (Fig. 1.13) also occurred, albeit at a slower rate. The trans and cis isomers of Phorwite were easily separable using TLC and paper electrophoresis. The aldehyde, carboxylic and dihydro derivatives were identified by comparison of their electrophoretic and chromatographic behaviour with those of standard samples.

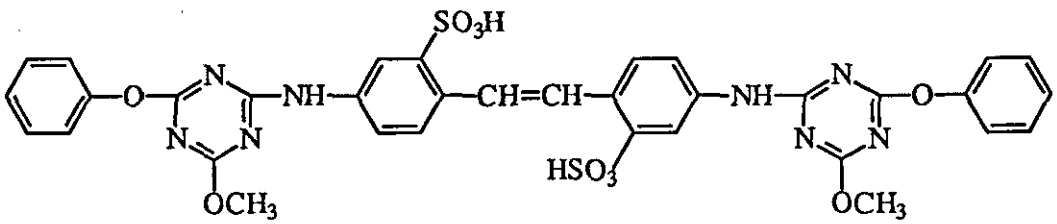
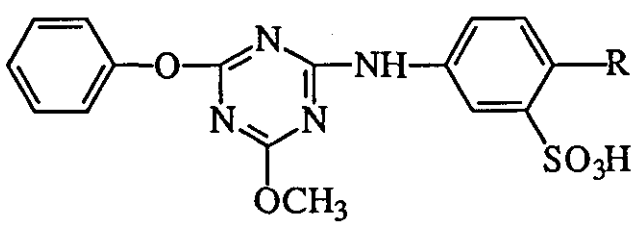


Fig. 1.12 Bis-4,4'-(4"-methoxy-6"-phenoxy-5-triazin-2"-ylamino)stilbene-2,2"-disulphonic acid [32].



R= CHO, COOH, CH_2CH_2-

Fig. 1.13 Structure of degradation products from stilbene derivative [32].

Several studies have looked at the degradation of fluorescent whitening agents on textiles, such as cotton and wool. Davidson *et al.* looked at the stability of whitening agents on wool [37]. It was noted that for stilbene whitening agents oxidative degradation occurred only in solution, on wool the stilbenes appear to be photoreduced rather than photooxidised. Hard and Reagan [38] looked at the exhaustion, photostability and photosensitising properties of triazinylaminostilbenes on cotton. It was concluded that photodegradation and photosensitising depended on the degree of substitution of the stilbene and the nature of the functional groups present.

1.5.6 Chemical properties of phenylenediamines

The literature contains a multitude of references to the properties of para-phenylenediamine derivatives, such as their deamination, study of intermediates, coupling reactions and other reactions of particular relevance to the present study. Various aspects of these properties have been reviewed in the present study.

1.5.6.1 Deamination

One of the earliest papers giving a comprehensive overview of the chemical constitution, electrochemical, photographic and allergenic properties of phenylenediamines [7] showed that the primary oxidation products of p-phenylenediamines are the semiquinones. This phenomena is used in colour photography whereby the quinonediimine oxidation product is coupled with a dye to give a coloured compound. Tong and Glesmann [39] explored the reaction between oxidised p-phenylenediamine and coupler in aqueous solution. The oxidation is shown in Fig. 1.14.

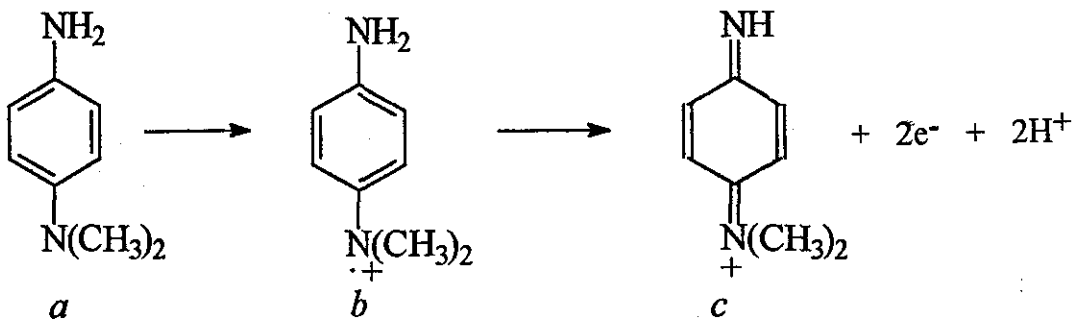


Fig. 1.14 Oxidation of p-phenylenediamine to p-quinonediimine [39].

The study sought to determine whether the structures *b* or *c* are involved in the coupling rate reaction. Phenylenediamine was oxidised using ferricyanide and then mixed with a coupler. At this point acetic acid and hydroquinone were added to stop coupling. The concentration of the semiquinone could be determined by coupling with a naphthol dye and spectrophotometric determination of the resulting compound. It was found that the stability of *b* is highest in acid solution where resonance forms are available. Above pH 8 dismutation was almost instantaneous and complete ($a + c = 2b$)[VV]. This study also found that the phenylenediamines underwent two major reactions, firstly coupling with the naphthol and secondly deamination to the corresponding quinone-monoimine.

Several studies on the deamination of phenylenediamines have been carried out. Tong [40] found that over the pH range of 7 to 12, the deamination involving the substituted amino group (*a* to *b*, Fig. 1.15) fitted a second order rate equation involving the product of hydroxide ion and quinonediimine, the oxidation product of phenylenediamine, (Fig. 1.15). The deamination of the unsubstituted amino group fits a first order rate equation and was insensitive to changes in pH,(*a* to *c*), see Fig. 115. The variations in reactivity due to substitution could be accounted for by inductive effects.

A similar method for the determination of rates of deamination as described previously were used [39]. Phenylenediamine was oxidised by addition of potassium ferricyanide which started the deamination reaction. After a predetermined interval the reaction was stopped by the addition of acid buffer to bring the pH to 6.7. Finally α-naphthol was added and the dyes formed by the coupling reaction were extracted with butyl acetate and their concentrations were determined spectrophotometrically.

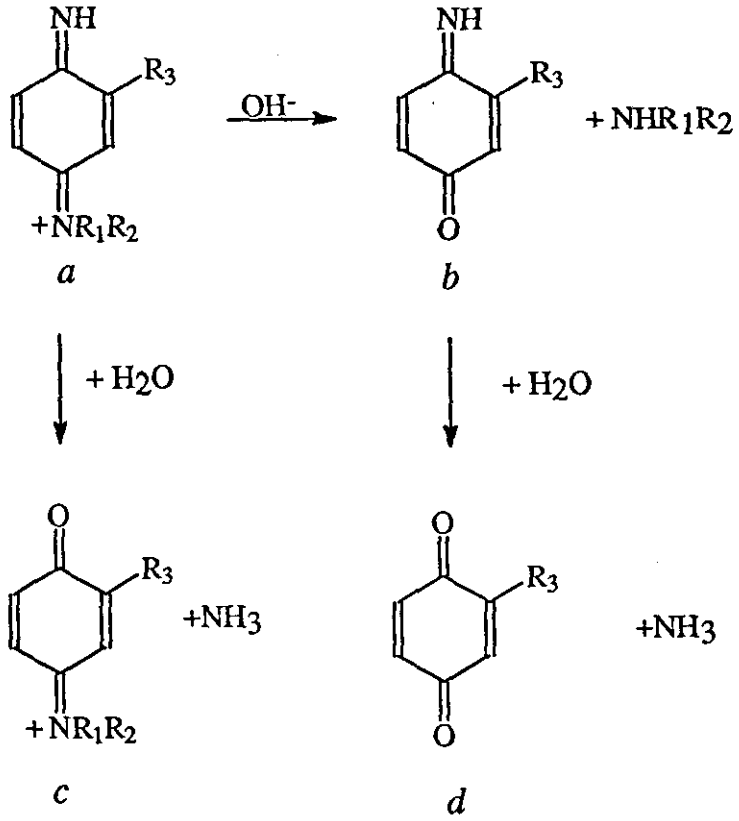


Fig. 1.15 Reaction pathways for deamination of quinonediimine[40].

The quinonediimine (see Fig. 1.16) formed from CD-3 is neutral at high pH. Being a zwitterion and having an overall neutral charge, its reaction with hydroxide ion was expected to be much slower and the reaction above pH 10 was suspected to be an uncatalysed hydrolysis.

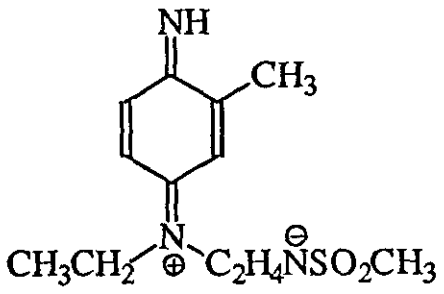


Fig. 1.16 Structure of p-quinonediimine derived from CD-3 at high pH [39].

Intermediate bases in the deamination of quinonediimines were studied by Tong *et al.* [41]. Hydroxyalkyl substituents on the tertiary nitrogen of the phenylenediamine can stabilise the quinonediimine (see Fig.

1.17) Equilibrium constants for the deamination were measured spectrophotometrically using the technique previously described [39]

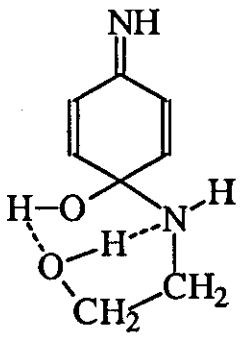


Fig. 1.17 Stabilisation of quinonediimine intermediate [41].

Tong and Glesmann [42] studied salt effects on the deamination rate of oxidised p-phenylenediamines. This is particularly important as photographic developer solutions contain high concentrations of salts such as carbonate, sulphate, sulphite and bromide. Very few conclusions were drawn from this study.

The kinetics and mechanism of acid deamination of substituted p-phenylenediamines has also been studied by Nickel *et al.* [43]. Seven substituted phenylenediamine derivatives were oxidised using iodine. Deamination was started by the addition of HCl, which initiated nucleophilic attack on the least substituted amino group by water, giving the substituted quinone monoimine. (In alkaline solution the quinonediimine is attacked by hydroxyl ion to form the unsubstituted quinone monoimine, see Fig. 1.15). A similar stopped flow system as that described by Tong [39] was used. The diimine and the monoimine resulting from deamination, produced different dyes on coupling, which were determined spectrophotometrically.

Nickel also studied the mechanism and kinetics of the conversion of N-substituted p-phenylenediamines, such as N,N-diethyl-p-phenylene-diamine, into p-aminophenols and hydroquinones in the presence of trace amounts of oxidants [44]. The primary oxidation product quinonediimine was deaminated, forming N-substituted quinone monoimine, which was able to react with the residual p-phenylenediamine giving p-aminophenol. The other reaction product quinone diimine was unstable and reacted with more developing agent to form more p-aminophenol. The residual quinone

monoimine deaminated slowly, giving quinone. This reacts with p-aminophenol to give hydroquinone. This means that, on degradation, p-phenylenediamine solutions still have some developing ability (hydroquinone is a developing agent) but their coupling ability is reduced as hydroquinone will not couple with the couplers in the emulsions.

Nickel has studied the influence of deamination on the stability of photographic dyes [45]. Normally p-phenylenediamine developing agents are stored in the solid form as acid salts, however, if the developing agent is not very dry, oxygen or any another oxidant can oxidise some molecules of the phenylenediamine salt. The p-quinonediimine formed can then undergo acid deamination as previously described [43][40], to form the quinone monoimine and so on, see Fig. 1.15. The deamination could be followed visually and the purple colour of the p-quinone monoimine could be seen. The p-aminophenols can result in loss of dye density, p-quinone monoimine can cause the formation of undesirable indophenol dyes, as shown in Fig. 1.18. The formation of specific dyes from reaction of the couplers in the photographic paper and the oxidised developing agent is crucial to the formation of the coloured image in colour photographic development. This study illustrated some of the problems which can occur when photographic developing agents and developer solutions are stored over time.

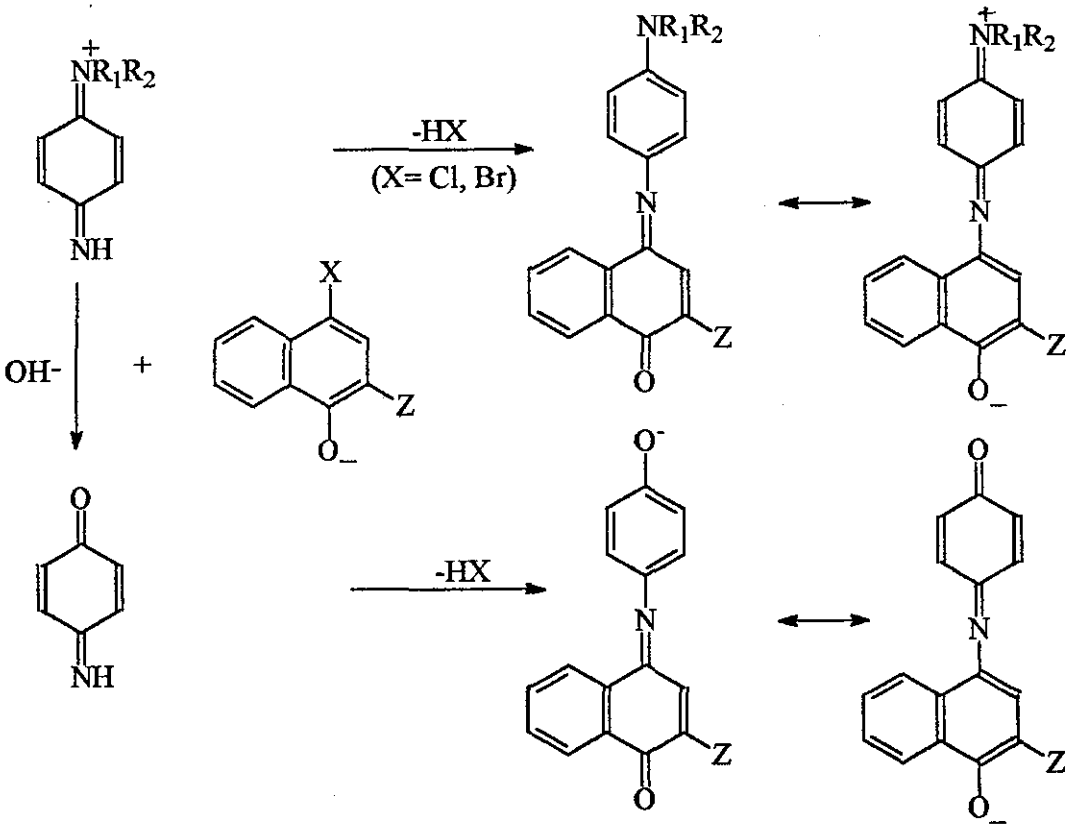


Fig. 1.18 Formation of indoaniline dyes from para-quinonediimine and of indophenol dyes from para-quinonemonoimine by coupling with α-naphthols [45].

The hydrolysis of p-benzoquinone monoimine and p-benzoquinone diimine have been studied by Corbett [46]. This study was similar to those undertaken by Tong [43] and Nickel [44] in that the kinetics of p-benzoquinone monoimine was investigated, this time over a pH range of 5.5 to 8. As indicated in the previous studies p-benzoquinone decomposes as it is formed. The study also showed that phosphate ions had a catalytic effect at pH less than 9. Experiments in the pH range of 6 to 8 indicated that the yield of benzoquinone was considerably lower and side reactions, producing significant brown coloration. It was postulated that polymerisation of the diimine is initially responsible for colour development at pH 6 to 8. It was also speculated that if any oxidation by the diimine would give rise to p-phenylenediamine and this leads to the formation of Bandroskis base [61][62].

Tong and Baetzold [47] undertook a study on the kinetics of the redox reactions of oxidised phenylenediamine species. This work looked at the

reaction of potassium ferrocyanide with quinonediimine. A phenylazide was photolysed to form the quinonediimine. This quinonediimine was reacted with p-phenylenediamine (see Fig. 1.3.). The rate of this reaction was studied by reaction of the quinonediimine with a dye.

A further study by Tong looked at the formation of partially oxidised para-phenylenediamines [48]. It was observed that although quinones form in solution they are extremely unstable at high pH and form addition complexes and undergo irreversible hydroxylation.

1.5.6.2 Coupling reactions

The kinetics of dye formation by oxidative coupling with a micelle-forming coupler has been studied by Tong and Glesmann [49]. These couplers are simultaneously ballasted with hydrophobic groups and solubilized with ionisable groups. They are useful in photographic technology as incorporated couplers because their low mobility during development prevents diffusion which would cause interlayer contamination in the coupler containing emulsion layer. The hydrophobic groups and ionisable groups confer detergent-like properties on these molecules. The rate of dye formation between quinonediimines and an anionic micelle-forming coupler was measured. One of the advantages of using a micelle-forming coupler is that the side reactions occur exclusively in the aqueous region and not in the micelle-forming region.

Further studies by Tong and Glesmann [50] explained the mechanism of oxidative coupling of p-phenylenediamines (Fig.1.18). It was shown that the coupling reaction is a two-step process whereby the phenylenediamine is firstly oxidised to the quinonediimine, which then reacts with the coupler. Coupling reactions were separated into two classes, depending on whether the second step requires oxidation (elimination of H) or proceeds by elimination of a substituent (X) other than hydrogen (H) from the reaction site of the coupler

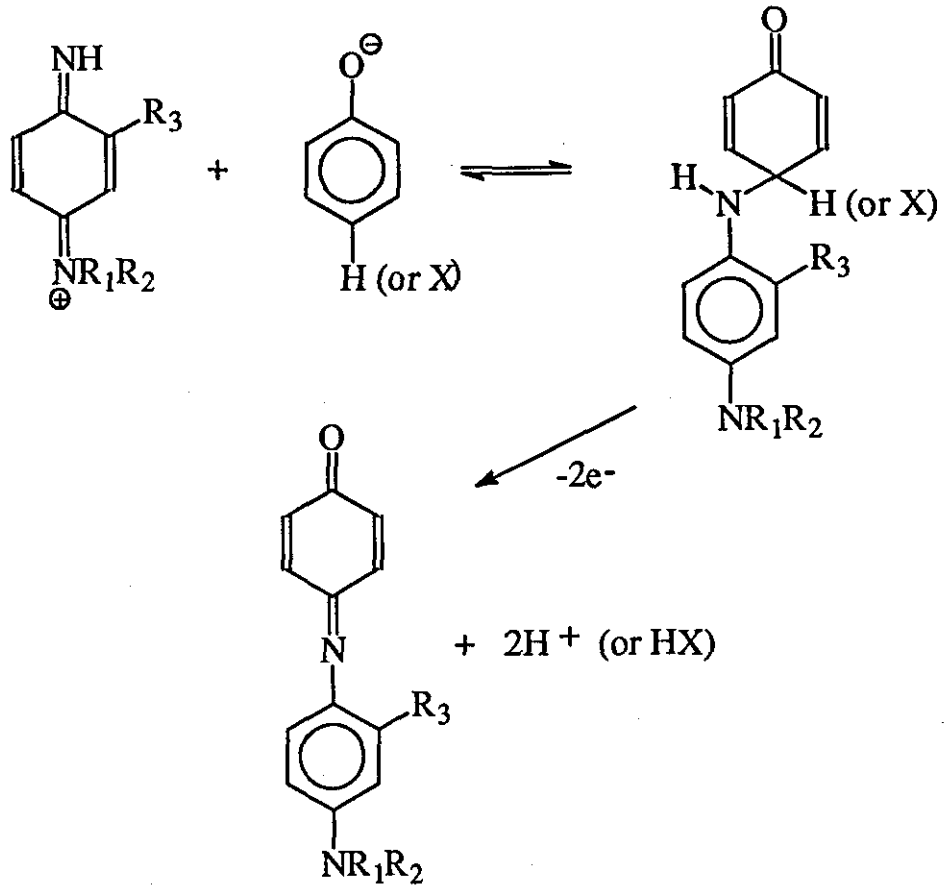


Fig. 1.18 Reaction of p-phenylenediimine with coupler [50].

Pelizzetti and Saini [51] undertook a similar study, looking at the reaction of quinonediimines with phenol and naphtholic compounds to form blue dyes and determined the rates of reaction.

1.5.6.3. Azo dye formation

As well as deamination and coupling reactions, other reactions of p-phenylenediamines include the formation of non-polar products such as azo dyes and indophenols.

Tong and Bishop [52] studied the formation of azo dyes from phenylenediamines. Azo dyes can form in alkaline solutions under oxidising conditions. A mechanism for their formation was postulated in the paper (Fig. 1.19).

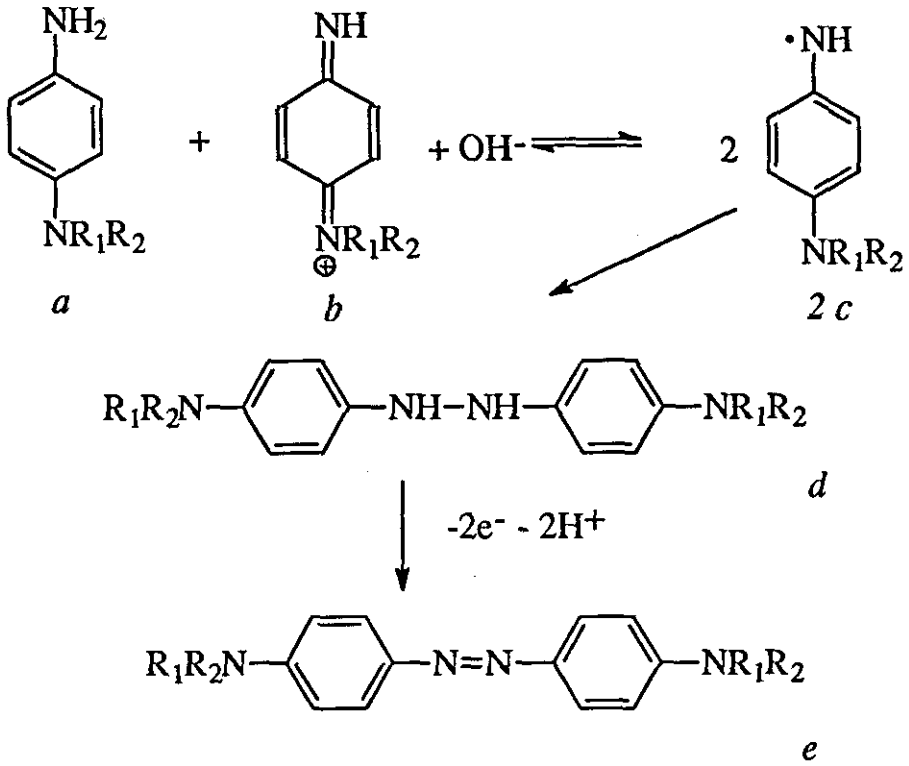


Fig. 1.19 Mechanism of azo dye formation [51].

An understanding of the conditions, which lead to azo formation, is of interest because even low yields of coloured by-products can seriously contaminate the azomethine and indoaniline image dyes [51]. The kinetics of azo dye formation were evaluated by using a p-phenylenediamine derivative, which formed very small amounts of azo dyes relative to the deaminated product. The rate of azo dye formation was deduced to be proportional to the hydroxide ion concentration and the product of the concentration of the p-phenylenediamine and its oxidised form, the quinonediimine. It was also shown that the more substituted the methyl groups o to the primary amino group the lower the yield of azo dye. This effect was probably caused by steric hindrance of the methyl groups. Electronic withdrawal from the ring also increases the azo dye yield, as the free radical reaction intermediates are stabilised (*c*).

Isolation of the azo-dye entailed dropping the pH of the oxidised phenylenediamine solution to about pH 6, then the mixture was extracted using butyl acetate. The extract was evaporated and dissolved in benzene and chromatographed on a Florisil column using benzene/ethyl acetate as eluent.

1.5.7 Phenazines and cinnolines from phenylenediamines

Very little work has been done on the identification of other possible degradation products from p-phenylenediamines. It may be possible for phenylenediamines to form complexes, phenazines, cinnolines and trimers through reactions with other compounds and self-condensation. Studies have investigated the photolysis of o- substituted azidophenyl , oxidation of o- and m-phenylenediamines and properties of phenazines and benzo[c]cinnolines.

Crankara and Makin reported the photochemical conversion of o-phenylenediamine and o-aminophenol into heterocyclic products [53]. o-Phenylenediamine was photooxidised in alcoholic potassium hydroxide and two main products were found. The 2,3-diaminophenazine was formed in 10% yield and the major product was the 2,2'-azobenzene. When o-phenylenediamine was photochemically oxidised in ethanol or dilute hydrochloric acid whilst air was bubbled through it, the phenazine-2,3-diamine was formed. Similar results were found using aqueous sodium hydroxide.

An autosensitized oxidation of o-phenylenediamine in an aqueous buffer was reported by Mekler and Bystryak [54]. The main product of the reaction was the 2,3-diaminophenazine. The oxidant, in this case, was molecular oxygen and the reaction was initiated by visible light.

A highly efficient oxidation of o-phenylenediamine to phenazine in the presence and absence of horseradish peroxidase was reported by Torcha *et al.* [55]. The oxidation of o-phenylenediamine in the absence of horseradish peroxidase took place at pH 11.2. Hydrogen peroxide was added and the precipitate formed was removed after 18 hours of stirring and recrystallised. The oxidation of o-phenylenediamine in the presence of horseradish peroxidase took place at pH 6.0. Again hydrogen peroxide was added, followed by addition of horseradish peroxidase, the resulting precipitate was filtered after 16 hours of stirring and recrystallised. The yield of pure product in the presence of horseradish peroxidase was 42%, whereas in its absence it was 5%. It was postulated that the oxidation took place

through a free radical mechanism whereby the free radicals couple with each other, rather than via diazo formation.

Watanabe *et al.* have studied phenazine derivatives as the mutagenic reaction products from the oxidation of o- or m-phenylenediamine derivatives [56]. A range of phenylenediamine derivatives including o-phenylenediamine, p-methyl-o-phenylenediamine, m-phenylenediamine and p-methoxy-o-phenylenediamine were treated with hydrogen peroxide and the precipitates filtered off and extracted with ethyl acetate after two days of standing. These extracts were column chromatographed on silica gel or activated alumina and eluted with chloroform-methanol, chloroform-ethanol and methanol. Brownish yellow crystals were isolated from oxidised p-chloro-o-phenylenediamine and the structure was found to be 2,3-diamino-7-chlorophenazine using mass spectrometry and proton NMR. Two reddish yellow crystals were isolated from para-methoxy-m-phenylenendiamine (*a*) and were deduced to be 2,7-diamino-3,8-dimethoxyphenazine (*b*) and 2,7-diamino-3-methoxy-phenazine (*c*) from the mass spectra and proton NMR (Fig. 1.20).

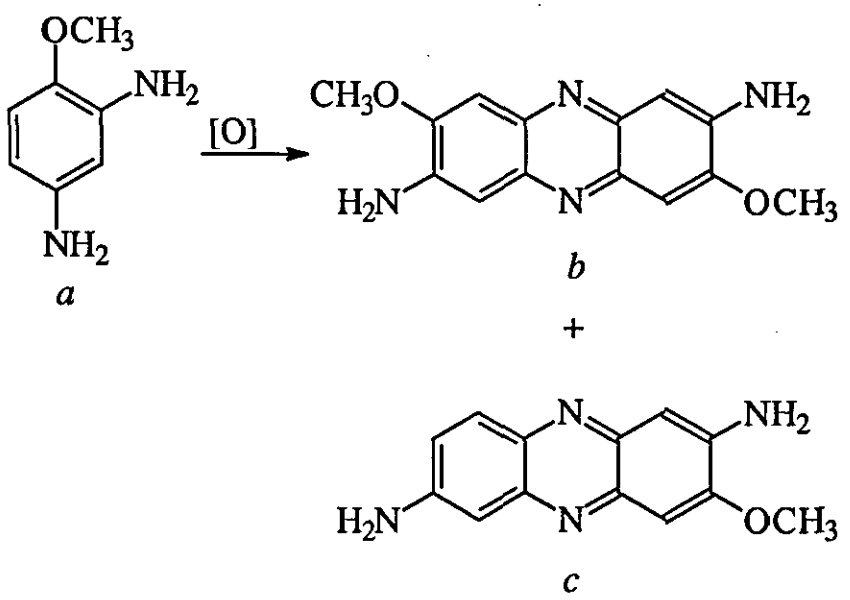


Fig. 1.20 Formation of phenazine derivatives from p-methoxy-m-phenylenediamine [56].

It was reported that the phenylenediamine compounds and their phenazine products were mutagenic.

The rates of formation of phenazines by cyclization of di and monoimines of N-(2-aminophenyl)-p-benzoquinone were studied by Loveless *et al.* [57]. This study proposed mechanisms for their formation. The diimines were formed by the oxidation of the corresponding diphenylamines with potassium ferrocyanide. Between pH 6 and 9 the diimines cyclised to form the corresponding phenazines. However, above pH 10, 2-hydroxyphenazine derivatives were formed rather than the corresponding 2-aminophenazine. Hydrolysis of the terminal imine could conceivably occur before or after cyclisation. It was found that hydrolysis preceded cyclization by allowing the conversion of diimine to phenazine to take place at a pH sufficiently low as to preclude hydrolysis of the diimine and then to increase the pH to 10.5 by addition of hydroxide ion. No hydrolysis of the phenazine was observed.

Low temperature photolysis of o-substituted azidobiphenyls led to the formation of phenazine or benzo[c]cinnoline derivatives [58]. When 2,2'-diazido-6,6'-dimethylbiphenyl (*a*, Fig. 1.21) was irradiated in a glassy matrix at 77K a tar like mixture was produced. This tar was examined by TLC. Two products were identified as the 1,10-dimethylbenzo[c]cinnoline (*b*) in 60% yield and the 1,6-dimethylphenazine (*c*) in 8% yield (Fig. 1.21).

The formation of the phenazine was found to be very temperature dependent and it was not formed at higher temperatures. A reaction pathway was put forward to explain the formation of the phenazine and benzocinnoline products (Fig. 1.22). The aziridine intermediates *c* and *d* are relatively strained but may be stable at low temperature, hence the temperature dependency.

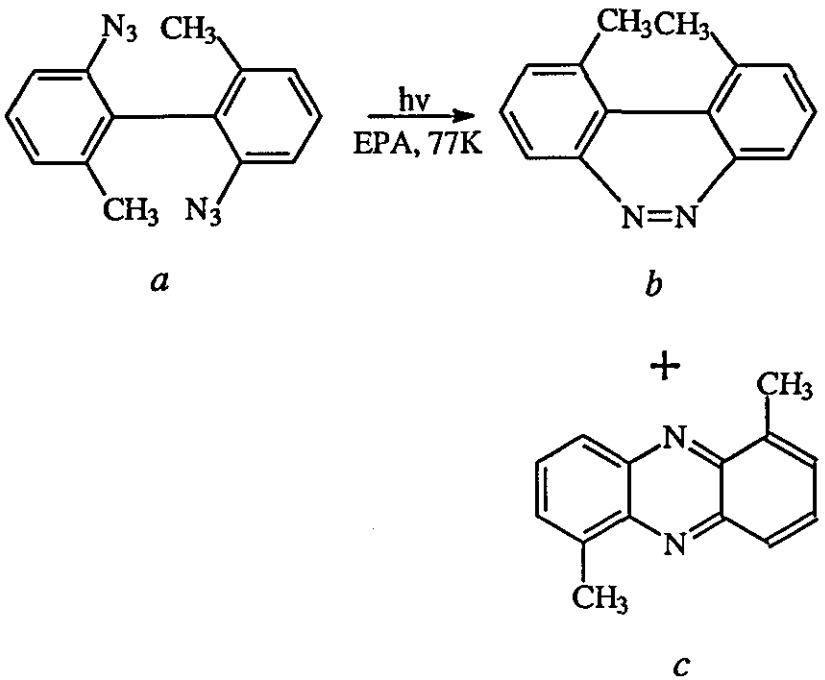


Fig. 1.21 Photolysis of 2,2'-diazido-6,6'-dimethylbiphenyl [58].

Triplet state resonance Raman spectroscopy of phenazine was studied by Kessler *et al.* [59]. The Raman spectrum of a solution of phenazine excited at 440 nm in the absence of an N_2 laser pulse exhibited a weak band at 1371 cm^{-1}. However, in the presence of the N_2 laser pulse this signal was increased twenty-fold in intensity. It was necessary to observe the Raman spectrum 200 ns after the N_2 pulse, as at shorter times a broad fluorescence overlapped the Raman bands.

Japar and Abrahamson studied the flash spectroscopy and photoreduction of phenazine [60] and indicated that it was possible for phenazine to form a dimer.

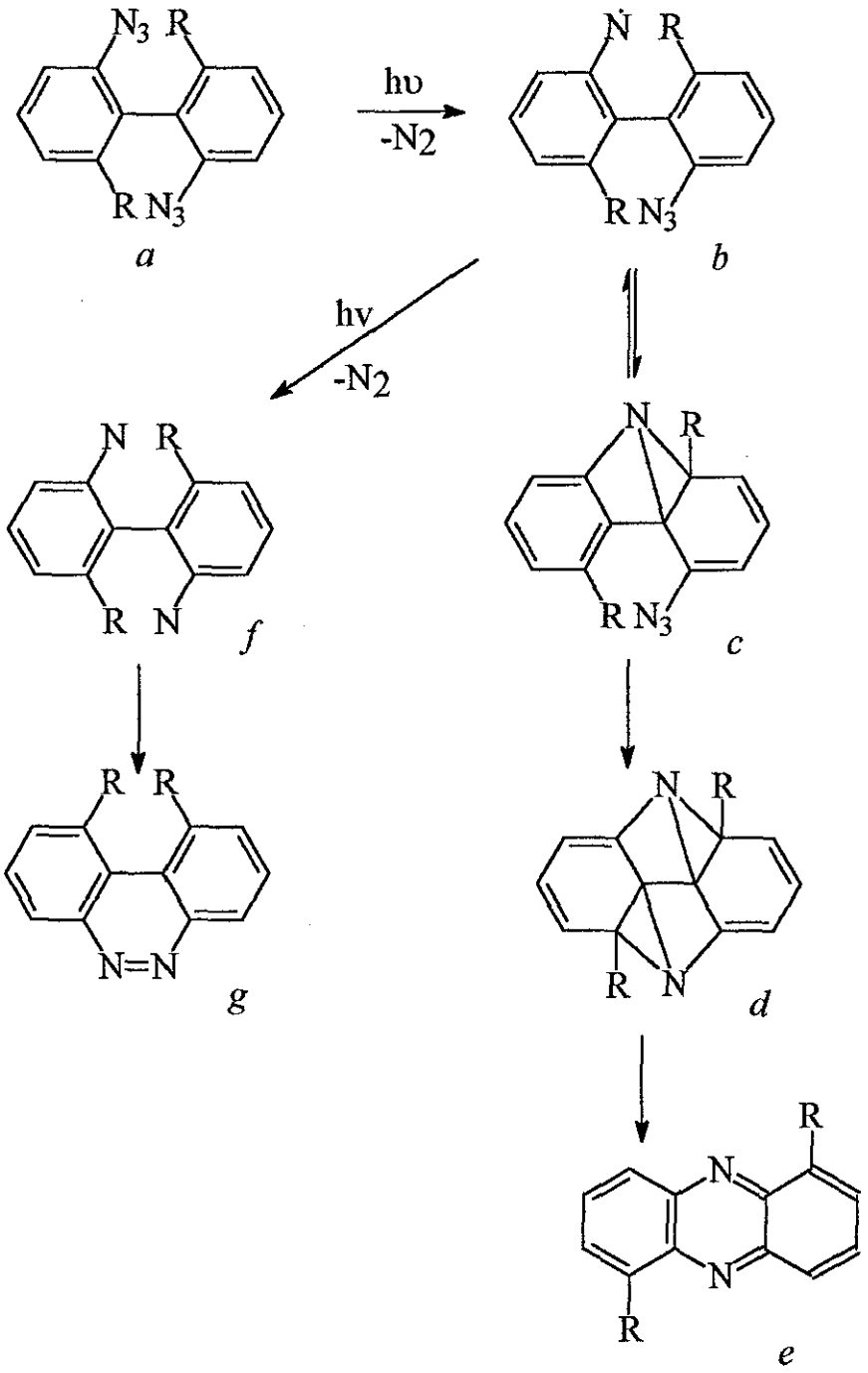


Fig. 1.22 Proposed pathway for formation of phenazines and benzo[c]cinnolines [58].

1.5.8 Formation of Bandroski's base from phenylenediamines

Lauer and Sunde reported on the formation and mechanism of formation of the trimeric Bandroski's base [61] from the oxidation of substituted p-phenylenediamine. However, the exact structure was unknown. In a later paper [62] Lauer and Sunde indicated that the structure shown in Fig. 1.23 was the correct structure for Bandroski's base. It may be possible for this type of compound to form in photographic solutions but steric hindrance due to the sidechains may limit its yield.

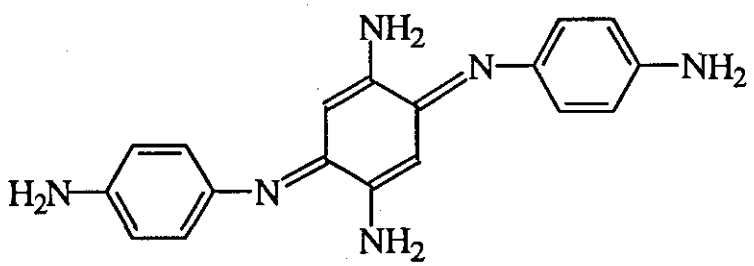


Fig. 1.23 Structure of Bandroski's base[62].

1.5.9 Reaction of phenylenediamine and benzoquinone

It is theoretically possible for p-phenylenediamine colour developing agents to undergo deamination to form benzoquinones in solution [40]. It may be possible for these benzoquinones to undergo reaction with the original p-phenylenediamines to form charge transfer complexes. A solid state reaction between p-phenylenediamine and p-benzoquinone has been documented by Singh and Singh [63]. When the two compounds were mixed in a mortar and pestle, a blue colour developed which ultimately changed to dark brown. Differential scanning calorimetric studies showed that the reaction products obtained from the solution and from the solid state were the same. Further studies showed that the reaction product was a 1:1 molecular complex of p-phenylenediamine and p-benzoquinone.

1.5.10 Electrochemistry of developing agents

The electrochemistry of phenylenediamines and related compounds have been studied in detail.

Pontius [64] gave an general overview of the electrochemistry of developing agents. Opallo looked at the solvent effect on the electro-oxidation of p-phenylenediamine [65]. A relatively stable radical cation is formed in aprotic solvents. It was shown that the solvent affects the electron transfer rate of the one electron electro-oxidation of phenylenediamine at a platinum electrode.

Bailey and Ritchie [66] looked at the cyclic voltammetry of the aqueous electrochemistry of p-benzoquinone. It was found that at high pH the doubly ionised hydroquinone product is formed.

1.6 Present study

The broad aims of the project were to investigate analytical methods for the determination of the degradation products and contaminants that might be found in used photographic developer solutions. This topic is of interest because of the possible environmental impact of the disposal of used developer solutions and because it might be possible to relate unexpected organic constituents of the solutions to anomalous or below standard performance of the developer solutions in processing laboratories. It was planned to examine the application of a range of analytical methods, including solid phase extraction, ion-pair chromatography and capillary electrophoresis for the extraction, isolation and identification of low levels of organic components from aqueous solutions used in the photographic industry.

These aims were formulated into a project which set out to:-

(a) develop separation methods for the separation of the components of colour developer solutions to provide a profile of the composition of the mixture.
(b) investigate the degradation of developer solutions and developing agents under different conditions,
(c) isolate and identify the major degradation products that are formed in these solutions.

Although the work would concentrate on processing solutions, it was intended that many of the analytical methods would also be applicable to environmental samples.

Chapter 2

Experimental

2.1 Liquid Chromatography

2.1.1 Chemicals

The developing agents CD-3 {methane sulphonamide, N-[2-(4-amino-3-methyl phenyl) ethyl amino] ethyl}-sulphate.H_2SO_4 (2:3 salt) and CD-4 (ethanol, 2-[(4-amino-3-methyl phenyl) ethyl amino] sulphate.HCl (1:1 salt)) were obtained from Kodak Ltd, Harrow, UK. HiPerSolv grade Sodium 1-heptanesulphonate and ammonium acetate were from BDH Ltd (Poole, Dorset, UK). HPLC grade ammonium acetate and tetrabutylammonium bromide from FSA (Loughborough, Leics., UK) were also used. Water of 18mΩ resitivity or less was purified on site using an Elgastat water deioniser system. Methanol and acetonitrile were HPLC grade from FSA (Loughborough, Leics., UK) and dichloromethane and acetic acid were laboratory grade.

Phorwite REU (Bayer, Cheshire, UK), Versa TL (lithium polystyrene sulphonate, National Adhesives) and BD-89 (diethylhydroxylamine, Honeywell Stein) were obtained from Kodak.

2.1.2 Developer solutions and developing agents.

Buffered solutions of CD-3 developing agent (4.8 g) and CD-4 developing agent (4.5 g) were prepared using laboratory grade potassium carbonate (BDH Ltd, Poole, Dorset) 25 g and 37.5 g, respectively, and diluted to one litre with deionised water. Similarly CD-3/sulphite and CD-4/sulphite solutions were prepared using CD-3 (4.8 g), CD-4 (4.5 g) and a two-fold molar excess of laboratory grade sodium sulphite (BDH Ltd, Poole, Dorset).

Commercial concentrates of RA4, RA100 and C-41 developing solutions and standards were obtained from Kodak. Formulations for

RA4/RA100 solutions are shown in Table 2.1 and C-41 developer solution in Table 2.2.

Component	RA4 formulation	RA100 formula
Water	800 ml	800 ml
Triethanolamine	5.5 ml	11 ml
Versa TL (lithium polystyrene sulphonate)	0.25 ml	0.25 ml
Potassium sulphite(45%)	0.5 ml	0.5 ml
N,N diethyl-hydroxylamine	3 ml	6 ml
Phorwite REU	2.3	1 g
Lithium sulphate	2.7	2 g
AntiCal No. 5	0.8	0.6 ml
AntiCal No. 8	0 ml	0.6 ml
Potassium chloride	1.8 g	6 g
Potassium bromide	0.02 g	0.028 g
CD-3	4.85 g	3.8 g
Sulphuric acid (20%, 7N)	0 ml	3.1 ml
Potassium carbonate	25 g	25 g

Table 2.1. Formulations of RA4/RA100 developer solutions [70]

Component	C-41 formulation
Water	800 ml
CD-4 developing agent	5.88 g
Sodium bromide	0.45 g
Pentetic acid penta-sodium salt (40%)	8.48 g
Sodium metabisulphite	3.6529 g
Potassium carbonate	37.5 g
Potassium hydroxide (48%)	0.9448 g
Hydroxylamine sulphate	3.403 g

Table. 2.2. Formulation of C-41 developer solution [70]

Fotospeed CKRA4/D (Fotospeed, Wilts, UK) colour paper processing solution was prepared according to the instructions. To prepare a working developer solution, solution A (10 ml) was added to water (454 ml) and stirred vigorously. Then whilst continuing to stir, solutions B (7 ml), solution C (20 ml) and the starter solution (9 ml) were added in that order.

Afga Color Process AP70 (C-41) (Agfa-Gevaert AG, Leverkusen 1, Germany) was also prepared according to the instructions. To prepare a working colour developer solution, solution A (50 ml) was added to water (420 ml) and stirred. Then whilst stirring, solution B (5 ml) and solution C (20 ml) were added, in that order.

Photocolor FP (Photo Technology, Herts, UK) C-41/RA4 compatible developing solution was prepared according to the instructions. To prepare a working developer solution one part of concentrate A (167 ml) was diluted with two parts of water (334 ml) and concentrate B (20 ml) was added whilst stirring continuously.

2.1.3 Samples for degradation

Samples used in the HPLC study of the degradation of developing agents and developing solutions (RA4, RA100 and C-41) were prepared by pipetting the fresh samples (2 ml) into individual stoppered clear glass vials (20 ml). Before analysis these samples were diluted with acetonitrile (5 ml). This mixture was then made up to volume in a volumetric flask (10 ml) with deionised water. The procedure used for the CD-3 sulphonates was slightly different. 100 mls of the CD-3/sulphite solution was degraded in a 250 ml covered beaker which was irradiated with sunlight and 2 ml portions were diluted with deionised water and analysed daily. A similar procedure was executed for CD-4/sulphite solutions

2.1.4 HPLC Instrumentation

Initial HPLC separations were performed using an LKB 2150 pump (LKB Produkter AB, Bromma, Sweden) and a Pye Unicam 4020 UV detector (Pye Unicam Ltd, Cambridge, UK) set at 254 nm. The eluents were pumped at 2 ml/min, later reduced to 1.3 ml/min . The samples (10 µl) were injected using a Rheodyne 7125 injector valve (Rheodyne Inc., Cotati, California, USA) fitted with a 20μl loop, onto a Supelco LC-18-DB analytical column (150 mm x 4.6 mm) packed with 5 μm base deactivated octadecylsilica (Supelco Inc., Bellefonte, PA, USA). A Supelco octylsilica guard column (20 mm) was fitted in front of the analytical column. A Shandon Hypersil BDS C_{18} analytical column (150 mm x 4.6 mm) (Shandon Scientific, Runcorn, Cheshire, UK) packed with 5μm base deactivated octadecylsilica was also used. The columns and injector were maintained at a constant temperature of 40°C using a Shimadzu CTO-6A column oven. The retention times were determined using a Hewlett-Packard HP3393 integrator (Hewlett-Packard, Waldbronn, Germany).

This system was superseded by a Hewlett-Packard HP1084B HPLC with a Hewlett-Packard HP1040A diode array detector (Hewlett-Packard, Waldbronn, Germany). The detector was controlled by a Hewlett-Packard HP-85B microcomputer. Data storage was facilitated by use of a Hewlett-Packard HP9133 hard drive, with a 3½ inch single-density single sided floppy disk drive. The detector recorded the chromatograms at up to eight different wavelengths ranging from 190 nm to 600 nm. Retention times and

integration areas were calculated by the HP1040A software and printed out on a Hewlett-Packard Thinkjet printer, together with the chromatograms for each specified wavelength. Chromatograms could also be plotted out on a Hewlett-Packard HP7470A plotter at a specified wavelength or a series of wavelengths. Clear glass sample vials of 2 ml capacity (Hewlett-Packard, Germany) with crimpable tops were obtained from Kodak. Sample injection volumes of 20 µl were used.

Chromatograms in the present studies were run and plotted out at 243 nm, unless otherwise specified. This wavelength was chosen because the absorption maxima for CD-3 and CD-4 were in this region.

The HP1040A DAD and HP1084B HPLC worked independently of each other, the only contact between them being the turning on and turning off signals, by means of solenoids, sent by the HP1084B to the HP1040A at the beginning and end of each analysis run. The same Supelco guard and analytical columns, as previously described, were used at an oven temperature of 40ºC.

A semi-preparative HPLC system consisting of the HP1084B chromatograph coupled to a Pye Unicam PU4020 detector set at 270 nm, was also used. A semi-preparative octadecylsilica column (250 mm x 10 mm), which had been packed at Kodak, was used. Injection volumes of up to 200 µl were possible, using the HP1084B. The mobile phase consisted of ammonium acetate buffer (3.08 g/l) adjusted to pH 4.8 with glacial acetic acid and acetonitrile was used as the organic modifier. A flow-rate of 3 ml/min was necessary for satisfactory separation of peaks and adequate retention times. The HP1040A detector was not used as such a high flow-rate could damage the flow-cell.

2.1.5 Mobile phases

A variety of mobile phases were prepared using different combinations of buffers and organic modifiers and evaluated.

Mobile phase ***I*** consisted of acetate buffer with ion-pairing agent:acetonitrile (83:17 v/v). The acetate buffer (pH 4.8) consisted of ammonium acetate (3.08 g), sodium heptanesulphonate (0.5 g) and glacial

acetic acid (3 ml) in deionised water to give a one litre solution. The organic modifier was composed of triethylamine (0.5 ml) diluted to one litre with acetonitrile.

Mobile phase *II* consisted of acetate buffer and organic modifier (80:20 v/v). The acetate buffer (pH 4.7) was composed of ammonium acetate (3.08 g) and glacial acetic acid (6 ml) made up to one litre with deionised water. The organic modifier used was methanol.

Mobile phase *III* was similar to *I* and consisted of ammonium acetate (3.08 g) and glacial acetic acid (3 ml) diluted to one litre with water (pH 4.7) but without ion-pairing agent. The organic modifier was triethylamine (0.5 ml) made up to one litre with acetonitrile.

Mobile phases *I, II* and *III* were used in the initial analyses of CD-3 developing agent, however, a modified mobile phase *IV* was used for the analyses of fresh and degraded developer solutions. This consisted of ammonium acetate (3.08 g) and tetrabutylammonium bromide (1 g) made up to one litre with water and adjusted to pH 5.6 with glacial acetic acid. The organic modifier was composed of tetrabutylammonium bromide (1 g) made up to one litre with acetonitrile but no triethylamine was used as all the columns used were base deactivated.

2.1.6 Chromatographic conditions

Various gradient elution conditions were used for the separation of developing agents, degradation products and other developer solution components.

2.1.6.1 Method *A*

The initial conditions used for the elution of CD-3 and its degradation products were, isocratic elution, acetate buffer/acetonitrile (83:17, mobile phase *III*), a flow-rate of 1 ml/min and detection at 243 nm.

2.1.6.2 Method *B*

The subsequent conditions used for the elution of CD-3 and CD-3 non-polar degradation products are shown in Table 2.3. The mobile phase used was mobile phase *III* (see 2.1.5). A flow-rate of 1.3 ml/min and detection at 243 nm were also used.

Time/mins	*% Organic modifier (acetonitrile)*
0	17
10	60
20	60

Table. 2.3 Gradient elution conditions for CD-3 developer solutions. (mobile phase I, see 2.1.5)

2.1.6.2 Method *C*

As the developing solutions, such as RA4 and RA100, contained components other than the developing agent and its degradation products, it was necessary to modify the HPLC elution conditions. The elution condition conditions for developer solutions containing CD-3, CD-3 degradation products and other components, such as Phorwite REU are shown in Table. 2.4. The mobile phase used here was mobile phase *IV* see 2.1.5. A flow-rate of 1.3 ml/min and detection at 243 nm.

Time/mins	*% Organic modifier (acetonitrile)*
0	11
7	28
15	32
16	62
25	62

Table 2.4 Elution conditions for the separation of CD-3, CD-3 degradation products and other components in developer solutions (mobile phase IV, see 2.1.5).

2.1.6.3 Method *D*

The elution conditions for developing solutions containing CD-4 and its degradation products are shown in Table. 2.5. The same conditions were used in the analysis of C-41 developer solutions. The mobile phase used here was also mobile phase *IV* see 2.1.5. A flow-rate of 1.3 ml/min and detection at 243 nm were also used.

Time/mins	*% Organic modifier (acetonitrile)*
0	5
7	28
15	32
16	62
25	62

Fig. 2.5 Elution conditions for the HPLC separation of CD-4 and CD-4 degradation products (mobile phase IV).

2.1.7 LC-MS

Liquid chromatography-mass spectrometry systems (LC-MS) were used at Kodak Ltd, Harrow for the analysis of developing agent and developer solutions using a pH 4.7 acetate buffer (7.7 g/l ammonium acetate, glacial acetic acid) with acetonitrile organic modifier. The chromatographic conditions used were similar to those in Table 2.5. Two different systems were used. The first was a Hewlett-Packard HP1090 coupled to a Finnegan quadrapole mass spectrometer, using a Finnegan thermospray interface. This system used a flow-rate of 1.3 ml/min and the same Supelco columns as previously described. The second LC-MS system consisted of a Hewlett-Packard HP1090 HPLC coupled to a VG Quattro quadrapolar mass spectrometer, by a VG electrospray interface (VG Scientific, Cheshire, UK). This system used a Shandon Hypersil BDS octadecylsilica column (150 mm x 2.1 mm) and a flow-rate of 0.4 ml/min.

2.2 Capillary electrophoresis

2.2.1 Chemicals

Several other chemicals were used in addition to the chemicals used in Chapter 2.1.1. General purpose potassium carbonate, sodium sulphite and disodium orthophosphate were from BDH (Poole, Dorset, UK). Phorwite REU (Bayer UK, Cheshire) was obtained from Kodak. Lithium Hydroxide and sodium lauryl sulphate were reagent grade from FSA (Loughborough, Leics, UK). General laboratory reagent grade benzamide was from Aldrich Chemical Co. Ltd. (Gillingham, Dorset, UK). Dilute hydrochloric acid (3 M) was prepared by diluting concentrated hydrochloric acid (BDH, Poole, Dorset, UK) with deionised water (1:4).

2.2.2 Buffers

The buffer solution used in the analyses of CD-3, Phorwite and RA4 consisted of potassium carbonate (6.0 g) made up to one litre and it had a pH of 11.5. The micellar forming buffer used in the analyses of CD-3, Phorwite and RA4 was composed of sodium dihydrogen orthophosphate (0.04 M, pH 7.48), pH adjusted using HCl (3 M), sodium dodecyl sulphate (0.0057 M) and acetonitrile (90:10 v/v). The buffer used in the analyses of CD-4, CD-4 degradation products and C-41 developer solutions consisted of disodium orthophosphate (5.68 g) made up to one litre with water and adjusted to pH 6.77 with HCl (3 M). The CD-3, CD-3/sulphite, CD-4 and CD-4/sulphite solutions were prepared as in Chapter 2.1.3. A benzamide marker solution was prepared by diluting benzamide (5 g) to one litre with deionised water. One or two drops were added to samples, prior to analysis to monitor the electroosmotic flow.

2.2.3 Samples for degradation

The solutions for the CE degradation studies were prepared as for the HPLC studies. Samples of developer solutions (C-41, RA4) were pipetted (2 ml) into individual stoppered clear glass vials (20 ml). Before analysis these samples were diluted and made up to volume in volumetric flasks (10 ml) with deionised water. Acetonitrile, which had been added in the HPLC studies was not added as the CE studies centred more on the separation and

analysis of polar products. The non-polar products were not separated and were eluted with the electroosmotic flow.

2.2.4 CE Instrumentation

The CE separations were carried out on a Beckman P/ACE System 2050 capillary zone electrophoresis system (Beckman Instruments, High Wycombe, UK), using a fused silica capillary (Beckman) with an internal diameter of 50μm, a total length of 57 cm and a length from inlet to detector of 50 cm. The samples were injected under pressure using a 1 to 3 second pressure injection, the time period depending on sample concentration. Separations were performed at 25°C using a voltage of +15 kV. Data was collected at 254 nm, using a P/ACE UV Absorbance Detector and a 2 Hz collection rate. The capillary was conditioned each day by rinsing with a lithium hydroxide solution (0.1 M) and between each run the capillary was washed with the appropriate running buffer solution for 30 seconds.

2.3 Isolation and identification of developing agent degradation products

2.3.1 Chemicals

Several other chemicals were used in addition to the chemicals used in Chapter 2.1.1. Laboratory grade dichloromethane and chloroform were used. Flash chromatography silica, HPLC grade methanol and HPLC grade acetonitrile were obtained from FSA (Loughborough, UK). Reagent grade potassium permanganate and TLC grade silica GF_{254} were from Merck (Poole, UK). TLC aluminium sheets coated with silica gel 60 F_{254} (20 cm X 20 cm) were from E. Merck (Darmstadt, Germany).

2.3.2 TLC and flash chromatography eluants

The TLC eluent used for the elution of CD-3 degradation products consisted of dichloromethane/acetonitrile (80:20 v/v). The TLC eluent for the elution of CD-4 degradation products consisted of dichloromethane/methanol (80:20 v/v), with potassium carbonate (5 g) added. These proportions were varied over repeated elution runs to give optimum separation of products.

Products were eluted from a flash chromatography column using dichloromethane/acetonitrile. The proportions were varied from a high percentage of dichloromethane to a high percentage of acetonitrile over time to allow a gradient elution of degradation products.

2.3.3 Apparatus

A large preparative scale TLC tank capable of holding five large TLC plates (1 m x 20 cm)was used. The TLC plates were prepared by mixing silica gel with water (2.4:1 w/v) and mixing well. This slurry was then spread, 0.2 mm thick, onto glass plates (1 m x 20 cm) using a TLC spreader (Shandon Scientific, Cheshire, UK). These plates were allowed to dry overnight and activated in an oven, at 130°C, for 1½ hours before use. TLC bands were eluted using 2.5 litres of eluant and scraped off the plates using a spatula.

A No. 4 sintered glass crucible was used for the filtration and collection of degradation products from degraded developer solutions. The

precipitates were filtered under vacuum using a water pump and washed with deionised water. They were then air dried using the water pump before being placed in a dessicator overnight.

A large glass flash chromatography column (50 cm x 7 cm) was wet packed with a flash silica-dichloromethane slurry to a depth of 25 cm. A glass wool plug was used to retain the silica and a thin layer (1 mm) of acid washed sand, mixed with dichloromethane, was placed on top of the silica. The sample, dissolved in a minimum amount of dichloromethane, was loaded onto this sand.

Analytichem Bond Elut solid phase extraction cartridges (Varian, Harbor City, USA) were used. These cartridges consisted of plastic syringes (1 cm^3 capacity) filled with silica bonded phases. The bonded phases included NH_2, OH, SAX, SCX, C_{18}, C_8, C_2. Before use the cartridges were conditioned by passing 2 ml of the elution solvent through them and then washing with water. At this point the cartridges were not allowed to dry out. The sample was then passed through the cartridge and the analyte of interest retained by the bonded phase. It was then washed using water or a similar solvent to elute off impurities. The analyte was subsequently washed off the bonded phase using a suitable elution solvent (e.g. methanol or acetonitrile).

2.3.4 Spectrometric instrumentation

The proton NMR spectra were recorded at Loughborough using a Bruker AC250 250 MHz NMR spectrometer. The instruments used to run proton NMR spectra at Kodak were a JEOL GX-400 with 5 mm C/H dual probe for spectra and decoupling and a Varian Unity +400WB with 5 mm indirect probe for COSY NMR spectra. All samples were run in deuterated chloroform except for the purple CD-3 compound, which was run in either deuterated chloroform, DMSO and deuterated acetone. Mass spectra were recorded on a VG Instruments (VG. Cheshire UK) ZAB-2F mass spectrometer. Raman spectra were recorded at Kodak on a Perkin-Elmer 2000 FT-Raman spectrometer. Fluorescence spectra were recorded on a Perkin Elmer LS 50B fluorescence spectrometer (Perkin Elmer, Beaconsfield, Bucks, UK) and UV spectra were run on a Unicam 8700 spectrophotometer (Unicam Ltd, Cambridge, UK).

Chapter 3

HPLC analysis of CD-3 developing agent and developer solutions

3.1 Introduction

CD-3 developing agent is used in RA4 and RA100 developer solutions to develop the latent image on photographic paper. RA4 and RA100 developers differ in the concentrations of the components used (Table.2.1). RA4 is the older formulation and is now being superseded by RA100 which has slightly lower concentrations of CD-3, Phorwite and diethylhydroxylamine. The only UV detectable species in developer solutions are the developing agent, stain reducer (Phorwite), the developing agent degradation products and Versa TL (lithium polystyrene sulphonate). Versa TL is present in small concentrations.

As the developer solution is used to process photographic paper the developing agent is consumed, some developing agent is lost to atmospheric oxidation, dyes are leached out of the paper, bromide builds up in solution and a multitude of other changes occur. The aim of the project was to develop a method of analysis for CD-3 developing agent and to apply this method to fresh and degraded RA4/ RA100 developer solutions for the analysis of developing agent, degradation products and other developer solution components such as Phorwite. A profile of CD-3 degradation, along with the degradation of the other components could then be constructed.

3.2 HPLC analysis of CD-3 developing agent

Before studies into the degradation of the colour developing agent CD-3 could be instigated, it was necessary to develop a method of analysis. This method could then be used to monitor developer solutions as they degraded. Several factors were taken into consideration, in particular resolution, retention time and peak shape. The separation used method *A* (Chapter 2.1.5) The initial mobile phase chosen consisted of acetate buffer/acetonitrile (83:17 v/v), this mobile phase was based on the method used in a Kodak method for the determination of CD-3 in developer solutions [23], see Chapter 2.2. A chromatogram of fresh CD-3 solution is shown in Fig. 3.1.

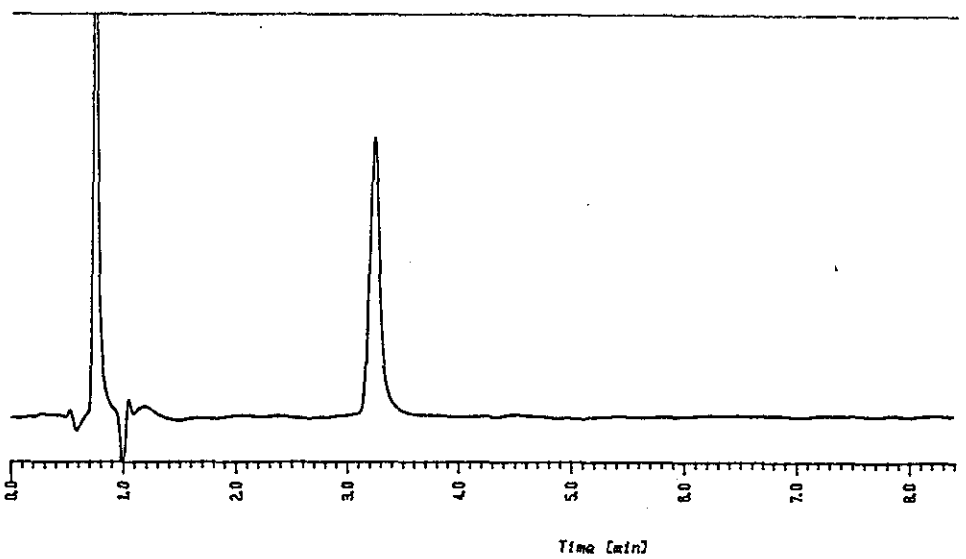


Fig. 3.1 Chromatogram of fresh CD-3 (method B, detection at 243nm, Supelco column)

As CD-3 has a primary amine group and a sulphonamide group, its overall molecular charge can be changed, depending on pH. The pH of the mobile phase was varied to increase or decrease the retention of CD-3. A chromatogram of CD-3 analysed using a pH 4.97 acetate buffer is shown in Fig. 3.2. The retention time of CD-3 was 3.2 mins. A pH 6.77 disodium hydrogen phosphate buffer was used to further increase retention time to 8.1 mins (Fig. 3.3). Finally a pH 8.2 disodium hydrogen phosphate buffer

increased the retention time still further to 8.6 mins by deprotonating the primary amine of the CD-3 molecule (Fig. 3.4).

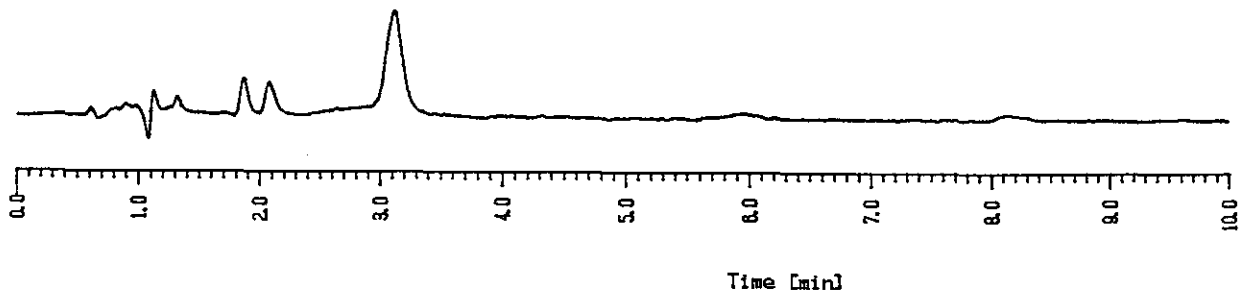


Fig. 3.2 Chromatogram of CD-3 (pH 4.97 acetate buffer/acetonitrile (83:17 v/v), Supelco column)

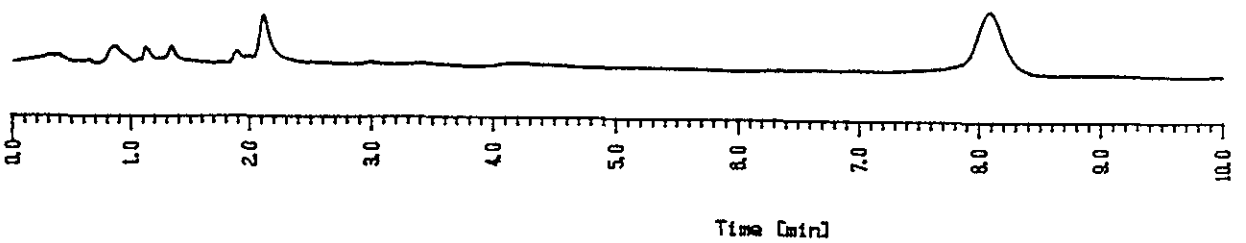


Fig. 3.3 Chromatogram of CD-3 (pH 6.77 phosphate buffer/acetonitrile (83:17 v/v), detection at 243 nm, Supelco column).

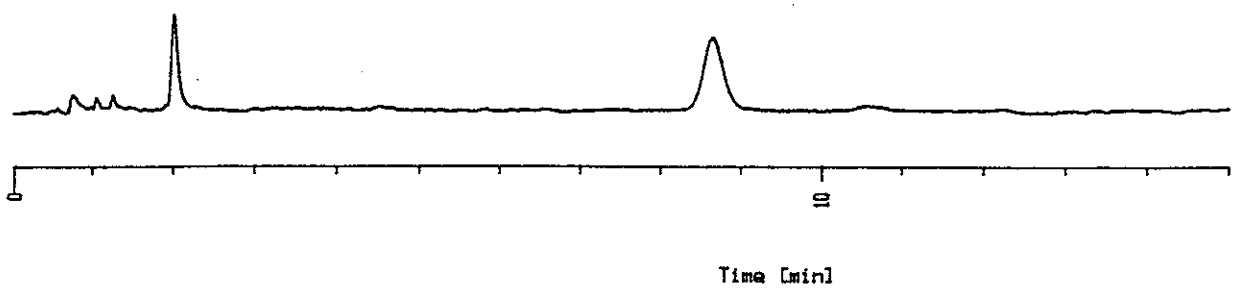


Fig. 3.4 Chromatogram of CD-3 (pH 8.2 phosphate buffer/acetonitrile (83:17 v/v), detection at 243 nm, Supelco column).

Peak shape was not ideal and tended to be broad using the buffers described and it was found that a pH 5.6 acetate buffer/acetonitrile gradient system, (method ***B*** see Chapter 2.1.5) gave a good separation of CD-3 with a good peak shape and adequate retention time. The % organic modifier was varied from 11 % to 28 % over 7 mins. This method was later modified for the analysis of CD-3 sulphonates and Phorwite, by the addition of t-butylammonium bromide ion-pair reagent (method ***C***, pH 5.6 acetate buffer, t-butylammonium bromide and acetonitrile organic modifier). A typical gradient elution of CD-3 eluted using method ***C*** is shown in Fig. 3.5. The developing agent elutes with a retention time of 7.0 minutes.

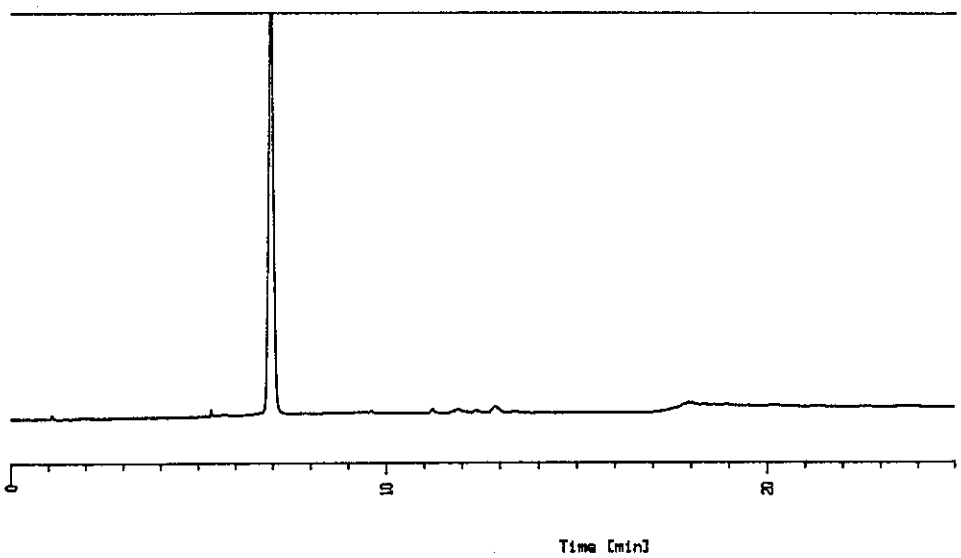


Fig. 3.5 Chromatogram of fresh CD-3 (method C, detection at 243 nm, Supelco column).

A calibration plot for CD-3, run under the above conditions, is shown in Fig 3.6. As CD-3 is subject to atmospheric oxidation it was necessary to add ascorbic acid to the prepared CD-3 solutions as a preservative. Various concentrations of CD-3, with ascorbic acid as antioxidant (0.7 g/l), were run in triplicate and the graph had a good correlation coefficient (r^2= 0.9993).

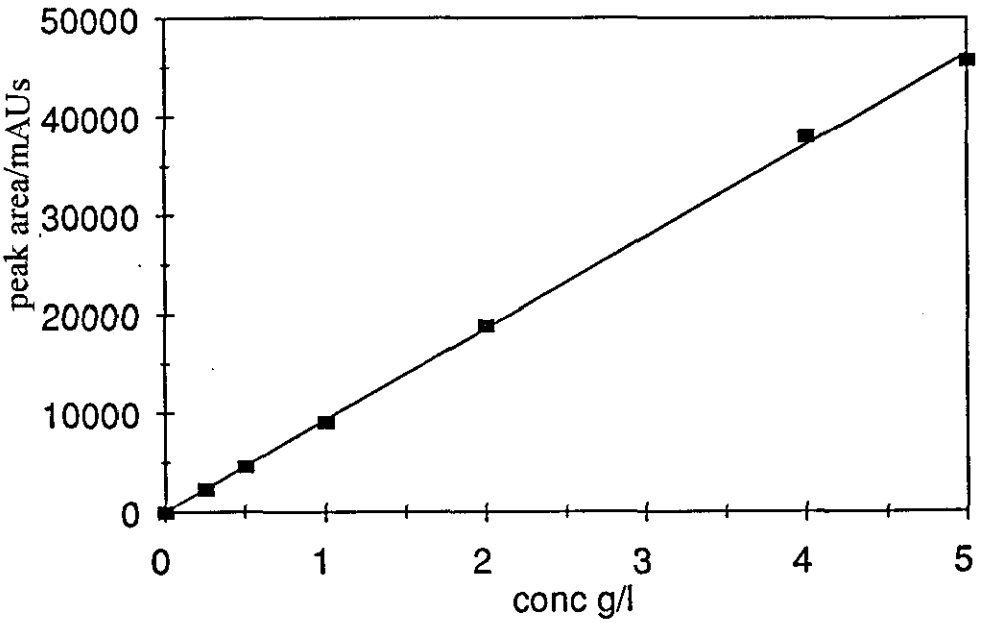


Fig. 3.6 Calibration plot for CD-3 (method C, 20 µl injection volume, detection at 243 nm)

3.3 HPLC analysis of Phorwite.

Phorwite REU is a stain-reducing agent found in RA4 and RA100 developer solutions (see Chapter 2.1.2). Very little is known about its chemistry in the context of photographic developer solutions but its optical brightening properties have been studied extensively. As sulphite has been largely removed from paper developer solutions, such as RA4 and RA100, the level of non-polar degradation products formed from oxidised developing agents has increased [9]. Such degradation products can build up in solution and cause staining of the photographic paper. Phorwite REU seems to counteract this staining but its role in this process is not fully understood.

In industry, Phorwite is routinely analysed by spectrophotometric methods rather than by chromatographic means [67]. However, a Kodak method for the liquid chromatographic separation of Phorwite has been published [30]. This method found up to nine peaks but was unpredictable as they were not reproducible.

The current studies undertook to develop a robust method for the analysis of Phorwite. The same method, method *C*, as used for CD-3, CD-3 sulphonates and RA4/RA100 (see later) developer solutions was used. The gradient elution which went from 11 % acetonitrile to 28 % acetonitrile over 7 mins, to allow for separation of CD-3 and then from 20 % acetonitrile to 32 % acetonitrile over 8 mins to allow separation of Phorwite which was ion-paired with tetrabutylammonium bromide. The Phorwite components eluted with retention times of between 9.5 mins and 14 mins. This method proved to be quantitative and reproducible for the analysis of Phorwite. A calibration graph for the three main peaks is shown in Fig. 3.7.

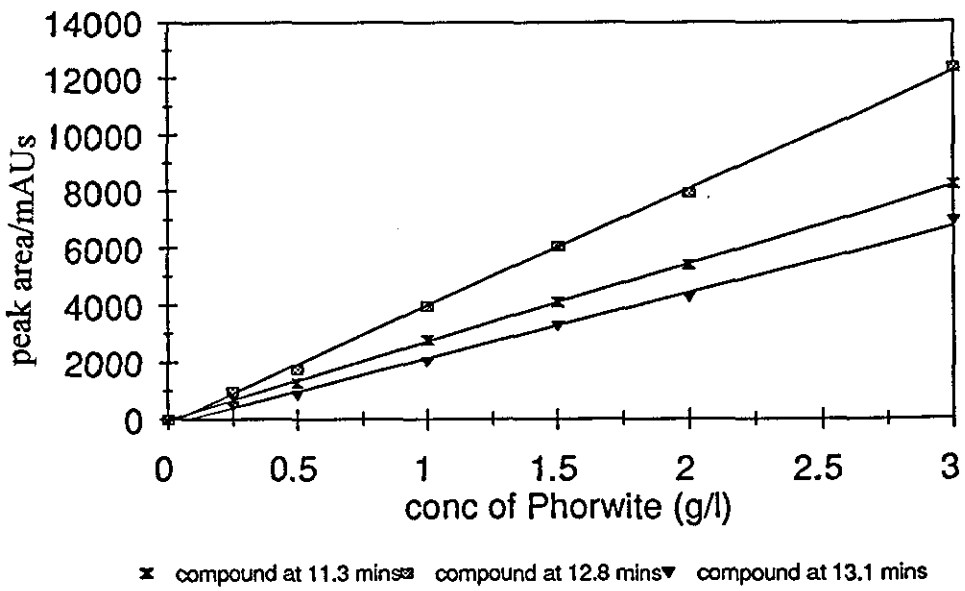


Fig. 3.7 LC calibration plot for main Phorwite peaks. (method C, 20 µ l injection, detection at 243 nm, Supelco column)

A chromatogram of a freshly prepared Phorwite is shown in Fig. 3 8. Nine (possibly ten) peaks can be seen. Phorwite is a bulk chemical which relies more on its optical properties rather than on exact composition. It may be that several different compounds are formed by the various steps used in the manufacture process [71]. All peaks have very similar UV spectra (see Fig. 3.9).

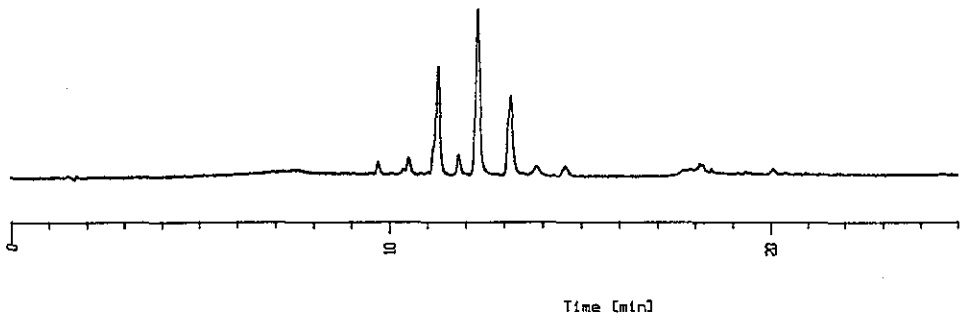


Fig. 3.8 Chromatogram of fresh Phorwite (method C, detection at 243 nm, Supelco column).

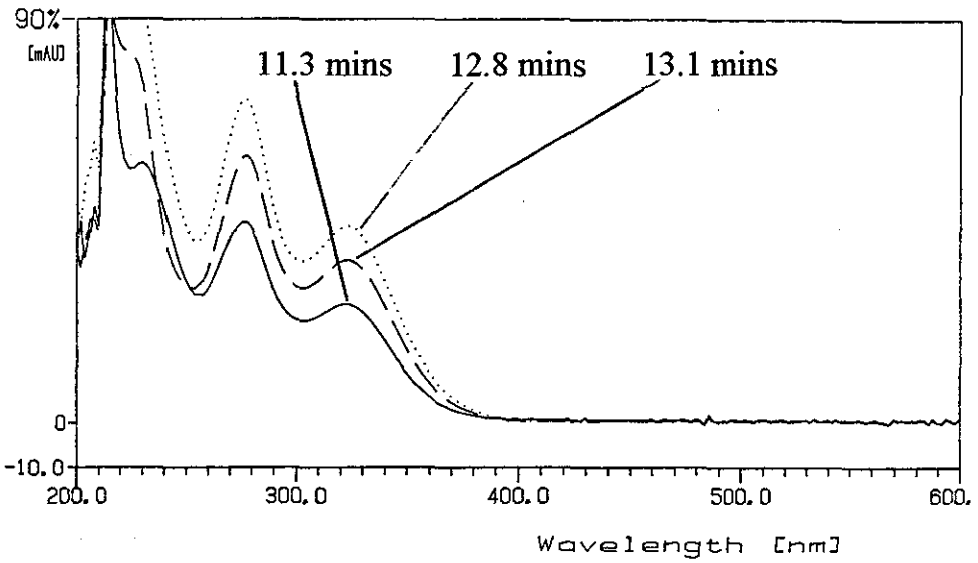


Fig. 3.9 Spectra of Phorwite peaks.

3.4 HPLC analysis of RA4 developer solutions.

The examination of RA4 developer solutions was based on the analysis of CD-3 developing agent and Phorwite. It was necessary to separate

the developing agent CD-3, the stain reducing agent Phorwite and any degradation products which may be formed.

The HPLC gradient for elution and separation of a typical RA4 developer solution is shown in Fig. 3.11 and a typical chromatogram of fresh RA4 is shown in Fig. 3.10. The developing agent CD-3 was eluted using a slow ramp of organic modifier, from 0 to 7 mins and had a retention time of 6.9 mins. As the stain reducing agent, Phorwite, (Fig. 1.6) contains ionisable sulphonate groups it was necessary to include an ion-pairing agent, tetrabutylammonium bromide (0.1 % w/v), in the mobile phase to facilitate retention on the non-polar octadecylsilica column. Phorwite needed a flatter gradient for separation of components and a ramp from 28 % acetonitrile to 32 % acetonitrile over 8 mins was used. Phorwite is present in RA4 as up to nine peaks, ranging in retention times from 9.5 mins to 14 mins. Finally a steep ramp, from 15 to 16 mins, followed by isocratic elution to 25 mins, was needed to elute the non-polar CD-3 degradation products which will be described in more detail in Chapter 6.

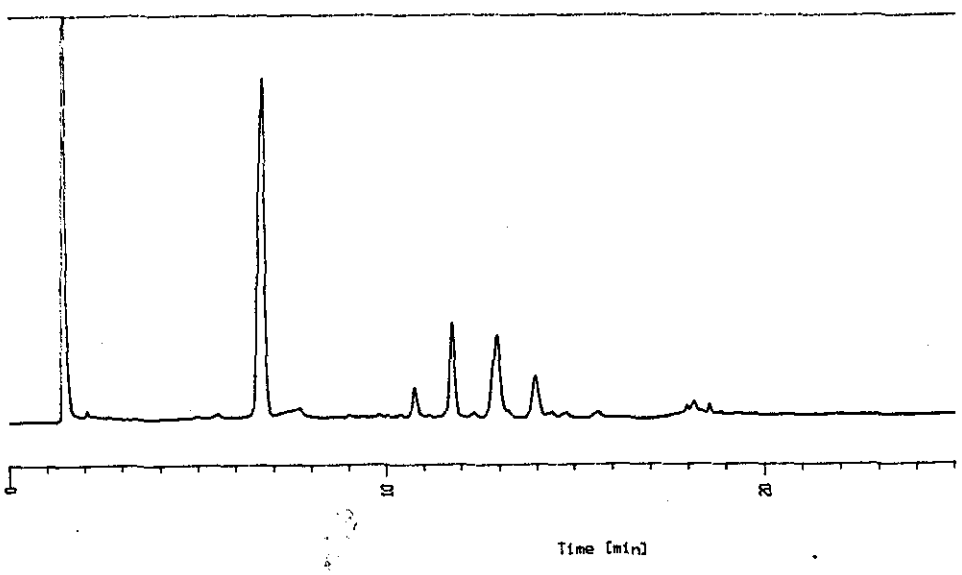


Fig 3.10 Chromatogram of fresh RA4 developer solution. (method C, detection at 243 nm, Supelco column)

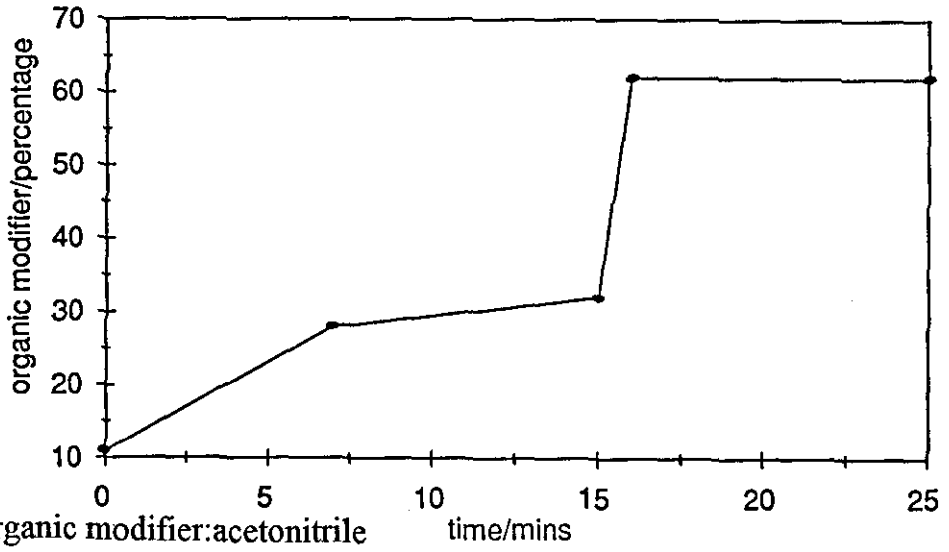


Fig. 3.11 HPLC gradient for RA4 developer solutions (see method C, chapter 2.1.6.2)

3.5 Degradation studies

After a method of analysis had been developed, the degradation of CD-3 was investigated. It was then necessary to investigate the reaction of CD-3 with other compounds in the developer solutions, such as sulphite, and finally to look at the degradation of other compounds, such as Phorwite. At this stage the degradation of RA4 developer solutions would be investigated.

3.5.1 Degradation of CD-3 solutions

Before conducting studies on the degradation of developer solutions. It was necessary to investigate the degradation pattern of CD-3 developing agent. Studies were instigated where different concentrations of CD-3 were allowed to degrade with time. Ascorbic acid was added to the solutions to slow down the rate of degradation and allow for more accurate monitoring. Various concentrations of CD-3 and ascorbic acid were prepared namely 150:100 mg/l; 450:250 mg/l; 1350:900 mg/l and 4050:2700 mg/l respectively. The degradation of these samples were studied over a number of days. Two 36 day old samples are shown in Figs 3.12 and 3.13. As they degraded, the solutions turned purple in colour. This corresponded to the purple colour of p-quinonemonoimine reported by Nickel [45] as p-phenylenediamine underwent deamination.

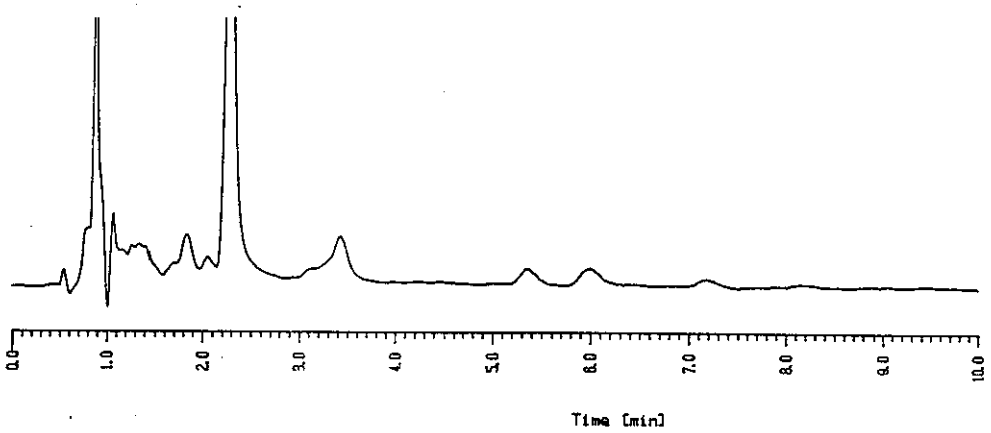


Fig. 3.12 Chromatogram of CD-3/ascorbic acid (450:250 mg/l. method A, detection at 243 nm, Supelco column).

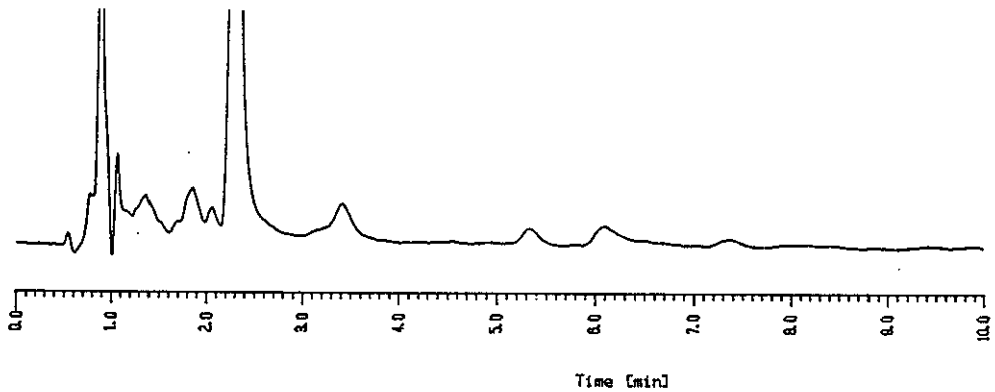


Fig. 3.13 Chromatogram of CD-3/ascorbic acid (1350:400 mg/l, method A, detection at 243 nm, Supelco column).

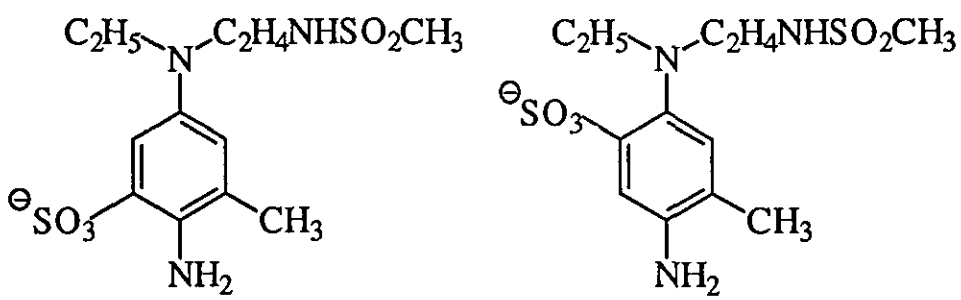
A range of degradation products was formed. Although these samples were over a month old, CD-3 could still be found in the more concentrated solutions. The more concentrated solutions also contained precipitated tar-

like material. A more detailed study of these precipitates is given in Chapter 4.

3.5.2 Formation of CD-3 sulphonates

Another important component of developer solutions is potassium sulphite, although it has now been replaced somewhat by diethylhydroxylamine in RA4 and RA100 solutions. Sulphite is used as an oxygen scavenger and oxidised developing agent scavenger. In this respect it can compete with the couplers found in photographic paper for the oxidised developing agent. Therefore it is preferential to keep sulphite concentration to a minimum level.

A method of analysis identical to that used for the analysis of RA4 developer solutions (see chapter 3.5) was used. CD-3 sulphonates contain sulphonic acid groups, as does Phorwite and can be separated by the same ion-pair chromatographic method. Previous studies [15] had indicated that the CD-3 m-sulphonate had a λ_{max} of 310 nm and the CD-3 o-sulphonate had a λ_{max} of 290 nm. This difference was used to identify peaks, using the diode array detector.



CD-3 m-sulphonate *CD-3 o-sulphonate*

Fig. 3.14 Structures of CD-3 sulphonates.

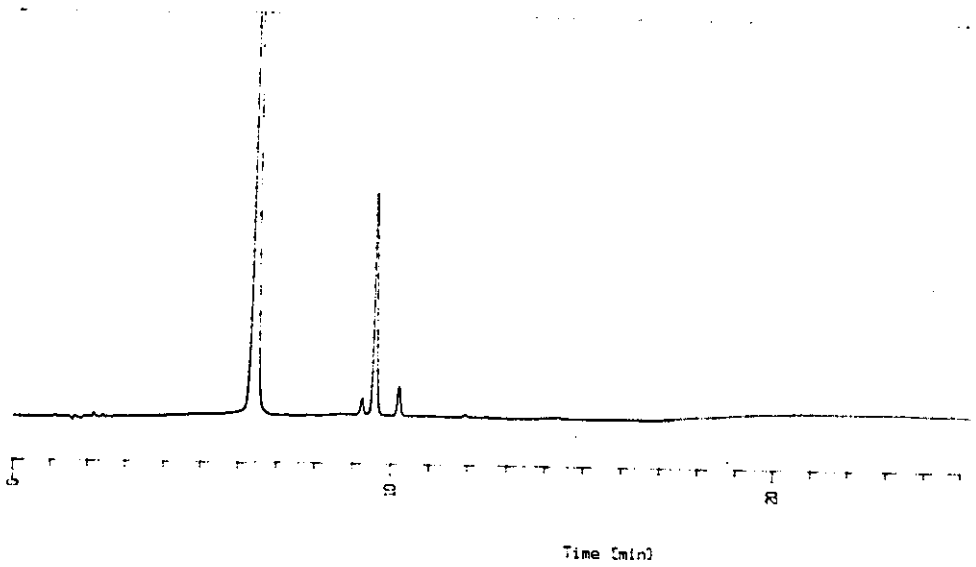


Fig. 3.15 Chromatogram of 1 day old CD-3/sodium sulphite solution (method C, detection at 243 nm, Supelco column).

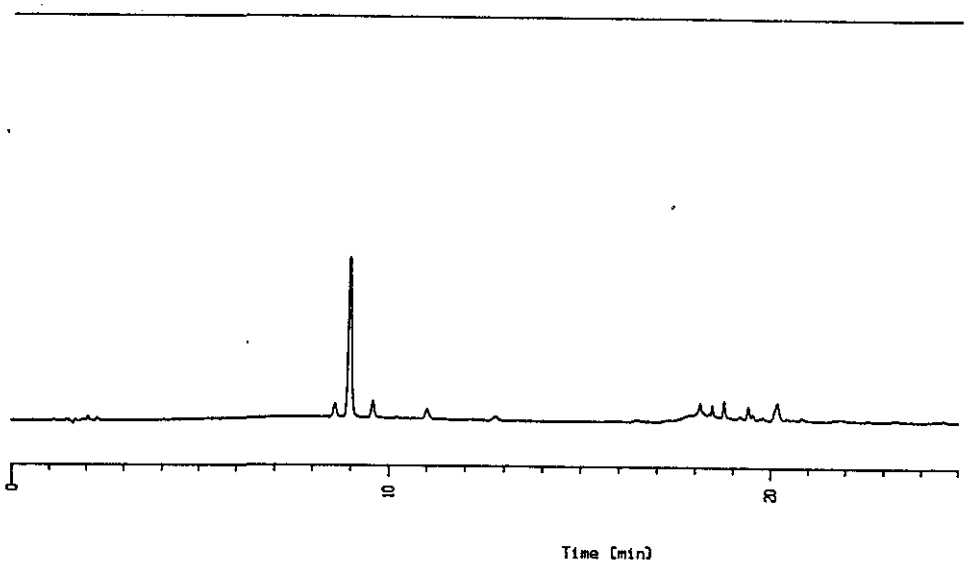


Fig. 3.16 Chromatograph of 14 day CD-3/sodium sulphite solution (method C, detection at 243 nm, Supelco column).

The m-sulphonate of CD-3 (Fig. 3.14) had a retention time of 9.0 mins and the o-sulphonate (Fig. 3.14) had a retention time of 8.6 mins (see Fig. 3.15). The m-sulphonate is formed first, followed by the o-sulphonate. Over prolonged time several other compounds are formed including another sulphonate with a retention time of 11 mins (see Fig. 3.16.). It may be that disulphonates are formed, as the UV spectra are similar to the m- and o-sulphonates, but this was only speculation. A graph of the degradation of a CD-3/sulphite solution with time is shown in Fig. 3.17.

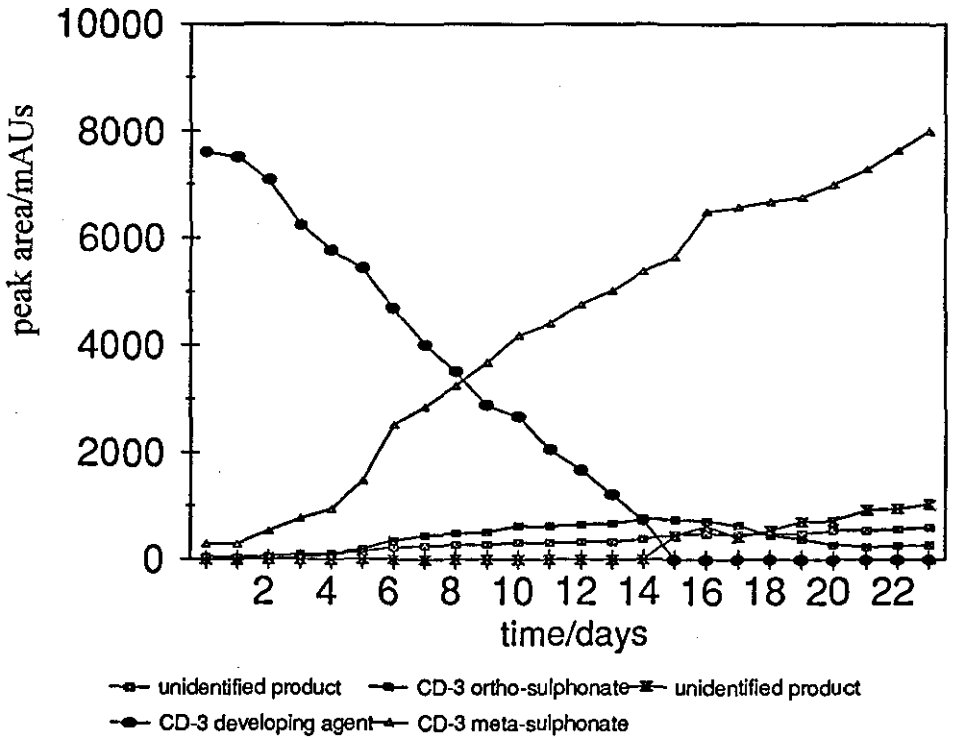


Fig. 3.17 Degradation of CD-3/SO_3 with time (method C, detection at 243 nm, Shandon column).

As sulphite has largely been replaced by diethylhydroxylamine in RA4 and RA100 developers no further studies were undertaken.

3.5.3 Degradation of Phorwite

A solution of Phorwite, irradiated by sunlight, was analysed over a time period of twelve days using the method already described for the analysis of Phorwite (chapter 3.4) and a graph of concentration of main Phorwite peaks (as in Fig. 3.8) versus time is shown in Fig. 3.18.

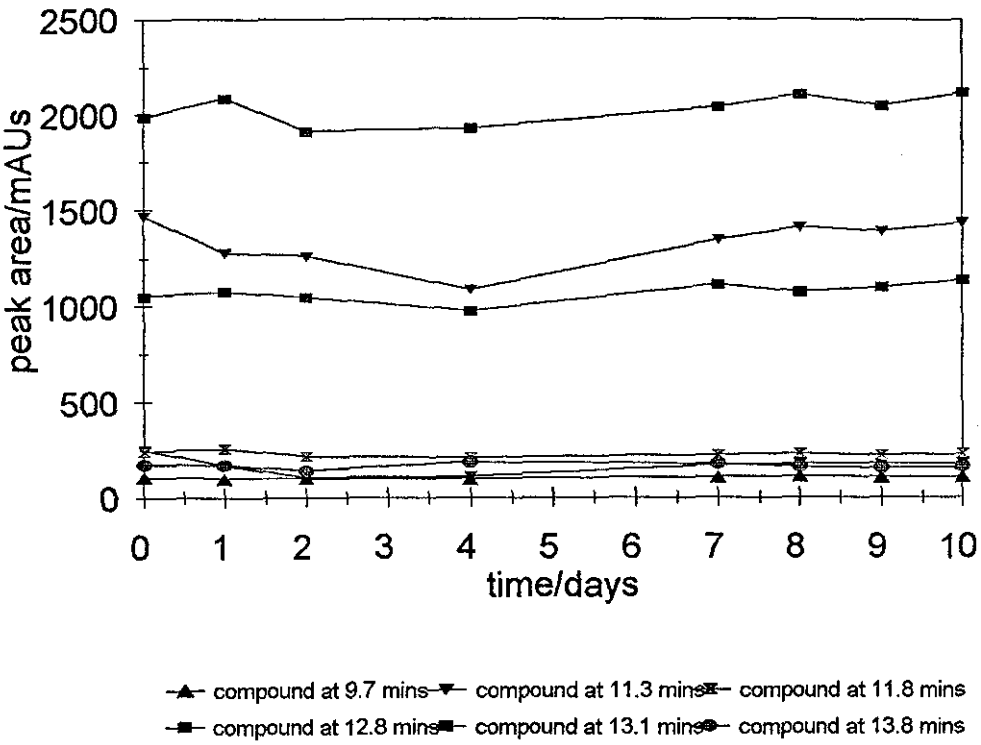


Fig. 3.18 Graph of peak area versus time for Phorwite (method C, detection at 243 nm, Shandon column).

All the peaks varied slightly over time in a random manner but this probably had more to do with sampling error than with variations in concentration. The findings, which show that Phorwite does not degrade, seemed to contradict those found previously [31].

3.5.4 Degradation of RA4 developer solutions

Degradation studies on RA4 solutions were undertaken. A number of samples of RA4 were placed in individual sealed vials (see Chapter 2.1.3) and allowed to degrade in sunlight, at room temperature, with time. Each day, a vial was made up to volume and this vial formed a representative sample of the degraded RA4 solution. A chromatogram of 24 day old RA4 is shown in Fig. 3.19.

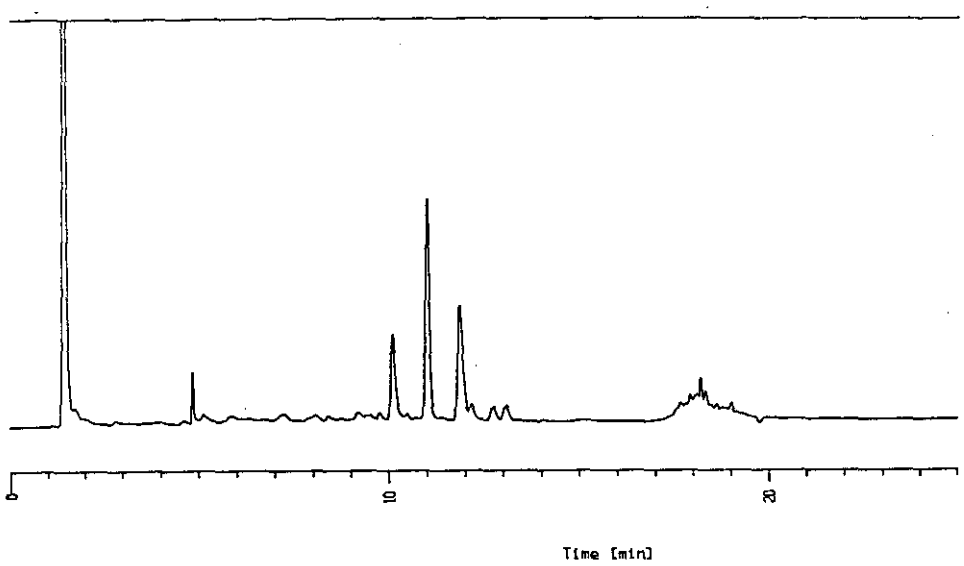


Fig. 3.19 Chromatogram of 24 day old RA4 developer solution (method C, detection at 243 nm, Supelco column).

By this time the developing agent CD-3 has completely degraded and a range of non-polar products with retention times ranging from 17 mins to 21 mins have appeared. The proliferation of products makes resolution of individual compounds difficult, however, several of these non-polar products were separated and isolated by TLC and identified using mass spectrometry and NMR (see Chapter 4). These products were yellow, blue and purple in appearance. As the absorption coefficients of the degradation products were not known, it was not possible to calculate a mass balance for the developing agent and the resulting degradation products.

As well as non-polar products the diode array scan showed several polar compounds eluting before and after CD-3 in the degraded RA4 developer chromatogram (see Fig. 3.22). It was also possible to see a compound eluting with the same retention time as CD-3 but with a slightly different spectrum. It may be that these compounds are aromatic polar derivatives of CD-3 but no evidence was available. The spectrum of a peak in the chromatogram of degraded RA4, which eluted with a retention time of 2 mins, is shown in Fig. 3.20. There is some similarity between this and the spectrum of methyl-1,4-benzoquinone shown in Fig. 3.21. Methyl-1,4-benzoquinone elutes with a retention time of 1.8 mins. It is possible to form

Attempts to isolate the polar degradation products from CD-3 using solid phase extraction cartridges were unsuccessful.

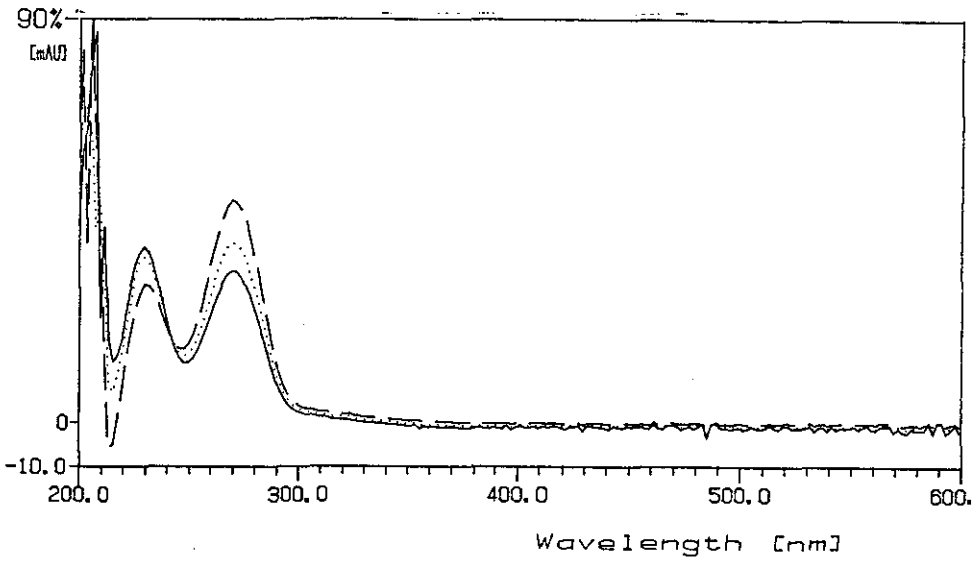


Fig. 3.20 UV spectrum of possible benzoquinone compound in RA4 (method C, Supelco column).

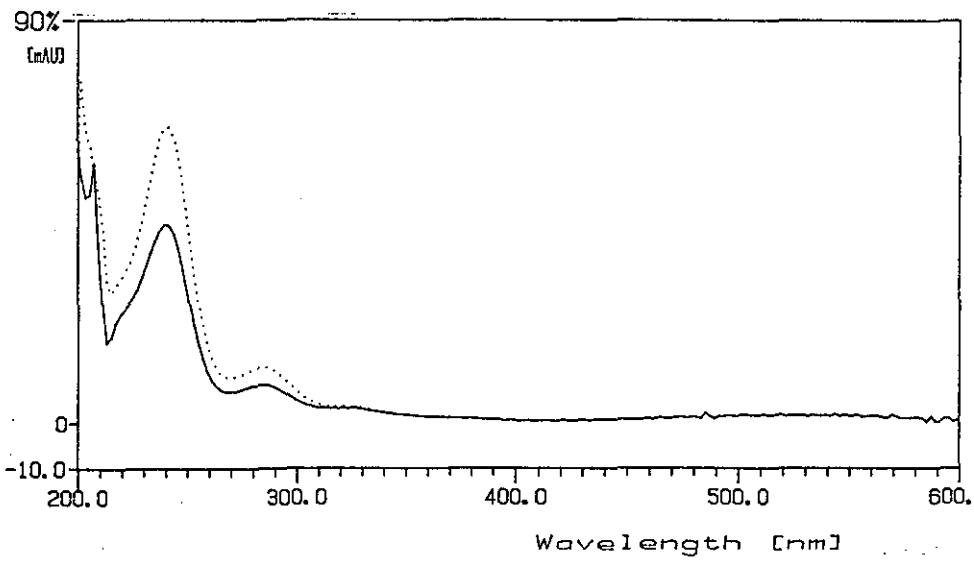


Fig. 3.21 UV spectrum of methyl-1,4-benzoquinone, run in acetonitrile.

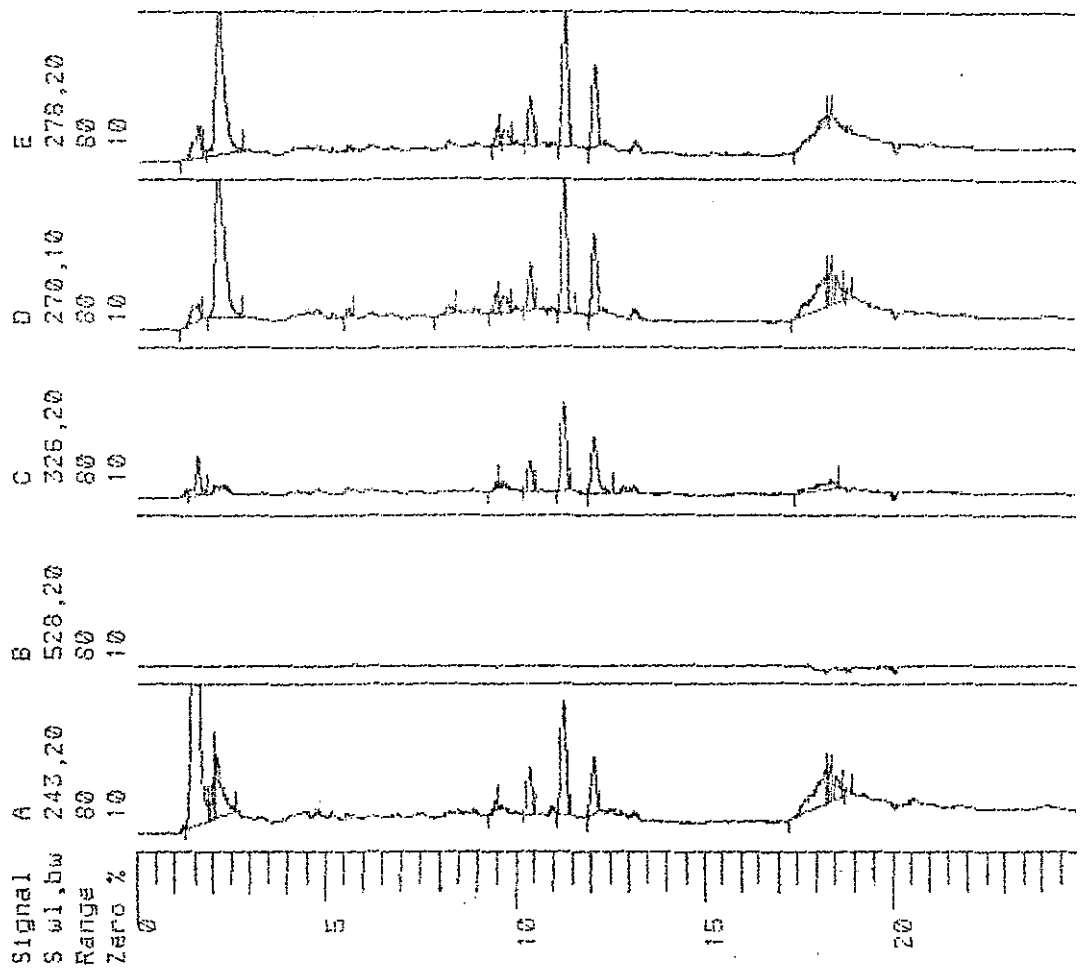


Fig. 3.22 Diode array scan of twenty four day old RA4 solution. (method C, detection at 243 nm, Supelco column)

The graph of degradation of RA4 developer with time is shown in Fig. 3.23 and Fig 3.23a. The variation of the Phorwite peak area with time was random and due to sampling error.

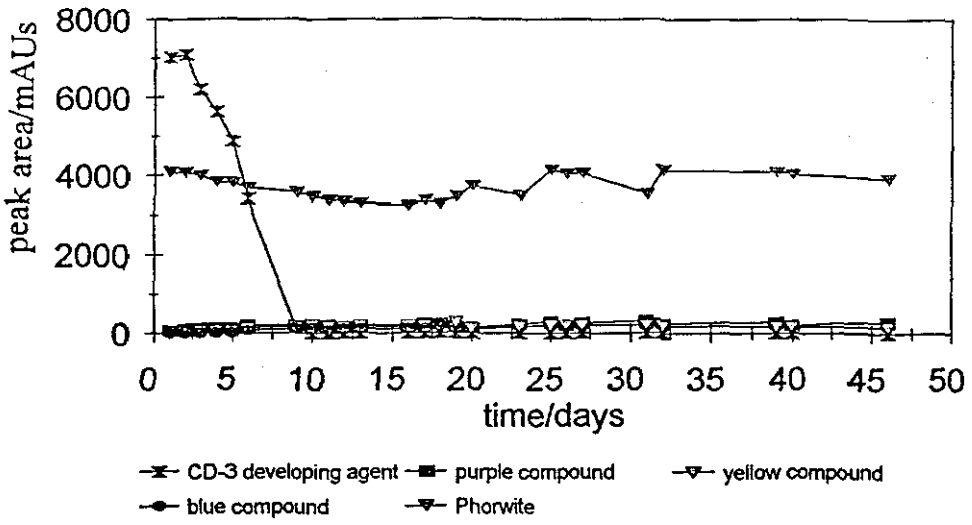


Fig. 3.23 Graph of RA4 degradation versus time (analysis conditions as Fig. 3.19).

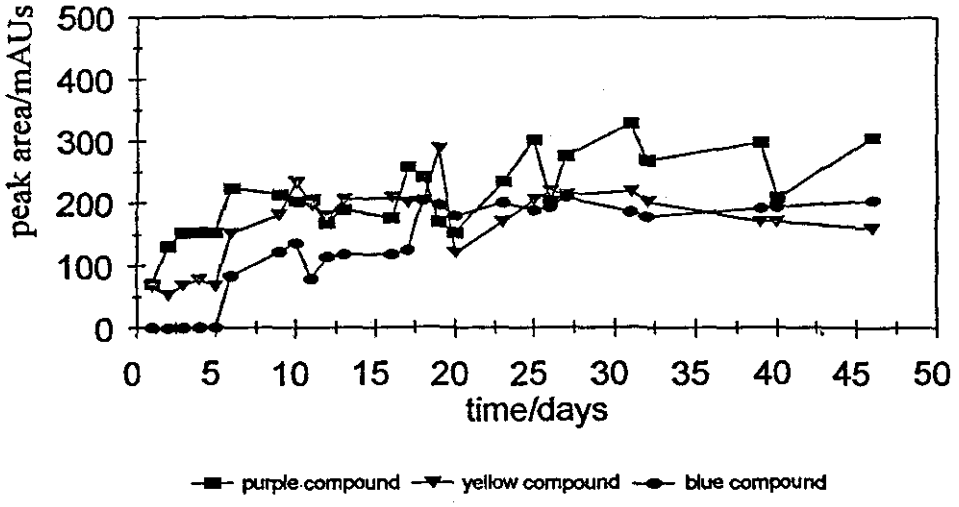


Fig. 3.23a Expansion of minor components in degradation of RA4 with time (analysis conditions as Fig. 3.19).

It was difficult to isolate individual peaks for the yellow, blue and purple compounds from amongst the multitude of non-polar peaks present and this gave rise to random errors in peak areas. Hence the scattering effect seen in Fig. 3.23a.

The developing agent CD-3 appears to have an initial stable period and then appeared to degrade via zero order kinetics, in that the rate of degradation was independent of concentration of developing agent. In the initial period the preservative diethylhydroxylamine was apparently preferentially oxidised. However, CD-3 started to degrade when all the preservative had been oxidised and CD-3 was completely oxidised after nine days. The rate of degradation increased when the developer solutions were stirred and this corresponded to results already seen [28][29]. The non-polar degradation products appeared to form as soon as all the preservative had been oxidised.

Despite literature evidence to the contrary the stain reducing agent Phorwite [31] does not appear to degrade in RA4 developer solutions.

It was indicated earlier that a proliferation of non-polar products formed and precipitated from solution (Fig. 3.22). As the graph in Fig. 3.24 showed, individual degradation products formed slowly but by weighing the precipitate from a known volume of degraded RA4 it was found that 2.63 g/l of precipitate was formed from 4.85 g/l of CD-3 developing agent (54.23 % yield of initial CD-3). The multitude of products was apparent in the TLC plate of Fig. 3.25. The bands were eluted by performing repeated runs of the TLC plate in the elution tank and acetonitrile:dichloromethane ratio was changed slightly between runs to optimise the separation of bands. The yellow, blue and purple degradation products are clearly seen and several other coloured compounds can be seen including a multitude of brown compounds. The products degraded quickly on the plates into a multitude of other unidentified coloured compounds.

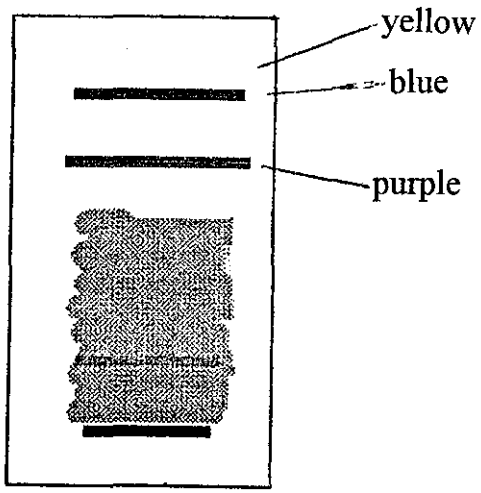


Fig. 3.25 TLC silica plate of RA4 precipitate.

The TLC plate also shows a brown band which is not eluted by polar or non-polar eluents. It is postulated that this is polymeric material but mass spectrometry studies were inconclusive as the material was involatile, see Chapter 4. TLC studies showed that there was a multitude of compounds present.

3.6 Used developer solution samples

A sample of RA100 (PS-003913) which had been used in a commercial laboratory to develop photographic paper was obtained from Kodak Ltd, Harrow for evaluation. A chromatogram is shown in Fig 3.26, the developing agent CD-3 can be seen along with the peaks already seen for Phorwite. However there are several unidentified peaks which had not been seen before, at 8.5 mins, 10.9 mins and several compounds at 18 mins to 20 mins. The compounds at 18 mins to 20 mins did not have similar spectra to those already seen. These may have been compounds which were leached from the photographic paper into the developer solution.

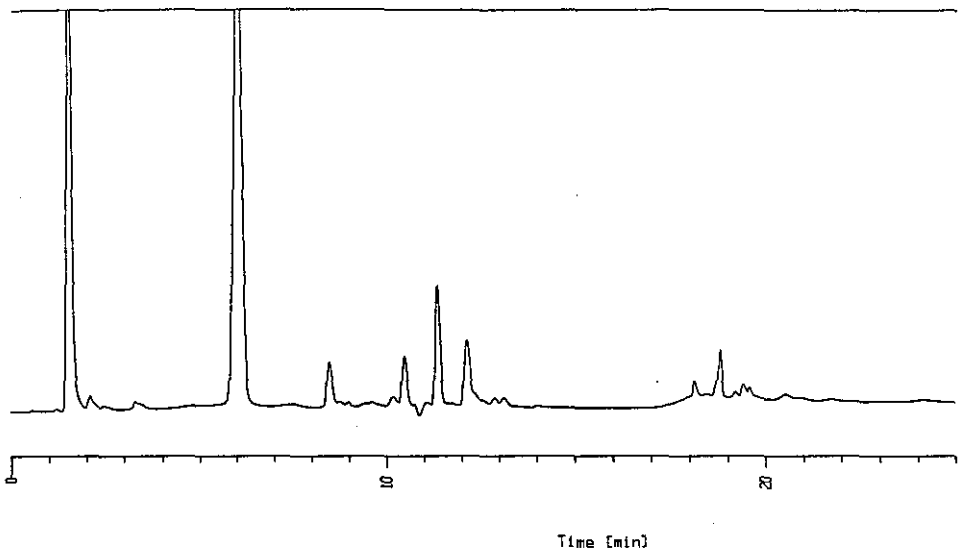


Fig. 3.26 Chromatogram of used RA100 developer solution (method C, detection at 243 nm, Shandon column).

3.7 LC-MS studies

Mass spectrometry studies were carried out on fresh and degraded samples of developer solutions. The results were disappointing and did not show any major components, apart from the signals corresponding to the developing agents. One of the characteristics of LC-MS is that it is not possible to use ion-pair agents in the mobile phase and new separation methods would have to be developed for the analysis of both fresh and degraded developer solutions.

3.8 Summary

Methods of analysis were developed for the analysis of CD-3 developing agent, Phorwite stain reducing agent and the degradation products present in RA4 and RA100 developer solutions. Investigations indicated that buffered solutions of CD-3 and solutions of RA4/RA100 developer solutions degraded to give precipitates of similar composition and multitude of components.

The monitoring studies undertaken showed that CD-3 degrades in RA4 developer solutions into a range of polar and non-polar compounds. The developing agent appeared to degrade by zero order kinetics whereas Phorwite did not appear to degrade.

Chapter 4

Isolation and identification of non-polar degradation products from CD-3 developing agent

4.1 Introduction

Precipitates were observed to form, with time, in RA4/RA100 solutions. They were also observed in aged buffered solutions of CD-3. As outlined earlier (Chapter 3) the yield of precipitate in a degraded RA4 developer was 54.4 %, a similar yield of 53.2 % was seen in a degraded RA100 sample. Initial TLC studies on the precipitates revealed them to be a complex mixture of non-polar products and polymeric material. The older the sample the more polymeric material seemed to be present. It was necessary to separate and isolate the products before identification could be facilitated.

The study therefore set out to isolate the most prominent non-polar degradation products by the most suitable method and to identify these products by HPLC, mass spectrometry, NMR, and any other appropriate spectrometric methods.

4.2 Isolation of CD-3 and RA4 degradation products

Various methods for the isolation and identification of the precipitated degradation products were investigated. The precipitate had a tar-like consistency which caused handling difficulties as it adhered to beakers and spatulas. The precipitates were sparingly soluble in water but were soluble in acetonitrile, methanol and to a lesser degree, dichloromethane. As they were only sparingly soluble in water the precipitates gave degraded developer solutions a turbid, brown coloured appearance.

Solid phase extraction was applied to the isolation of precipitation products from the bulk developer solutions. It was hoped that by using specific bonded silica phases, such as C_8 and C_2, it would be possible to isolate individual non-polar degradation products from the bulk developer solution. The method was tested initially on solutions of degraded CD-3 (0.4 g/l) adjusted to pH 7 with sodium hydroxide. The solid phase was activated by washing with deionised water, this was followed by a representative

sample of the CD-3 solution which contained both precipitate and supernatant liquid. This was then washed with more deionised water. The retained degradation products were eluted using acetonitrile and evaporated to dryness under nitrogen. This procedure worked well with the model system of solutions of CD-3 alone.

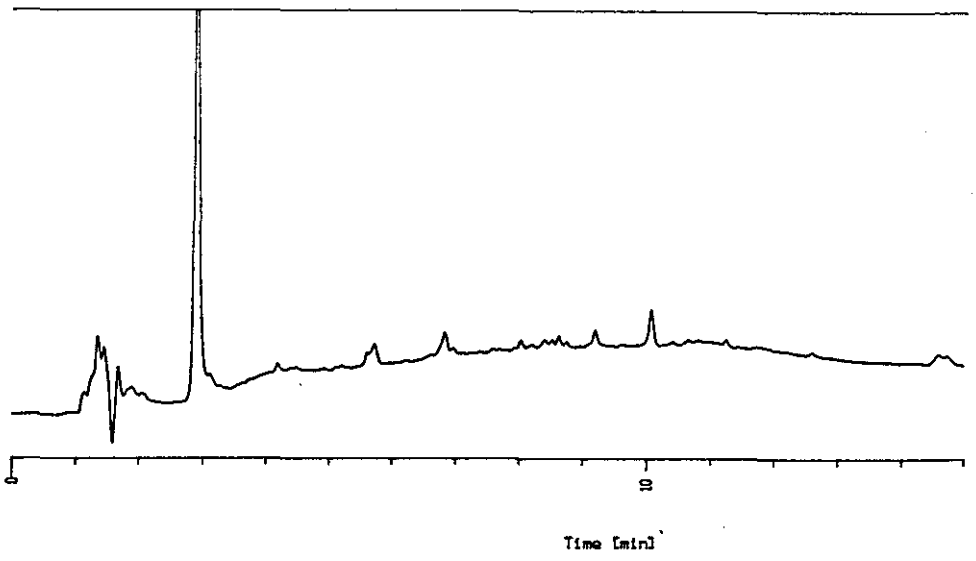


Fig. 4.1 Chromatogram of CD-3 solution (pH 7, 0.4 g/l)(method B, detection at 243 nm, Supelco column)

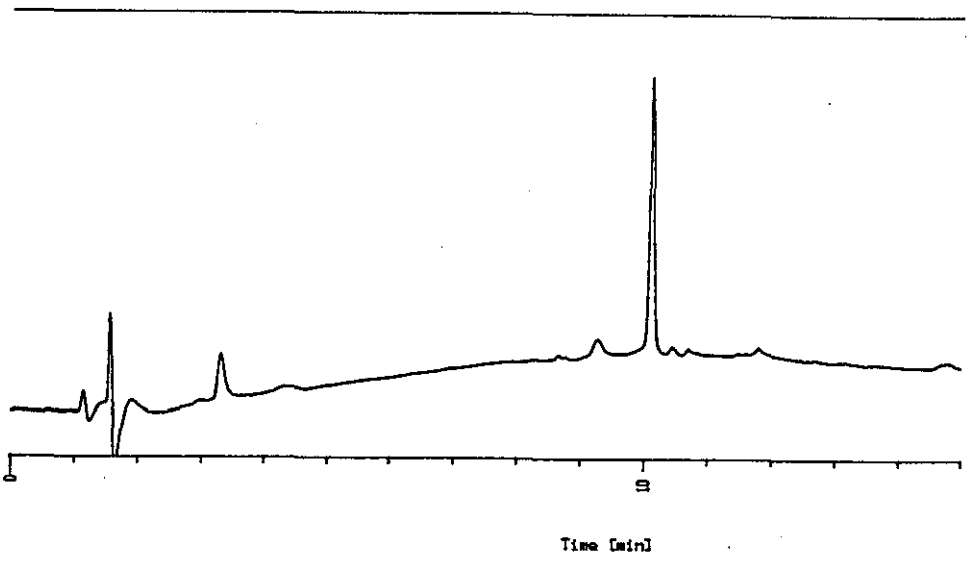


Fig. 4.2 Chromatogram of fraction, from CD-3 solution, on elution (pH 7, 0.4 g/l)(method B, detection at 243 nm, Supelco column)

A chromatogram of CD-3 solution (0.4 g/l) is shown in Fig. 4.1. and a chromatogram of a residue, which was extracted from a degraded CD-3 solution using a C_8 bonded phase cartridge is shown in Fig. 4.2. Note enhancement of the degradation products at retention times of 9.2 mins and at 10 mins.

The procedure developed for isolation of CD-3 non-polar degradation products was applied to an RA100 developer solution. The RA100 developer solution contained 3.8 g/l CD-3 and had a pH of 10.5. It was thought that the high pH would affect the silica bonded phase, which is only stable between pH 2 and 8. The pH of the RA100 solution was adjusted to pH 6 with dilute HCl to avoid this problem. One of the disadvantages of dropping the pH was that the stability of some of the degradation products may be pH dependent. A chromatogram of the RA100 solution is shown in Fig. 4.3. Fig. 4.4. shows a chromatogram of the fraction obtained, on elution with acetonitrile, from a C_8 solid phase extraction cartridge, after 2 ml of degraded RA100 was passed though the cartridge.

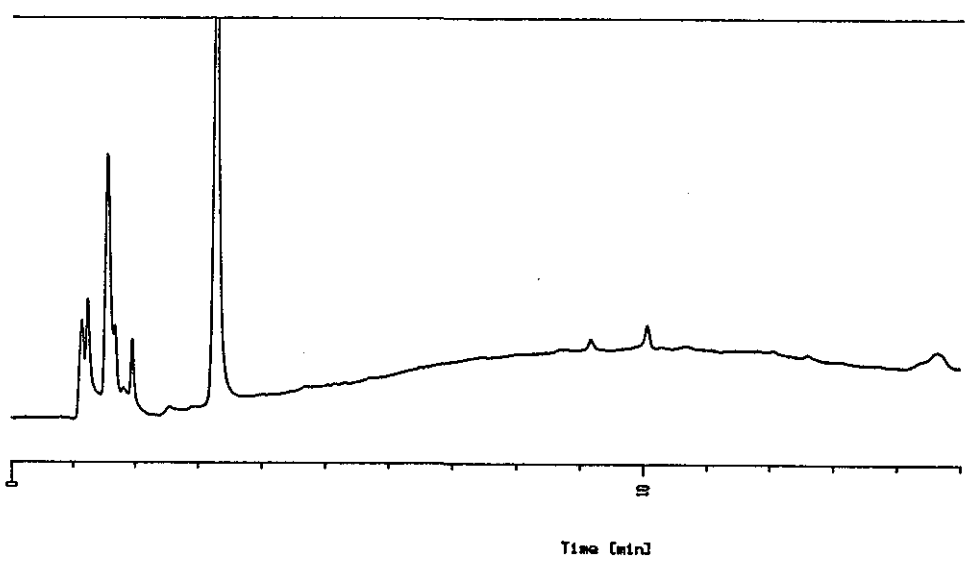


Fig. 4.3 Chromatogram of RA100 developer solution (pH 6)(method B, detection at 243 nm, Supelco column)

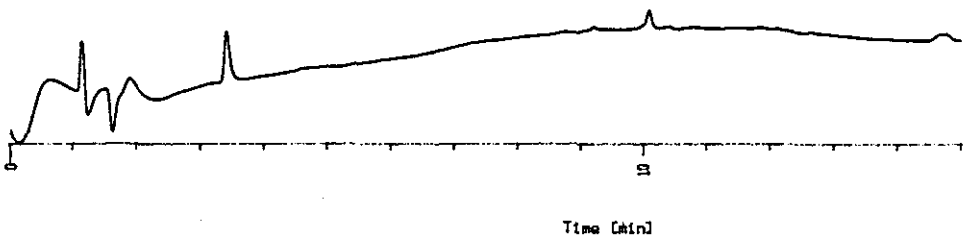


Fig. 4.4 Chromatogram of fraction from RA100 developer eluted from C_8 SPE cartridge (method B, detection at 243 nm, Supelco column)

However, the results were disappointing. Very little preconcentration of degradation products seemed to have occurred on the C_8 cartridge(Fig. 4.4). Also, at this pH, CD-3 had surprisingly been retained on the cartridge and has eluted off with the degradation products.

Further work was carried out to try to optimise the conditions for the specific retention of the degradation products on non-polar cartridges. At pH 6 CD-3 was neutral or a zwitterion and was being retained by the non-polar solid phase cartridge, along with the degradation products of interest. When a dilute HCl solution was passed through the cartridge the primary amino group on CD-3 was protonated and so CD-3 was eluted. The cartridge was then washed with water/acetonitrile (80:20 v/v). Finally the non-polar degradation products were eluted using acetonitrile and evaporated to dryness under nitrogen. As well as isolating these degradation products this method preconcentrated them (Fig. 4.5). See Fig. 4.3 for a chromatogram of the original RA100 developer solution.

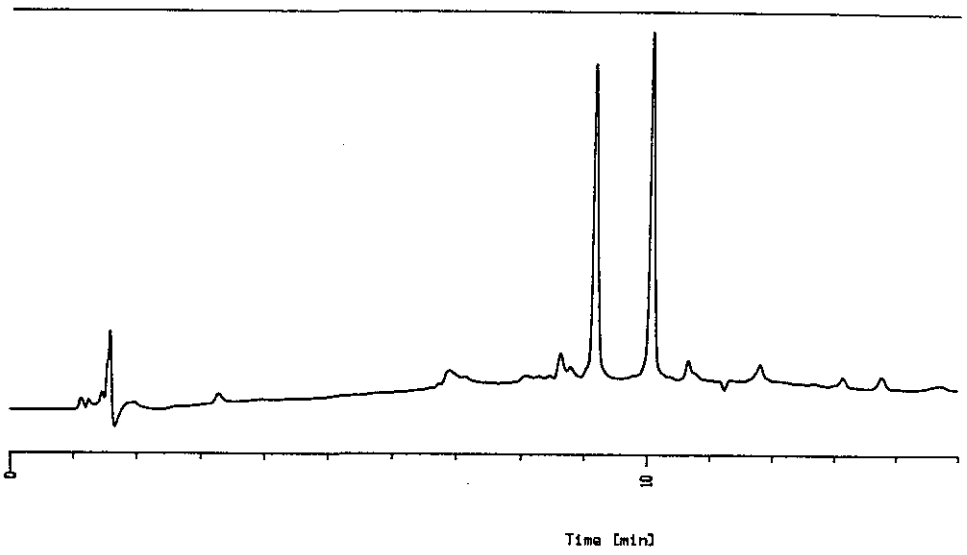


Fig. 4.5 Chromatogram of fraction from RA100 developer solution using selective elution from C_8 cartridge (method B, detection at 243 nm, Supelco column)

Although solid phase extraction worked well, it was decided the quantities of degradation products recoverable were too small. Larger solid phase extraction cartridges were available but even with these, insufficient quantities of material was recovered for further investigation. It was decided to attempt to separate and identify compounds from the precipitates in CD-3 and RA4/RA100 developer solutions rather than from the small amounts of non-polar products soluble in solution. The precipitates were shown to contain similar non-polar products as those found in solution.

4.3 Separation and isolation of precipitates

Chromatograms of the precipitates from RA4, RA100 and a buffered solution of CD-3 (pH 10.5) in (Fig 4.6), (Fig. 4.7) and (Fig. 4.8) showed that the precipitates were very similar in composition.

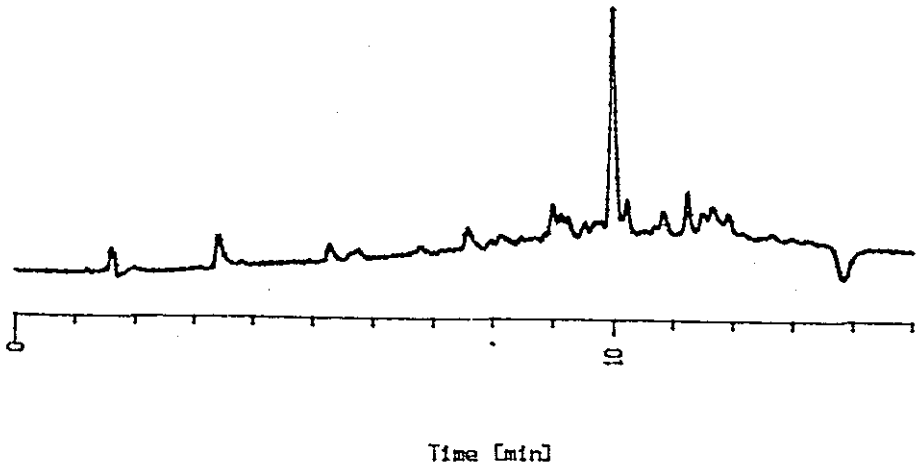


Fig. 4.6 Chromatogram of CD-3 precipitate (method B, detection at 243 nm, Supelco column)

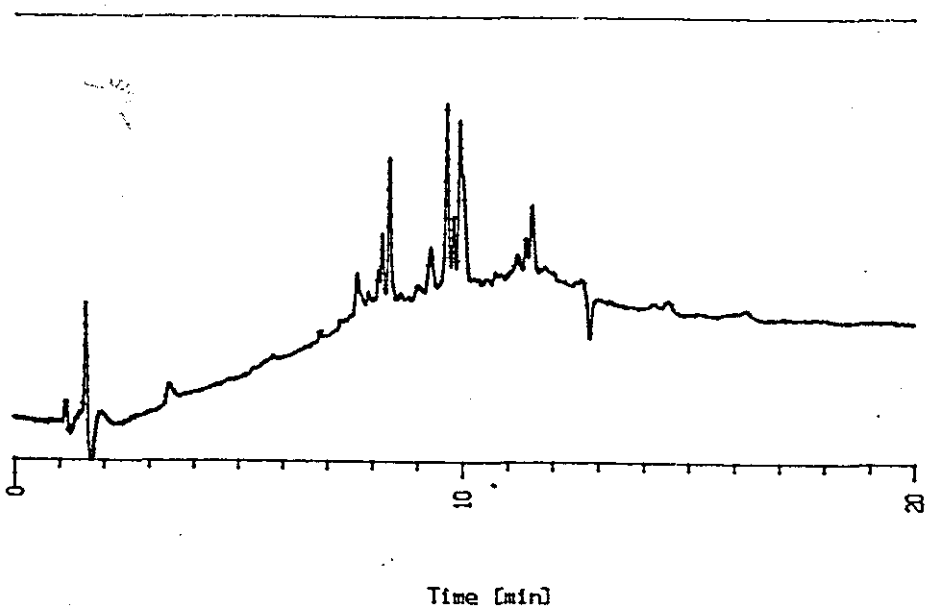


Fig. 4.7 Chromatogram of RA4 precipitate (method B, detection at 243 nm, Supelco column)

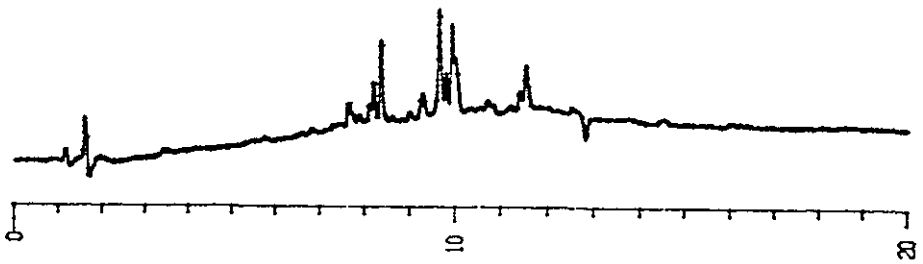


Fig. 4.8 Chromatogram of RA100 precipitate (method B, detection at 243 nm, Supelco column)

Various methods for the separation of the non-polar compounds in the developer precipitates were investigated. Firstly, semi-preparative HPLC was attempted using an octadecylsilica column (250 x 10 mm). The precipitates were dissolved in acetonitrile and injected using the 200 µl syringe of the HP1084B HPLC, method *A* as eluent and detection at 243 nm. However, the quantity of material involved (100 µg) tended to grossly overload the column. Injection needles became clogged with the precipitate and the precipitates caused pressure build-up in the guard columns, possibly due to precipitation. The proliferation of degradation products did not make for facile isolation of individual components.

Flash column chromatography was also investigated. A large glass column packed with flash silica was used (see Chapter 2.3). Large quantities of precipitates (3-4 g) could be loaded onto the flash column which could make this method ideal for isolation of compounds. Nevertheless, the resolution of bands eluting from the column was poor and most bands were indistinguishable from each other. Brown, insoluble, possibly polymeric materials precipitated onto the flash column making identification of individual bands extremely different as the silica was stained brown. Also the method used large quantities of acetonitrile and dichloromethane solvents.

Finally thin layer chromatography was investigated as a method for the separation of non-polar degradation products from CD-3, RA4 and RA100. Separation was achieved using the large silica plates (1 m x 20 cm) described in Chapter 2.3.3. The degradation products were coloured and so were easily distinguishable on the plates. Precipitated polymeric material did not elute and stayed at the bottom of the plate, not interfering with the separation of the other degradation products. Furthermore resolution could be improved by varying the ratio of acetonitrile/dichloromethane with repeated elution runs from 0 % acetonitrile to 20 % acetonitrile to give an optimum separation. An example of a TLC plate of RA100 and CD-3 precipitates is shown in Fig. 4.9.

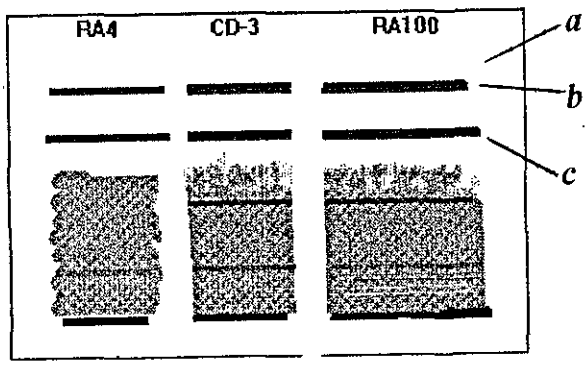


Fig. 4.9 Typical TLC elution order of CD-3/RA100 precipitate (dichloromethane/acetonitrile (90:10 v/v) eluent)

The TLC of an RA100 developer precipitate, RA4 developer precipitate and CD-3 precipitate (Fig. 4.9.) were very similar in appearance. Several of the bands were removed from the TLC plates, eluted from the silica with acetonitrile and evaporated to dryness under nitrogen. The fractions were further purified using smaller TLC plates (20 cm x 20 cm) to give yellow (*a*), blue (*b*) and purple (*c*) compounds which were examined in subsequent chapters by spectroscopic methods .

4.4 Mass spectrometry of precipitates.

A sample of the total CD-3 precipitate was analysed using mass spectrometry and the EI mass spectrum is shown in Fig. 4.10. The sample contained a large number of components (see Fig. 4.6 for the HPLC chromatogram) and was largely involatile. A high temperature mass spectrum was obtained and this showed a number of strong signals (Fig. 4.11) but assignment of the signals proved difficult.

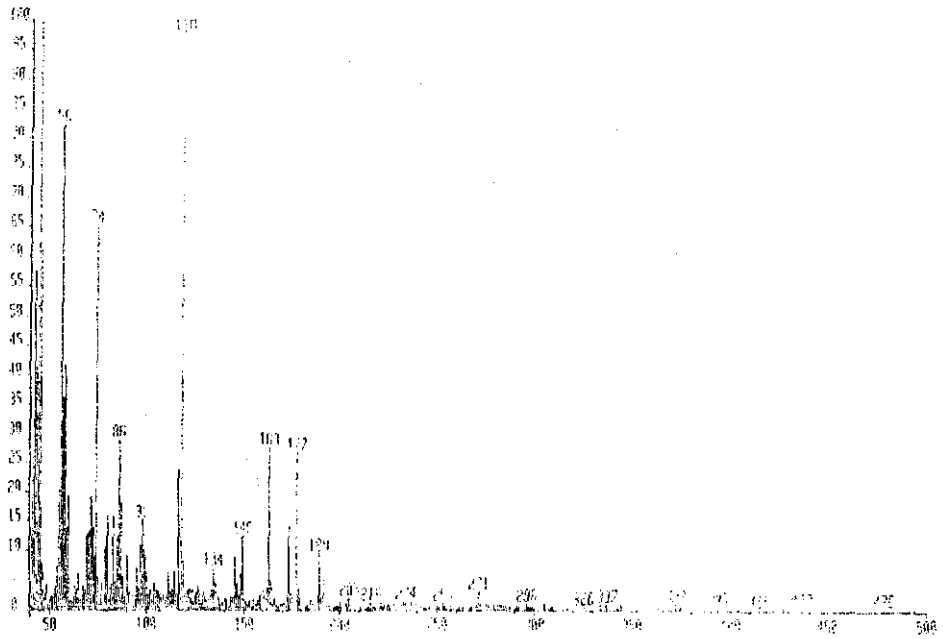


Fig. 4.10 EI mass spectrum of CD-3 precipitate.

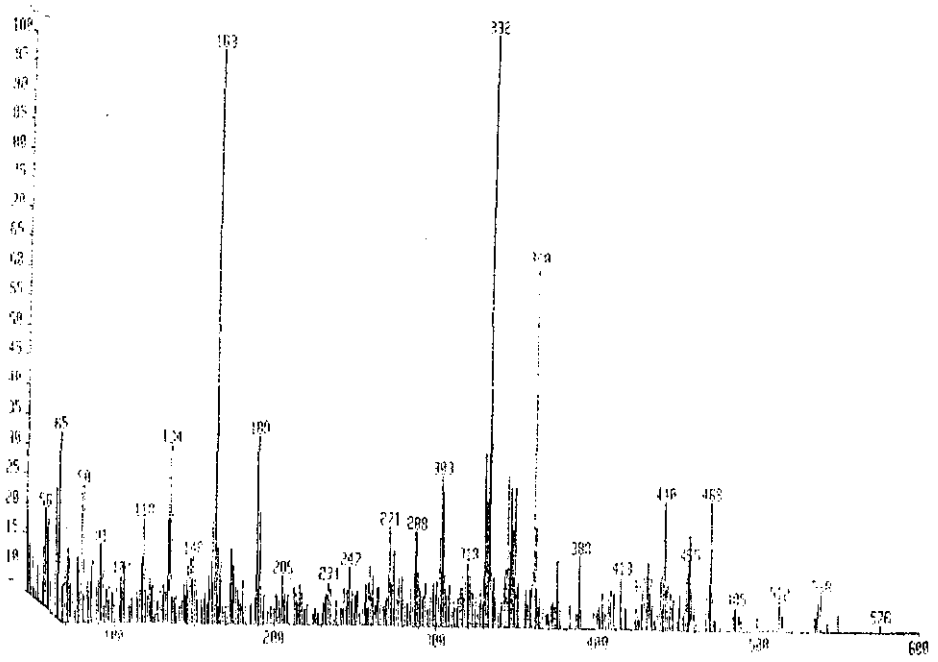


Fig. 4.11 High temperature mass spectrum of CD-3 precipitate.

4.5 Proton NMR of CD-3 developing agent.

The ^1H NMR of CD-3 was recorded to aid in the interpretation of the ^1H NMR spectra of non-polar CD-3 degradation products. As CD-3 is stored as the acid salt, it was deprotonated using high pH and then liquid-liquid extracted into dichloromethane so that the ^1H NMR of the free base could be run in deuterated chloroform. Deuterated chloroform was the preferred solvent because all degradation products were soluble in it and spectra were simpler than in other solvents.

The ^1H NMR of CD-3 is shown in Fig. 4.12., along with the tabulation of the main peaks in Table. 4.4. The structure of CD-3 is shown in Fig. 4.13.

Chemical shift δ/ **ppm**	Description	Integration ratio	Assignments
1.00	triplet, 6.7 Hz	3H	CH_3, *E*
2.15	sharp singlet	3H	CH_3, *J*
2.85	very sharp singlet	3H	CH_3, *A*
3.15	multiplet, 6.7 Hz	6H	CH_2's, *C, D*
3.38	broad singlet	2H	NH_2, *I*
4.95	broad singlet	1H	NH, *B*
6.60	multiplet	3H	ArH's, *F, G, H*
7.24	sharp singlet	impurity	$CHCl_3$

Table. 4.1 Tabulation of ^1H NMR data for CD-3.

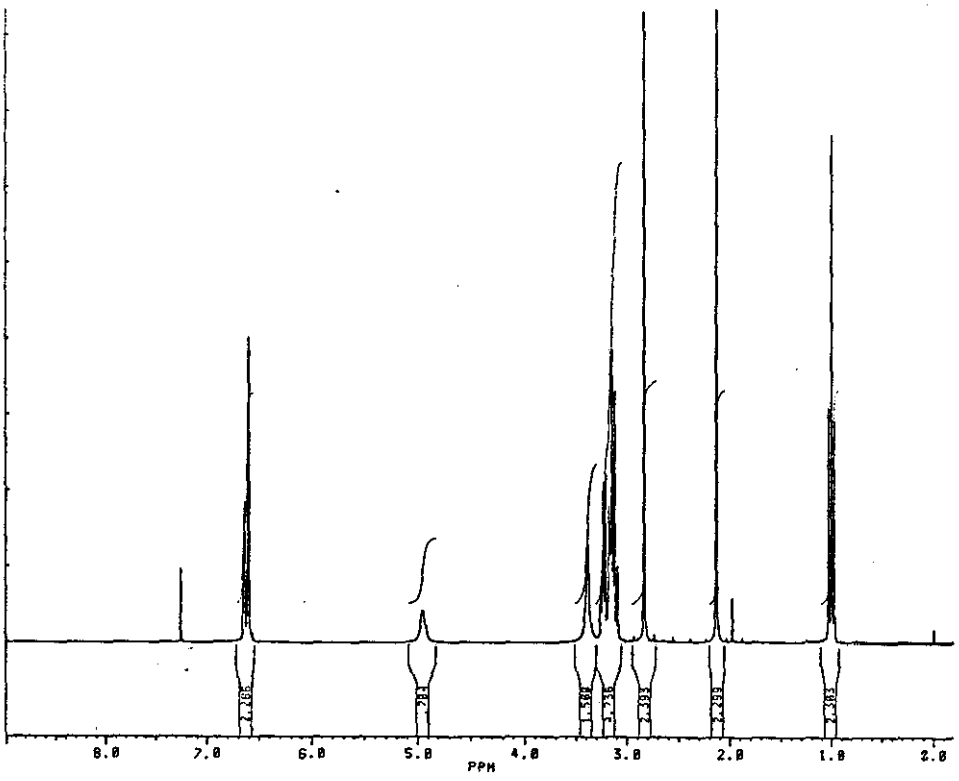


Fig. 4.12 ^1H NMR of CD-3, run in deuterated chloroform (250 MHz).

A CD-3 molecule is shown in Fig. 4.13.

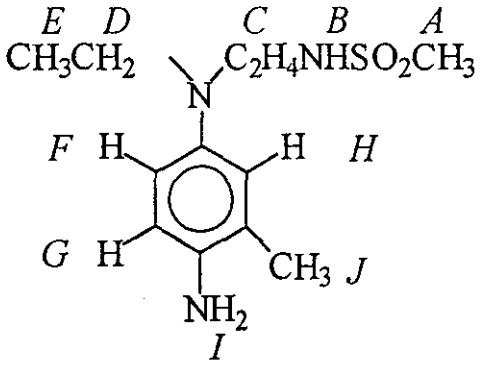


Fig. 4.13 Structure of CD-3 molecule.

The sharp triplet (6.7 Hz) at 1.00 ppm integrated to three protons which were coupled to two other protons. The integration and chemical shift were typical of a methyl group adjacent to a CH_2 group. Therefore this corresponded to the methyl group E. The sharp singlet at 2.15 ppm was a more deshielded methyl group which did not appear to be coupled to any further protons. This is characteristic of a methyl group attached to an aromatic ring (J). A very sharp singlet at 2.85 ppm also integrates to a methyl group and corresponded to that bonded to the sulphonamide group (A).

There is a complex multiplet centred at 3.15 ppm. It was difficult to deduce the coupling relationships but there is an apparent quartet centred on 3.12 ppm and this has a coupling constant of 6.7 Hz, this is consistent with the triplet at 1.00 ppm which also has a coupling constant of 6.7 Hz and so they are adjacent .The relative integrations and chemical shifts indicated that the multiplet was due to the three methylene groups (C and D) attached to the tertiary amine and sulphonamide of the sidechain. The broad singlet at 3.38 ppm, which integrated to two protons, was assigned to the primary amine group attached to the aromatic ring (I). The broadness of the signal at 4.95 ppm would seem to indicate some degree of proton exchange. This, along with the integration (1H) meant that the signal was due to the amine of the sulphonamide group (B). The multiplet at 6.6 ppm had a chemical shift typical of aromatic protons. This fact, along with the relative intensity (3H) indicates that it was due to the ring protons (F,G,H).

4.6 Identification of yellow CD-3 degradation compound

4.6.1 HPLC of yellow CD-3 degradation compound

A typical chromatogram for the yellow degradation compound isolated from RA100 precipitate is shown in Fig. 6.14. The yellow product eluted with a retention time of 13.0 mins. The yellow compound isolated from the precipitate of a degraded CD-3 solution was identical in retention appearance.

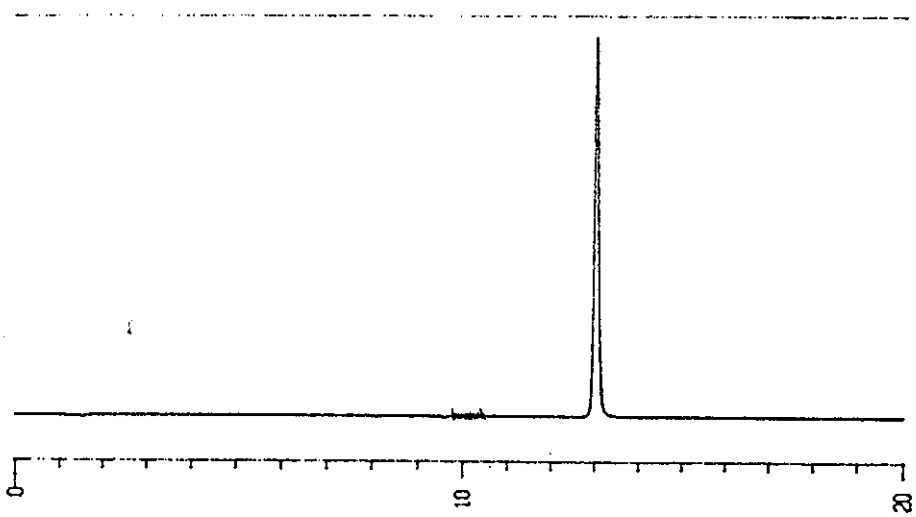


Fig. 4.14 Chromatogram of yellow CD-3 degradation compound (method B, detection at 243nm, Supelco column)

4.6.2 UV spectra of yellow CD-3 degradation compound

The UV spectral data for an ethanolic solution of the yellow compound are shown in Table 4.2. The solutions were yellow in appearance and this agrees with the observations of Bishop and Tong who also saw yellow azo compounds [52].

Absorbance maxima ethanol λ_{max} **(nm)**	**Absorbance maxima** ethanol + two drops of dilute HCl λ_{max} **(nm)**	**Absorbance maxima** ethanol + two drops of dilute NaOH λ_{max} **(nm)**
203.5	205.5	225.3
266.2	264.8	-
324.3	317.6	-
434.5	432.8	466.4

Table 4.2 UV spectral data for yellow CD-3 degradation product.

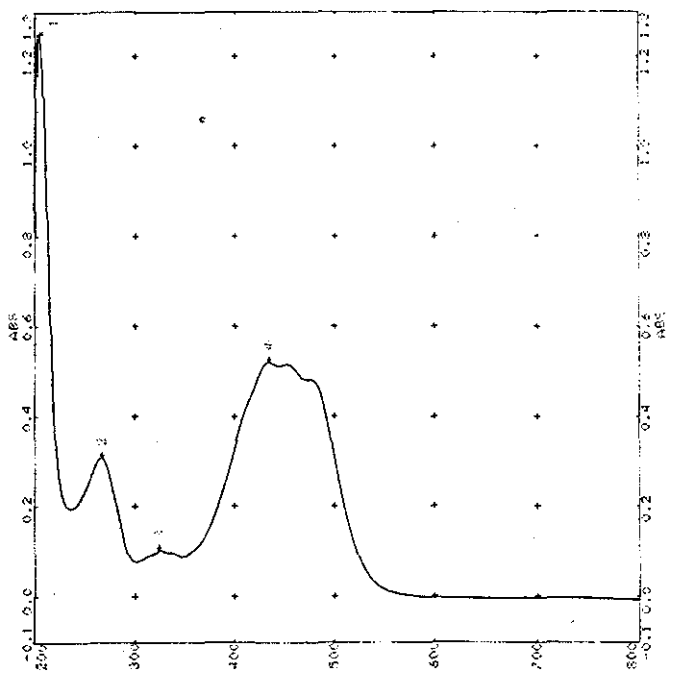


Fig. 4.15 UV spectrum of an ethanolic solution of yellow CD-3 degradation product

Bishop and Tong reported spectral data for a series of azo compounds in benzene [52], the azo compounds reported had absorbance maxima ranging from 425 nm (4,4'-bis (ethyl-β-hydroxyethylamino) azobenzene to 440 nm (4,4'-bis (diethylamino) azobenzene. They also reported the presence of three peaks in the 400 nm to 500 nm region and this characteristic can be seen for the yellow CD-3 degradation product, in Fig. 4.15.

As the pH of the ethanolic solution of the yellow CD-3 degradation product was changed, a noticeable variation in absorbance maxima occurred. This was probably due to protonation of the tertiary amine groups on the sidechains (see Fig. 4.20 for postulated structure). This protonation reduced the conjugation in the molecule as the protonated nitrogens were unable to donate electrons into the ring system. At high pH the nitrogen atoms are unprotonated, the nitrogen lone pairs can donate electrons into the ring system and there is a higher degree of conjugation. This conjugation results in the absorbance maxima shifting to longer wavelengths.

4.6.3 Mass spectrometry of yellow CD-3 degradation compound

The yellow products isolated from the precipitates of RA100 developer solution and degraded CD-3 solution were analysed using mass spectrometry and found to be identical in nature. The electron ionisation mass spectrum of the yellow CD-3 degradation product is shown in Fig. 4.16.



Fig. 4.16 Electron ionisation mass spectrum of yellow CD-3 degradation product.

Major fragments appeared at m/z 163 and 430. The molecular ion appeared at m/z 538. The fragment at 163 was characteristic of CD-3 and CD-3 derived species [72] and involved the loss of 108 mass units from CD-3 from the sidechain, corresponding to a $\cdot CH_2NHSO_2CH_3$ group. The structure of this fragment is shown in Fig. 4.17

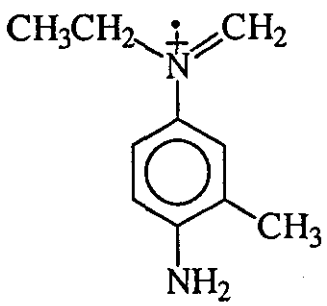


Fig. 4.17 Structure of fragment from CD-3 with m/z 163.

The fragment at m/z 430 was due to the loss of a $\cdot CH_2NHSO_2CH_3$ group from the molecular ion of the yellow compound at m/z 538. The molecular ion at m/z 538 corresponded to a dimeric compound of CD-3

which is four mass units less than two CD-3 molecules. Looking at a CD-3 molecule it can be seen that it can react with another CD-3 molecule at the primary amino group to form an azo dimer [52]. The NH_3 DCI mass spectrum gave an MH+ or higher molecular species. The CI mass spectrum of the yellow compound gave an MH+ of 539, as shown in Fig. 4.18, which confirmed the molecular weight as 538. This would mean that the fragment at m/z 430 had the structure shown in Fig. 4.19, whilst the azo dimer had the structure shown in Fig. 4.20.

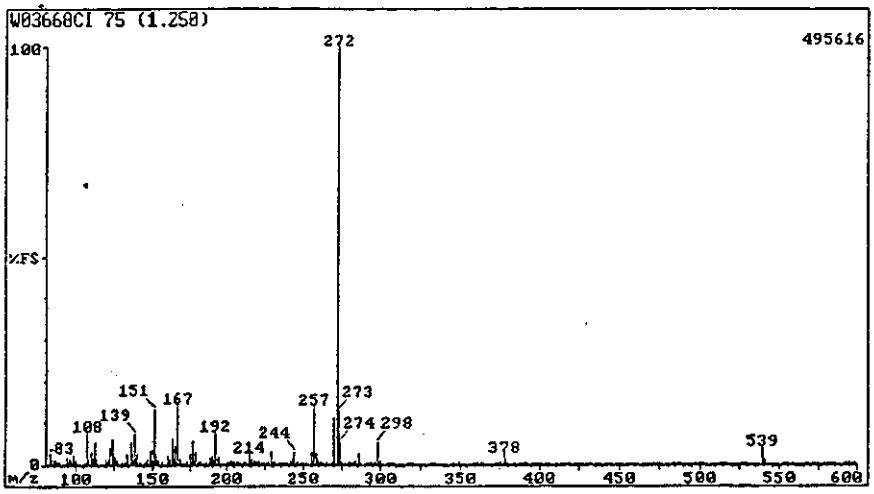


Fig. 4.18 NH_3 Direct Chemical Ionisation mass spectrum of yellow CD-3 degradation product

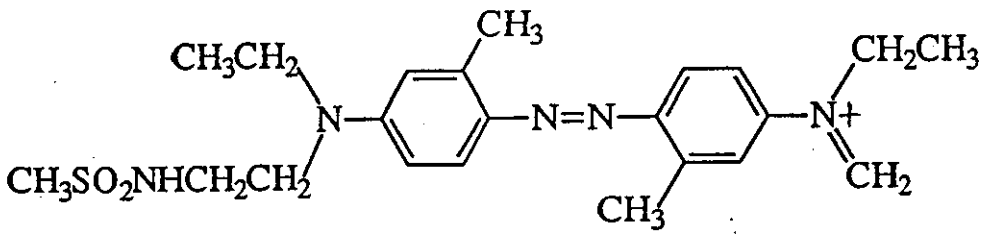


Fig. 4.19 Structure of fragment at m/z 430.

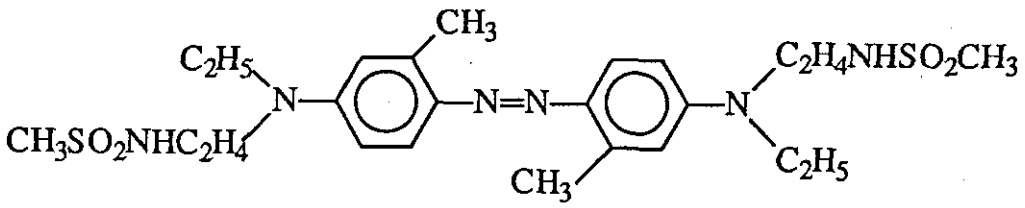


Fig. 4.20 Structure of yellow CD-3 compound (m/z 538)

4.6.4 Proton NMR of yellow degradation CD-3 compound

Proton NMR analysis was carried out on the yellow compound isolated from the precipitate of a degraded RA100 developer solution. The ^1H NMR spectrum of this compound is shown in Fig. 4.21 together with the chemical shifts and integration ratios in Table. 4.3.

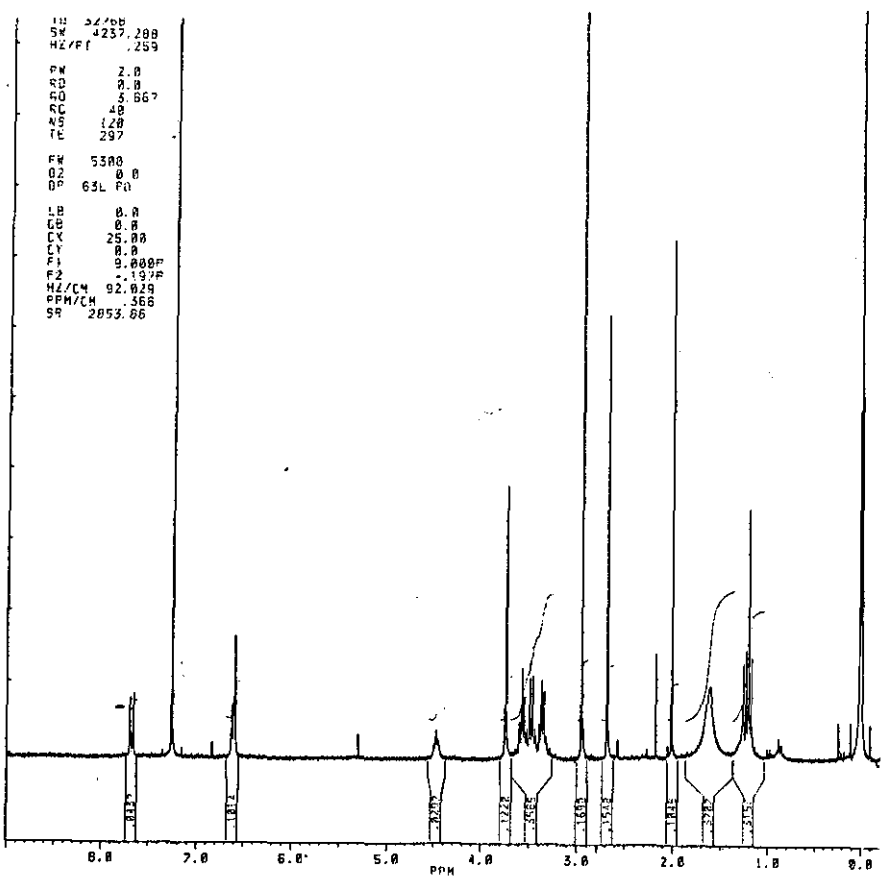


Fig. 4.21 ^1H NMR spectrum of yellow CD-3 degradation compound run in deuterated chloroform (250 MHz).

Chemical shift δ/ ppm	Description	Integration ratio	Assignment
1.20	sharp triplet, 6.9 Hz	3H	CH_3
1.50	singlet,	impurity	H_2O
2.70	sharp singlet	3H	CH_3
2.95	sharp singlet	3H	CH_3
3.50,	multiplets, 6.9 Hz	6H	CH_2's
4.45	possible triplet,	1H	$NHSO_2$
6.50	multiplet	2H	ArH's
7.25	sharp singlet	impurity	$CHCl_3$
7.67	possible doublet, 9.6 Hz	1H	ArH

Table. 4.3 Tabulation of ^1H NMR data for yellow CD-3 degradation product

The ^1H NMR spectrum of this yellow compound is similar to the ^1H NMR spectrum of CD-3 in appearance and integration. The singlet at 1.5 ppm is characteristic of water which is present as an impurity. The sharp singlet at 7.25 ppm is characteristic of $CHCl_3$ which is present as an impurity in the solvent system used for elution of the TLC plates.

The sharp triplet (6.9 Hz) at 1.2 ppm integrates to 3H and corresponds to a methyl group coupled to two protons, as indicated by the multiplicity. Comparison with the CD-3 ^1H NMR means that this signal is probably a methyl group of a CH_2CH_3 attached to the tertiary amine of a sidechain similar to that on CD-3.

The chemical shift and relative integration (3H) of the signal at 2.7 ppm would indicate a relatively deshielded methyl group. This would probably indicate the methyl group attached to the aromatic ring, as the methyl group attached to the SO_2 group (as in CD-3) would be even more deshielded. The large change from 2.15 ppm in CD-3 (Fig. 4.13 and Table 4.2 would agree with a dimerisation via the adjacent amino group. The singlet at 2.95, which integrated to 3H, corresponded to the methyl group

attached to the SO_2 group. A series of multiplets appeared at 3.5 ppm. Comparison with the ^1H NMR spectrum of CD-3 showed that this are probably due to the methylene groups attached to the tertiary amine and secondary amine of the sidechain. The coupling (6.9 Hz) indicated that one of the multiplets was coupled to the methyl group of the ethyl sidechain..

The signal at 4.45 ppm, which integrated to 1H, seemed similar to the NH of the sulphonamide group of CD-3 (4.95 ppm) but it had been shifted upfield slightly. The signal was probably a triplet and this would be reinforced by its proximity to a methylene group. The signal at 6.60 ppm was an unresolved multiplet and integrated to 2H. The signal was not clearly resolved and did not allow for a coupling constant to be calculated. This chemical shift was typical of aromatic protons. A possible doublet (9.3 Hz) at 7.67 ppm integrated to 1H, indicating that it was very deshielded. The original CD-3 molecule contained three aromatic protons centred on 6.60 ppm so it would seem logical that the signal at 6.60 ppm in the RA100 yellow product ^1H NMR was due to two aromatic protons in a similar environment to that in the original CD-3 molecule. However, the signal at 7.67 ppm was not apparent in CD-3 and must be due to a proton in a different chemical environment to that in CD-3 (Fig. 4.13, *G*), possibly due to its proximity to the N=N group and which is coupled to an adjacent aromatic proton. These assignments would seem to point towards a symmetrical azo dimeric type structure as shown in Fig. 4.20. This postulation agrees with the mass spectral data and the studies undertaken by Bishop and Tong [52].

Smith [79] reported the effect on the o- and m- aromatic protons of changing an amino group on p-aminophenol to a phenylazo group. In the p-aminophenol, the proton ortho to the hydroxyl group had a chemical shift shifted 0.7 ppm upfield relative to a similar proton in unsubstitiuted benzene. This was caused by the electron donating effect of the adjacent hydroxyl group. The proton meta to the hydroxyl group was moved upfield by a similar shift, caused by the electron donating effect of the amino group. However, in the corresponding phenylazophenol, the proton meta to the hydroxyl group and adjacent to the azo group was shifted downfield by 1.30 ppm relative to unsubstituted benzene, due to the electron withdrawing effect of the azo group. Whereas the proton ortho to the hydroxyl group was shifted upfield by 0.4 ppm due to its electron donating effect.

A similar shift of 1.07 ppm was seen for the aromatic proton ortho to the amino group in CD-3 (6.60 ppm) on forming the yellow CD-3 degradation product where the azo group had an electron withdrawing effect on the aromatic proton ortho to the azo group(7.67 ppm).

Smith recorded the data on a 40 MHz NMR spectrometer and the accuracy of the shifts are questionable but the general trend is apparent.

4.7 Identification of blue CD-3 degradation product

4.7.1 HPLC of blue CD-3 degradation compound

A chromatogram of the blue CD-3 degradation product isolated from CD-3 precipitate is shown in Fig. 4.22. The blue compound eluted with a retention time of 11.5 mins.

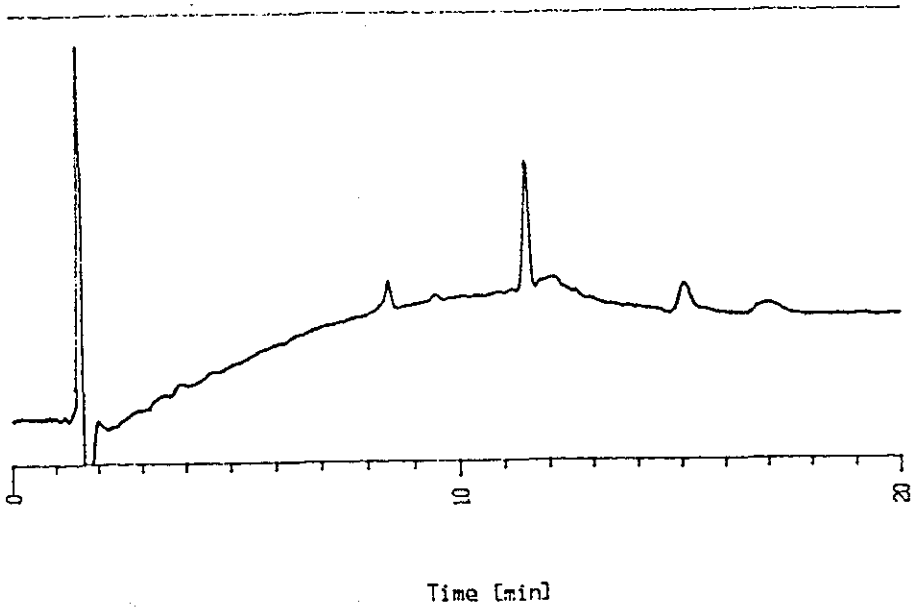


Fig. 4.22 Chromatogram of blue CD-3 degradation compound (method B, detection at 243 nm, Supelco column)

4.7.2 UV spectra of blue CD-3 compound

The UV spectral data of an ethanolic solution of the blue degradation product isolated from CD-3 precipitate is shown in Table 6.4. The UV spectrum of an ethanolic solution of the blue CD-3 degradation product is shown in Fig. 4.23.

Absorbance maxima ethanol λ_{max} (nm)	Absorbance maxima ethanol + two drops of dilute HCl. λ_{max} (nm)	Absorbance maxima ethanol + two drops of dilute NaOH. λ_{max} (nm)
201.7	203.5	218.0
282.4	282.8	272.6
380.4	381.6	-
637.6	632.8	664.0

Table 4.4 UV spectral data for blue CD-3 degradation product

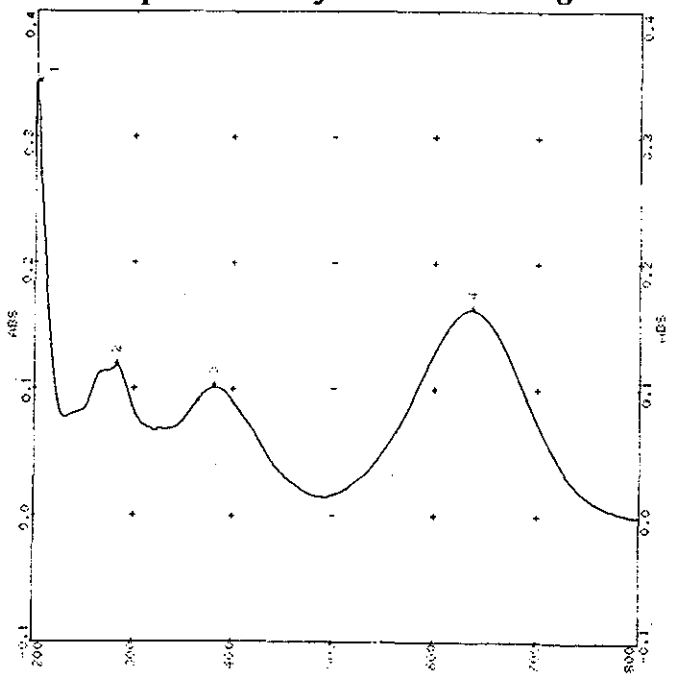


Fig. 4.23 UV spectrum of ethanolic solution of blue CD-3 degradation product

At low pH the nitrogen of the tertiary amine was protonated and unable to donate electrons into the ring system (see Fig. 4.26 for proposed structure). This reduced conjugation and lead to shorter absorbance maxima. However at high pH the tertiary amine was unprotonated and thus donated electrons into the ring system, thus increasing conjugation and the absorbance maxima. Pelizzetti and Saini [51] reacted CD-3 with 2-methylphenol to form a blue coloured solution with an absorbance maximum in aqueous alkaline buffer (pH 9) of 665 nm (cf. Table 4.40 664 nm). They postulated that a compound identical to the blue CD-3 degradation product was formed.

4.7.3 Mass spectrometry of blue CD-3 degradation compound

The electron ionisation mass spectrum of the blue compound is shown in Fig. 4.24. Apparent molecular ions can be seen at m/z 375 and 377. It was suggested that the species at m/z 377 originates from reduction of the sample in the mass spectrometer [73]. Fragments can be seen at m/z 240 and 269. The fragment at m/z 269 could be due to a loss of $CH_2NHSO_2CH_3$ (108 mass units) from a sidechain of the species at m/z 377. The species at m/z 240 involves the loss of a further CH_2CH_3 group.

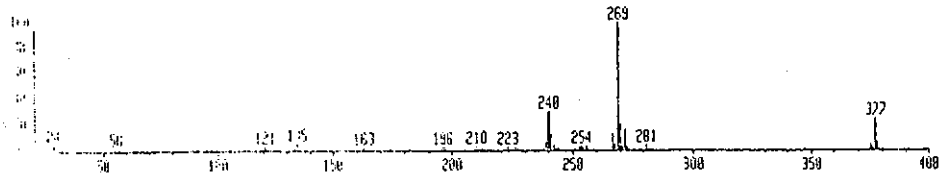


Fig. 4.24 Electron ionisation mass spectrum of blue CD-3 degradation compound

The NH_3 DCI mass spectrum has intense MH+ peaks at m/z 376 and 378. This would appear to confirm the molecular weight as 375, see Fig. 4.25. This information together with the ^1H NMR data shown later would confirm the structure as being a quinoneimine as shown in Fig. 4.26.

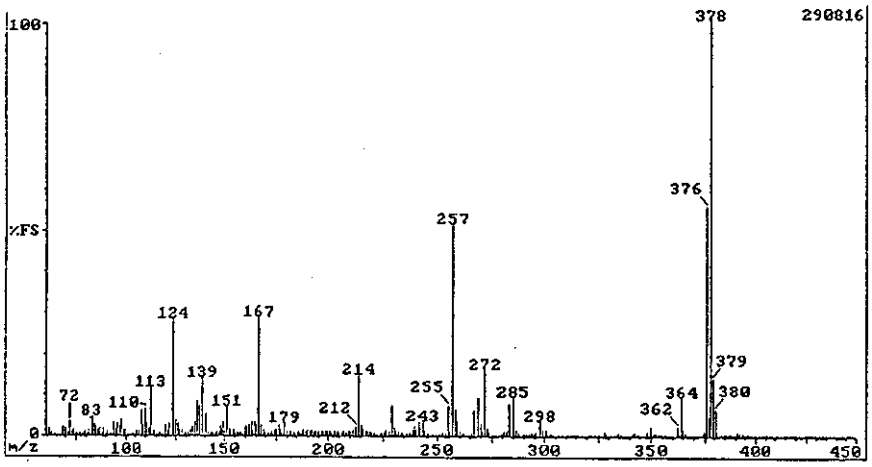


Fig. 4.25 NH_3 DCI mass spectrum of blue CD-3 degradation compound

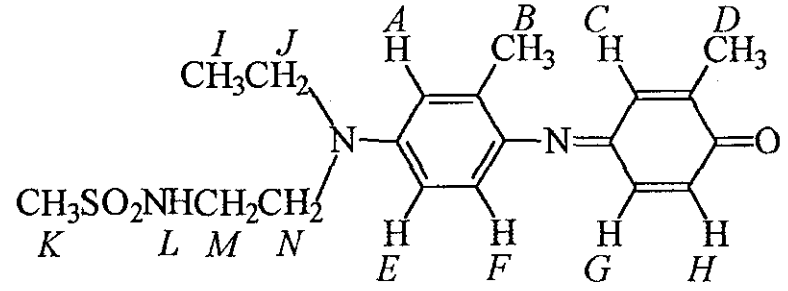


Fig. 4.26. Proposed structure of blue CD-3 degradation product.

4.7.4 Proton NMR of blue CD-3 compound

The blue CD-3 degradation product isolated from the precipitates of degraded CD-3 solution and RA100 was analysed by proton NMR. The ^1H NMR spectrum is shown in Fig. 4.27. The main signals, along with descriptions and integration ratios are shown in Table. 4.5. A proposed structure for the blue CD-3 compound is shown in Fig. 4.26.

Chemical shift δ/ ppm	Description	Integration ratio	Assignment
1.00	triplet, 7.5 Hz	3H	CH_3, *I*
1.55	singlet	impurity	H_2O
2.30	singlet	3H	CH_3, *B*
2.35	singlet	3H	CH_3, *D*
2.95	singlet	3H	SO_2CH_3, *K*
3.45	multiplets, 7.5 Hz	6H	CH_2CH_2, *J, M, N*
3.75	singlet	impurity	$C_2H_4(OH)_2$
4.45	broad singlet	1H	NH, *L*
6.60	multiplets, 10 Hz	5H	ArH's, *A, E, F* RCH=CHR, C, H, G
7.10	doublet, 10 Hz	1H	ArH
7.15	singlet	impurity	$CHCl_3$

Table. 4.4 Tabulation of ^1H NMR data for blue CD-3 degradation product

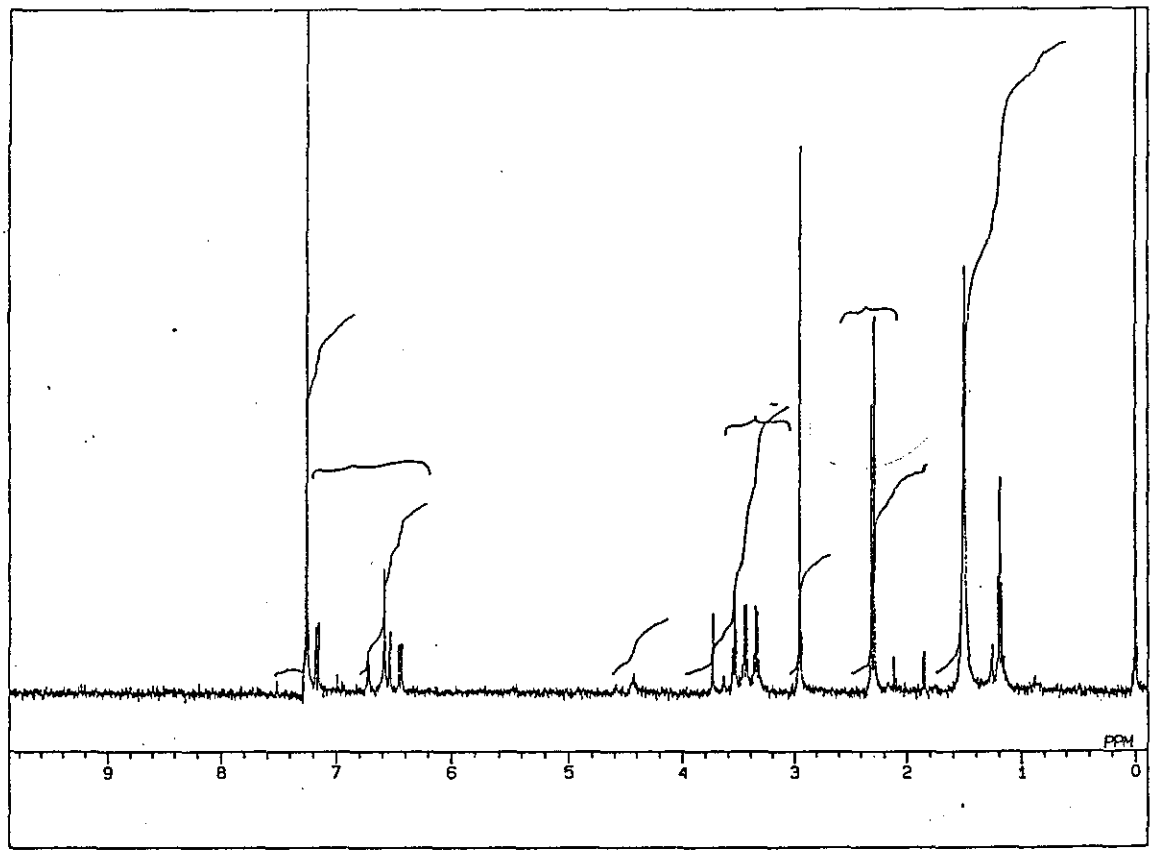


Fig. 4.27 ^{1}H NMR of blue CD-3 degradation product run in deuterated chloroform (400 MHz).

Impurities were present at 1.55 ppm due to water, 3.75 ppm due to ethylene glycol from the solvent system used in the isolation process and 7.15 ppm due to chloroform in the deuterated chloroform.

The triplet at 1.00 ppm (7.5 Hz) integrated to a methyl group and was assigned as the CH_3 of an N-ethyl group (*I*). The singlets at 2.30 ppm (*B*) and 2.35 ppm (*D*) integrated to methyl groups and were a methyl group attached to an aromatic ring and a methyl group attached to a conjugated system, which had been deshielded by an electron withdrawing group such as a carbonyl. The signal at 2.95 ppm (*K*) integrated to three protons and was probably part of an SO_2CH_3 system because of its chemical shift. This agreed with CD-3. The signals in the 3.00 ppm to 3.60 ppm region appeared to be a triplet of quartets and integrated to 6H. These signals probably originated from the amine bonded methylene groups of a sidechains (*J, M, N*). The coupling is difficult to see, but was estimated at 7.5 Hz. This was reinforced by the proximity of *J* to *I*, the triplet *I* had a coupling constant of 7.5 Hz.

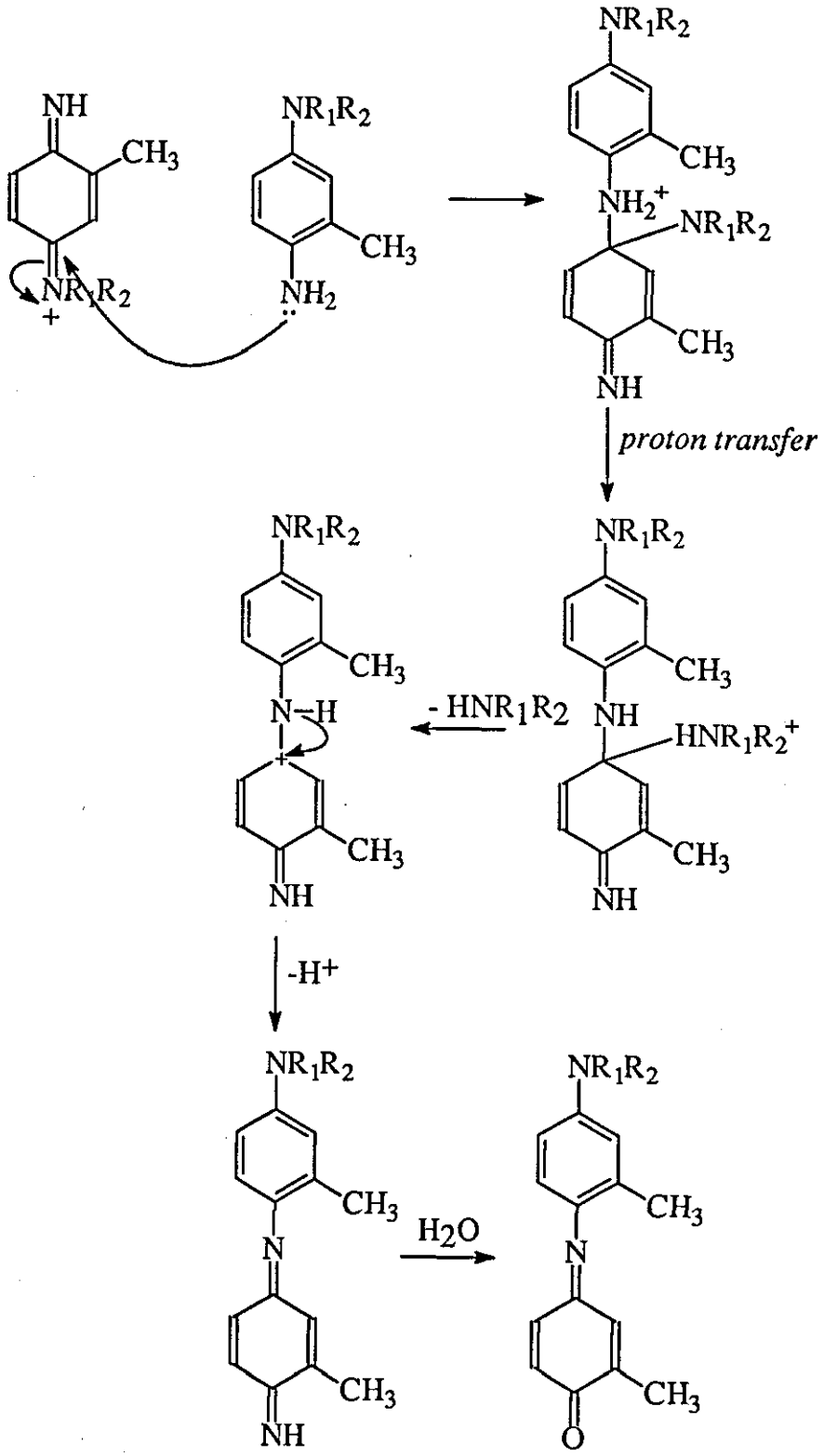
At 4.45 ppm there was a broad signal which integrated to a single proton. The broadness of this signal indicated that proton exchange was occurring, typical of an NH or OH system. In this case it was probably an NH (*L*), as the NH was relatively deshielded by the SO_2 group of the sidechain and its chemical shift was higher than that of an aliphatic amine. The signals from 6.60 ppm to 7.15 ppm were more complex than those seen in the yellow compound. The $CHCl_3$ impurity at 7.15 ppm may be obscuring some signals. Integration gave a total of 7H, one contributed by the $CHCl_3$. Therefore six protons remained and due to their chemical shift may be part of an aromatic ring system and an unsaturated ring system. The coupling was difficult to interpret but the pattern was similar to that seen for the blue CD-4 compound (Chapter 6.6.4)

Corbett investigated the proton NMR of indoaniline compounds which were similar to the blue CD-3 degradation product [78]. The chemical shifts for a series of methylated indoaniline dyes were reported. In the parent unsubstituted inoaniline dye, the aromatic protons corresponding to *E*, *F* (Fig. 4.26), had chemical shifts of 6.92 ppm and 6.65 ppm. The quinonoid ring protons corresponding to *C*, *G*, *H*, had chemical shifts of 7.32 ppm (*G*, *C*) and 6.50 ppm (*H*). In the o-methyl-indoaniline the aromatic ring protons had chemical shifts of 6.97 ppm and 6.73 ppm, corresponding to *E* and *F* in Fig. 4.26. The quinonoid protons had chemical shifts of 7.32 ppm (*G*), 7.24 ppm (*C*) and 6.61 ppm (*H*). The o-methyl group had the effect of moving the signals slightly upfield, probably due to the electron donating effect of the methyl group and splitting *G* and *C*, which had been chemically equivalent in the unsubstituted indophenol, into two signals. The o-methyl-indoaniline had the structure most similar to the blue CD-3 degradation product and the signals due to the aromatic and quinonoid protons in the 6.60 ppm to 7.00 ppm are similar to those seen in the blue CD-3 degradation product. The signals in the 7.20 ppm to 7.40 ppm range are also similar to those seen in the blue CD-3 degradation product (cf. 7.15 ppm in Table. 4.4. There is a more indepth analysis of the coupling given for the blue CD-4 degradation product, where expansions of the aromatic region were available (see Chapter 6.6.4).

The mass spectrum (see Chapter 4.3.3) gave a molecular weight of 375. Bearing this in mind and the fact that the NMR was more complex than the yellow azo compound seen previously then the ring structures must be unsymmetrical. The mass spectral data confirmed the structure (see Chapter 4.7.3).

4.7.5 Postulated mechanism for the formation of blue CD-3 degradation product

A postulated mechanism for the formation of this blue compound is shown in Fig. 6.28. The compound is formed by initial oxidation of the p-phenylenediamine to form the quinoneimine. This quinoneimine undergoes nucleophilic attack by the amino group of a p-phenylenediamine molecule. The resulting compound looses a sidechain and undergoes oxidation to eventually form a quinoneimine. Oxidative coupling of this type was also postulated by Tong and Glesmann [50].



R_1:- CH_2CH_3, R_2:- $CH_2CH_2NHSO_2CH_3$

Fig. 4.28 Postulated mechanism for the formation of blue CD-3 degradation compound.

4.8 Identification of purple CD-3 degradation compound

4.8.1 HPLC of purple CD-3 degradation compound

A chromatogram of the purple CD-3 degradation product is shown in Fig. 4.29. The purple product elutes with a retention time of 11.6 mins.

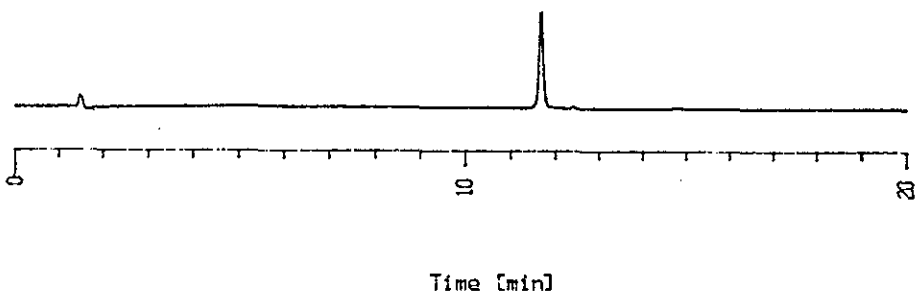


Fig. 4.29 Chromatogram of purple CD-3 degradation product (method B, detection at 243 nm, Supelco column)

4.8.2 UV spectra of purple CD-3 degradation product

UV studies were carried out on the purple CD-3 degradation product. The UV spectra of this compound, were run in ethanol (Fig. 4.30), acidified ethanol (Fig. 4.31) and basic ethanol (Fig. 4.32). The absorbance maxima are shown in Table 4.6.

Absorbance maxima acidified ethanol λ_{max} (nm)	Absorbance maxima methanol + two drops dilute HCl. λ_{max} (nm)	Absorbance maxima methanol + two drops dilute NaOH. λ_{max} (nm)
203.5	201.8	219.7
236.0	248.8	-
307.9	313.5	311.1
378.6	412.0	-
533.6	555.2	545.6

Table. 4.5 UV spectral data for purple CD-3 degradation product.

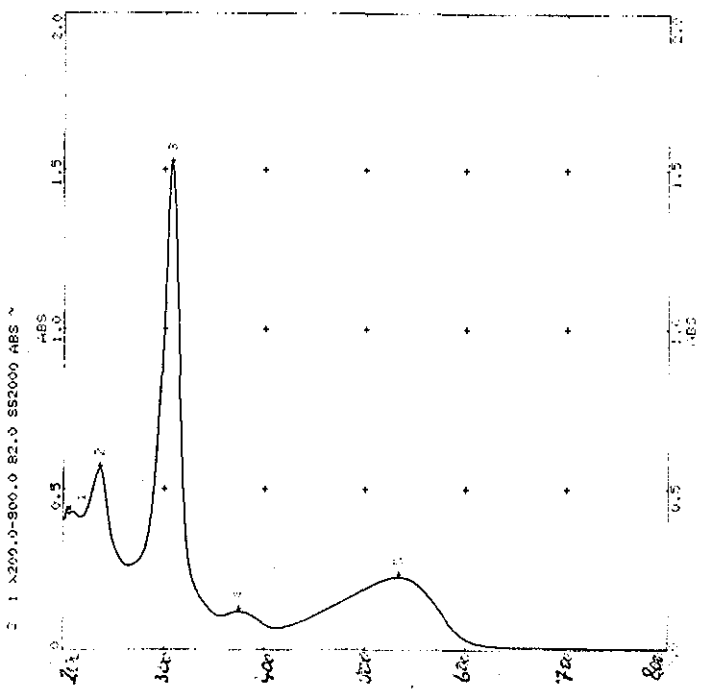


Fig. 4.30 UV spectrum of purple CD-3 degradation compound, run in ethanol.

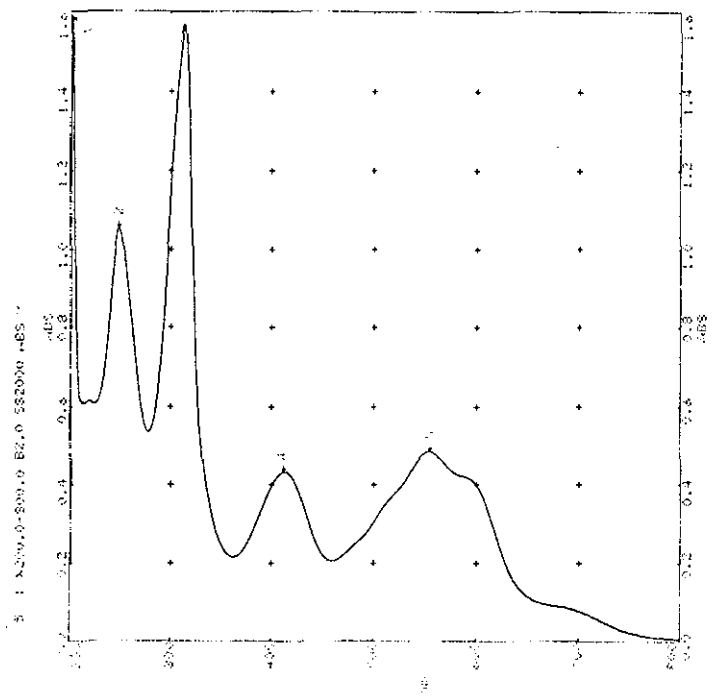


Fig. 4.31 UV spectrum of purple CD-3 degradation product, run in ethanol + two drops of dilute HCl

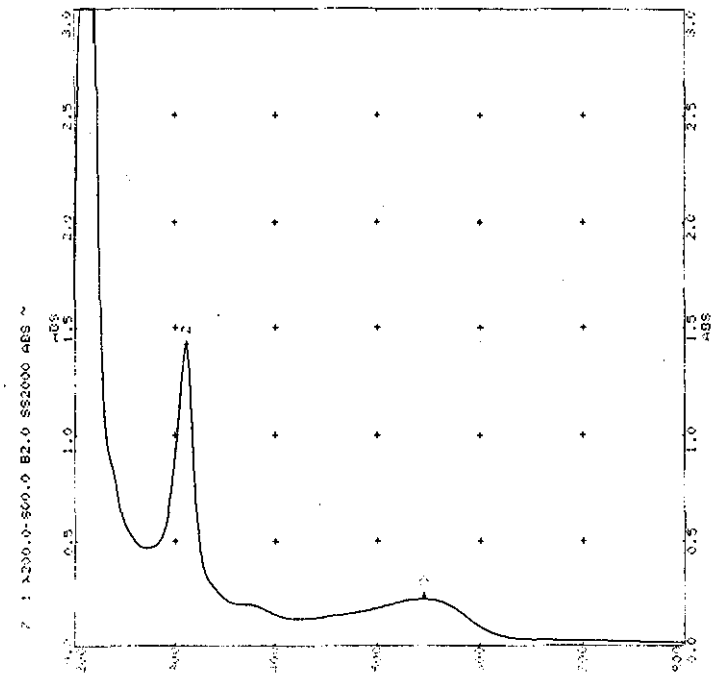


Fig. 4.32 UV spectrum of purple CD-3 degradation compound, run in ethanol + two drops of dilute NaOH.

4.8.3 Mass spectrometry of purple CD-3 degradation compound

A purple compound was isolated from RA100 precipitate and also from CD-3 precipitate. The EI mass spectrum of the purple compound is shown in Fig. 4.33. The mass spectrum shows a molecular species at m/z 534, with major peaks at m/z 455 and m/z 348. This corresponds to four mass units less than that seen for the yellow azo CD-3 degradation product. The signal at m/z 455 seems to be due to the loss of SO_2CH_3 from a sidechain. The signal at m/z 348 involved the loss of 186 mass units and could not easily be assigned. The NH_3 DCI mass spectrum of the purple compound gives an MH+ signal at m/z 535, confirming the mass as 534, see Fig. 4.34. Accurate mass measurements, using EI mass spectrometry, gave the molecular mass as 534.2081 corresponding to a molecular formula of $C_{24}H_{34}O_4N_6S_2$ (required 534.2083).

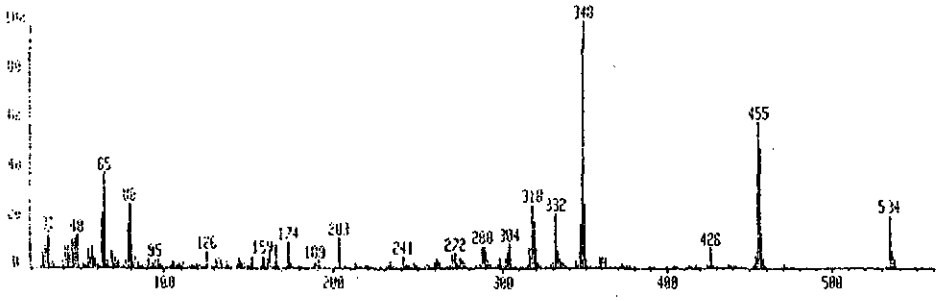


Fig. 4.33 EI mass spectrum of purple CD-3 degradation compound

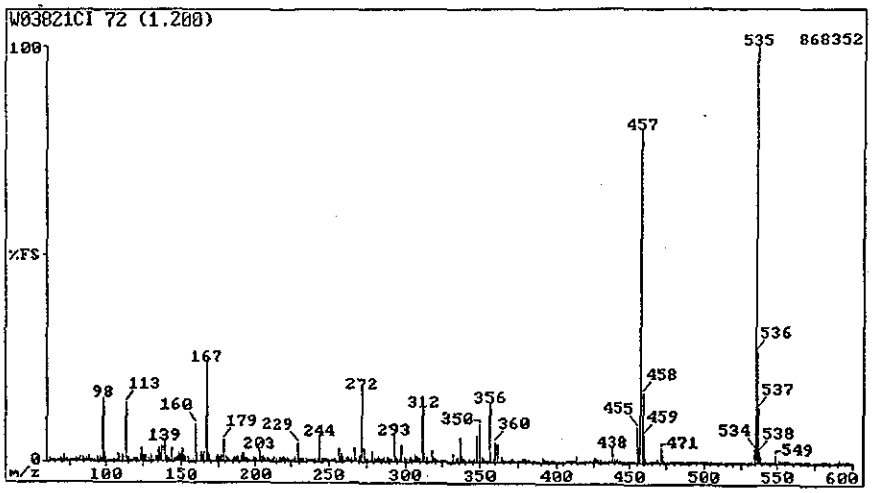


Fig. 4.34 NH_3 DCI mass spectrum of purple CD-3 degradation compound

4.8.4 Proton NMR of purple CD-3 degradation compound

The purple compound, isolated from RA100 precipitate and also from CD-3 precipitate, was analysed by proton NMR. This compound proved to have a very complex ^1H NMR spectrum and a mass spectrum which proved very difficult to interpret. A large quantity was isolated from the degraded CD-3 solution and ^1H NMR data, including a ^1H-DQCOSY spectrum were obtained before the sample degraded.

The ^1H NMR spectrum is shown in Fig. 4.35, along with the ^1H-DQCOSY spectrum in Fig. 4.36. The major signals with their relative integrations and their descriptions are shown in Table. 4.7. Analysis of these results led to a postulated structure (Fig. 4.37) and this was used as a basis of discussion.

Chemical shift δ/ **ppm**	Description	Integration	Assignment
1.25	triplet, 7.5 Hz	3H	CH_3, *N*
1.35	triplet, 7.5 Hz	3H	CH_3, *R*
1.70	singlet	impurity	H_2O
2.80	sharp singlet	3H	CH_3, *A*
2.90	sharp singlet	3H	CH_3, *F*
2.95	sharp singlet	3H	CH_3, *G*
2.97	sharp singlet	3H	CH_3, *H*
3.45	multiplet	4H	CH_2,CH, *C, L, K*
3.58	quartet with multiplet, 7.4 Hz	4H	CH_2, *M, Q*
3.65	possible triplet, 6.3 Hz	2H	CH_2, *D*
3.83	doublet of triplets, 6, 12 Hz	1H	CH, *I*
4.40	doublet of doublets, 14.7, 5.2 Hz	1H	CH, *J*
4.85	triplet, 6.7 Hz	1H	NH, *B*
6.95	doublet, 2 Hz	1H	ArH, *E*
7.10	sharp singlet	1H	ArH, *O*
7.28	sharp singlet	impurity	$CHCl_3$
7.30	doublet, 2 Hz	1H	ArH, *P*

Table. 4.7 Tabulation of ^1H NMR data for purple CD-3 degradation compound run in deuterated chloroform

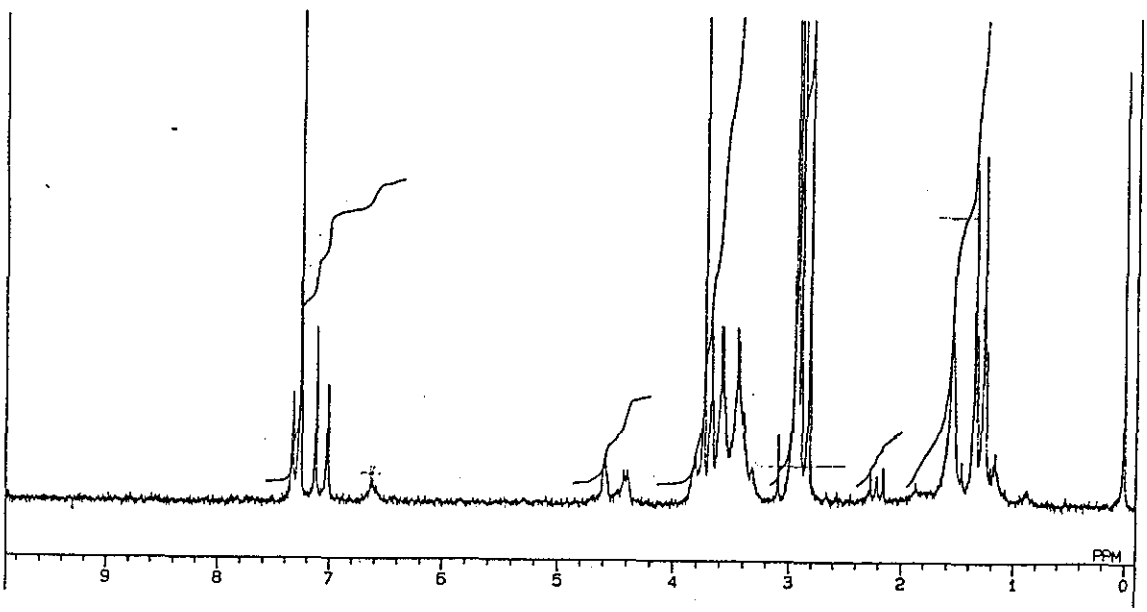


Fig. 4.35 ^{1}H NMR spectrum of purple CD-3 degradation compound run in deuterated chloroform (400 MHz).

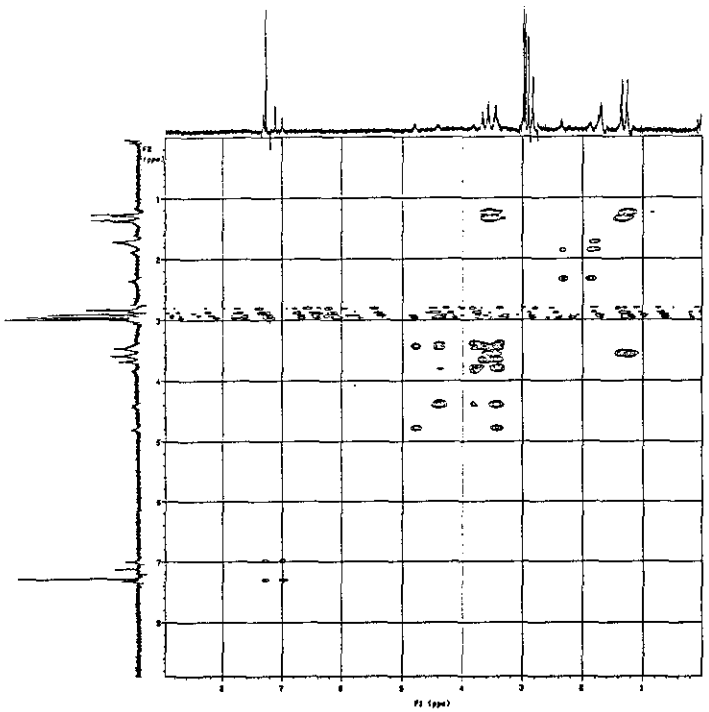


Fig. 4.36 ^{1}H-DQCOSY spectrum of purple CD-3 degradation compound run in deuterated chloroform(400 MHz).

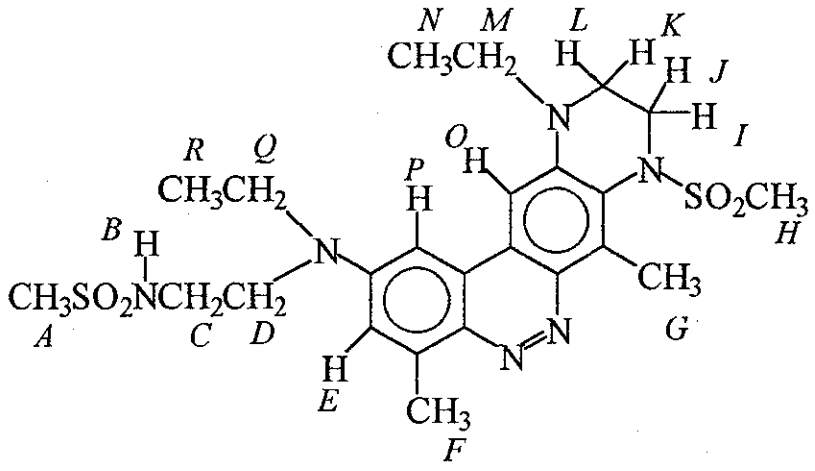


Fig. 4.37 Postulated structure of purple CD-3 degradation compound.

In the spectrum in deuterated chloroform, the signals at 1.25 ppm and 1.35 ppm both integrated to methyl groups. Their chemical shifts, triplet nature and relative integrations would identify them as methyl groups of the N-ethyl sidechains (***N***, ***R***). The difference in chemical shift seemed to indicate that their chemical environments are different as indicated in the postulated structure. Both triplets have similar coupling constants (7.5 Hz). The signal at 1.7 ppm was due to water which was present as an impurity.

At 2.8 ppm to 3.7 ppm there were four sharp singlets which each integrated to 3H, which along with their chemical shifts seemed to indicate four methyl groups in different chemical environments. Referring to the structure shown, these would be the methyl groups ***A***, ***F***, ***G*** and ***H***, with ***A*** and ***H*** more deshielded owing to their proximity to SO_2 groups and they are in positions to those in CD-3.

The signals at 3.4 ppm to 3.83 ppm involved a complex system of overlapping multiplets and the integration was not a great help. The complex nature of the multiplet did not allow the calculation of coupling constants. The signals at 3.45 ppm were very difficult to interpret but by looking at the ^{1}H-DQCOSY spectrum (Fig. 4.36) it was possible to see that one of the signals at 3.45 ppm was coupled to the signal at 4.8 ppm, which was assigned to the N-H (***B***) and also to the signal at 3.7 ppm, which was due to the protons (***D***). This indicated an NH-CH_2 group as in the original CD-3 molecule. There also appeared to be another signal at 3.45 ppm which was coupled to the signals at 3.9 ppm (***I***) and 4.4 ppm (***J***) and this was probably

due to the protons (*K, L*). A quartet (7.4 Hz) and multiplet, centred at 3.58 ppm was coupled to the CH_3's (*N, R*) of the ethyl groups which appear at 1.25 ppm and 1.35 ppm, indicating that it was due to the methylene protons (*M, Q*) of the ethyl groups. A triplet (6.3 Hz) at 3.65 ppm was coupled to that at 3.45 ppm and was due to the methylene of the $NHCH_2\underline{CH_2}N$ (*D*) group. The signal at 3.83 ppm, which integrated to a single proton, was coupled to the signals at 3.45 ppm and 4.4 ppm (see ^{1}H-DQCOSY), which indicated a CH group (*I*), which was coupled to other inequivalent protons. Because there were some impurities present from the solvent system used for isolation and the deuterated chloroform and the coupling is not very helpful, it is difficult to correctly assign this signal.

The signal at 4.4 ppm was coupled to the signals at 3.4 ppm and 3.9 ppm. This integrated to a single proton and was probably the proton *J*, which appeared as a doublet of doublets. The triplet (6.7 Hz) at 4.85 ppm, which integrated to a single proton was possibly the NH group (*B*) as its chemical shift indicated. This was coupled to the signal at 3.4 ppm (see COSY) which was a methylene (*C*) and this would confirm that the signal at 4.85 ppm was an NH group.

The three signals at 6.95, 7.20 and 7.30 ppm were all assigned to aromatic protons, as their chemical shifts indicated. The COSY spectrum showed that the signals at 6.95 ppm and 7.30 ppm were coupled to each other, albeit long range coupling, as the coupling constant, in each case, was small (2Hz). This could mean that two aromatic protons were on one ring (*E, P*) but were not adjacent to each other as this would lead to a much larger coupling constant. The signal at 7.1 ppm gave a sharp singlet and the ^{1}H-DQCOSY did not show any coupling, leading to the conclusion that the proton (*O*) was on a second highly substituted ring.

The complex coupling pattern of the *I, J, K, L* protons is indicative of a rigid alicyclic system in which the protons on individual methylene groups are held in fixed axial or equatorial positions.

However it was clear that the structure of the ring system was not clearly discernible from the NMR data and may be either the benzo[c]cinnoline type structure (I) or the phenazine type structure (II) shown in Fig. 4.38.

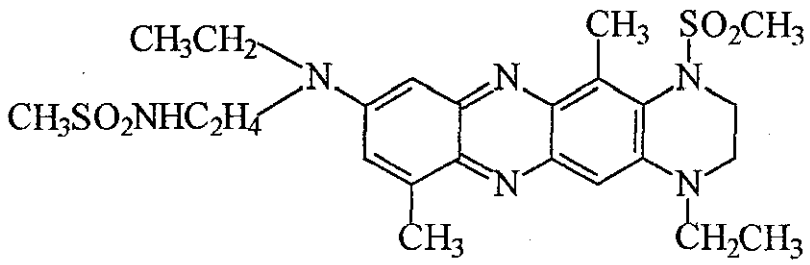


Fig. 4.38 Possible alternative structure of purple CD-3 compound.

From the number of protons, it is clear that there must be a four ring structure with 4 less protons than the azo compound. Initially it was difficult to assign a structure but the limited aromatic proton signals show the substitution pattern and indicate the position of linkage. The structure in Fig. 4.37 was proposed by cyclisation of the azo analogue, resulting from azo ring and sulphonamide cyclisation. However the spectra data does not distinguish the alternative phenazine structure as this only affects the central ring.

The purple compound was also run in deuterated acetone where a shift of signals occurred (Fig. 4.39). The methylene groups in the 3.50 ppm to 3.90 ppm range sharpen and become more clearly resolved. The aromatic protons in the 6.90 ppm to 7.30 ppm range are also affected and the signals at 7.30 ppm and 7.05 sharpen whilst the signal at 6.93 broadens. The signals at 7.30 ppm and 6.93 ppm appear to decouple from each other. There is a broad singlet at 6.30 ppm which may be assigned to an N-H group. Temperature also affected the spectra, sharpening the aromatic signals in the 6.90 ppm to 7.3 ppm range and simplifying the methylene signals. This could be due to increased flexing of the cyclised sidechain which may reduce the coupling.

The effect of adding deuterated trifluoroacetic acid (dTFA) was to protonate the cyclised sidechain nitrogens and improve the sharpness in the methylene region. The ^{1}H NMR spectrum of the purple CD-3, run in deuterated acetone with two drops of dTFA added is shown in Fig. 4.40. A simulation of the coupling pattern seen in the 3.5 ppm to 4.5 ppm region was run at Kodak. The observed chemical shifts together with the coupling constants between each proton were used to simulate the coupling pattern in the 3.5 ppm to 4.5 ppm region is shown in Fig. 4.41. The simulation agrees

well with the actual ^{1}H NMR spectrum seen (Fig. 4.41). Some of the signals seen in the simulation are obscured in the real sample.

It was thought that a Nuclear Overhauser ^{1}H NMR spectrum of the purple compound might help in identification whereby ring protons (*O* and *P* in Fig. 4.37) could be selectively decoupled and coupling patterns could become more clear. However, the protons in question had very similar chemical shifts in the benzo[c]cinnoline and so selective decoupling was not possible. In the phenazine type structure, the protons are too distant for the Nuclear Overhauser Effect to aid in interpretation.

Decoupling experiments were run in deuterated acetone, this led to a slight shift in the doublet of triplets at 3.83 ppm to 4.05 ppm but did not seem to effect the other signals. Decoupling of the methyl groups at 1.35 ppm led to the quartet and multiplet at 3.58 ppm collapsing into two singlets. This indicted that the quartet and multiplet contained the methylenes ***M*** and ***Q***, as these methylenes are adjacent to the methyl groups. Partial decoupling of the signal at 3.45 ppm or 3.58 ppm (***C***, ***K***, ***L***) led to the doublet of doublets at 4.40 ppm (***J***) collapsing to a broad singlet and the doublet of triplets at 3.83 ppm (***I***) reducing to a doublet, the triplet at 3.65 ppm (***D***) reduced to a singlet. Close examination of the coupling constants showed that the signal at 3.45 ppm had a coupling constants of 4.2 Hz and 14 Hz, the doublet of triplets at 3.83 ppm had coupling constants of 5.5 Hz (doublet) and 12.5 Hz (triplet), whilst the doublet of doublets had coupling constants of 5.2 Hz and 14.7 Hz. Decoupling of the doublet of doublets (***J***) at 4.40 ppm (5.5 Hz, 14.7 Hz) led to the collapse of the doublet of triplets at 3.83 ppm (5.5 Hz, 12 Hz) into a triplet and a partial collapse of the multiplet at 3.45 ppm. Decoupling of the doublet of triplets at 3.83 ppm (5.5 Hz, 12.5 Hz) led to the doublet of doublets at 4.40 ppm reducing to a doublet (14.7 Hz) and the multiplet at 3.45 ppm partially collapsing. This decoupling helped in confirming the structure of the cyclised sidechain as an ABCD pattern.

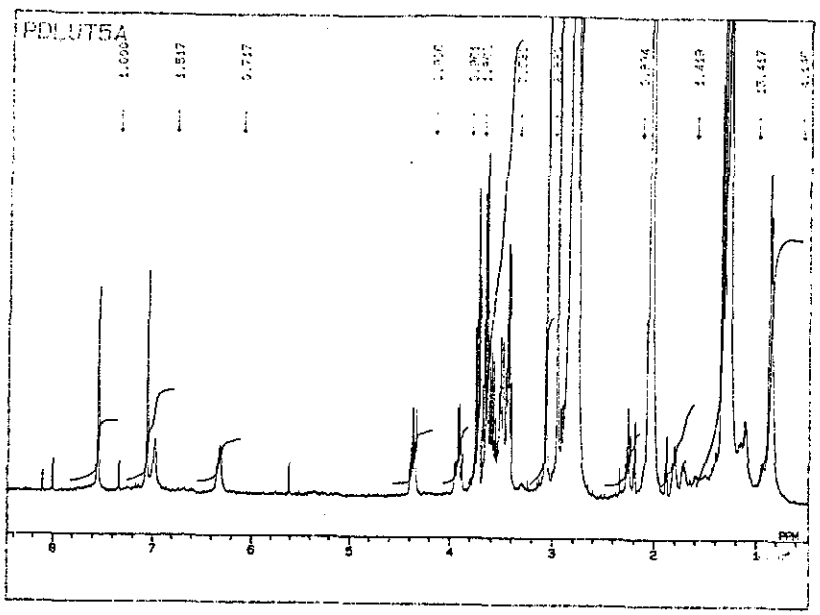


Fig. 4.39 Proton NMR of purple CD-3 degradation product, run in deuterated acetone (400 MHz).

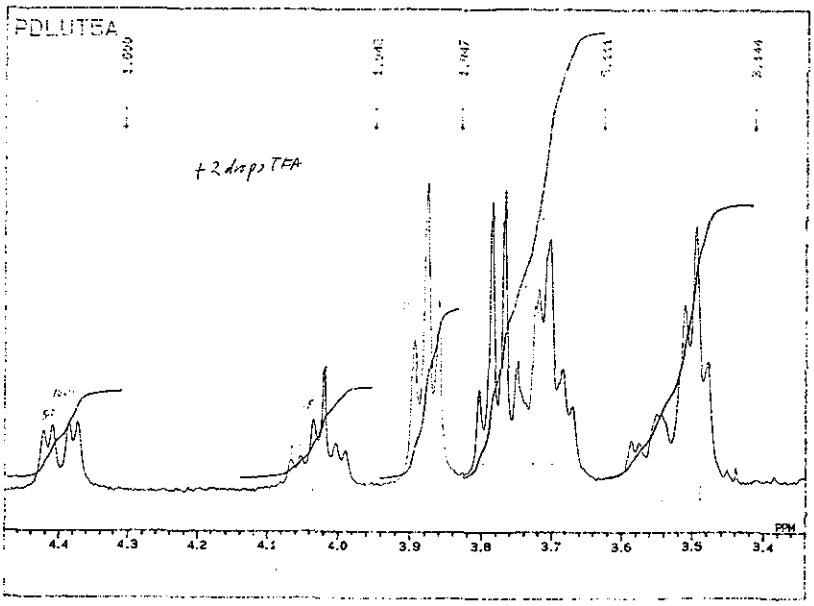


Fig. 4.40 ^{1}H NMR of purple CD-3 compound, run in deuterated acetone with two drops of TFA added (400 MHz).

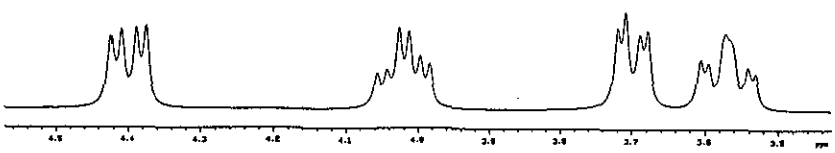


Fig. 4.41 Simulation of coupling in 3.5 ppm to 4.5 ppm region of proton NMR of purple CD-3 degradation product.

4.8.5 Spectroscopic analysis of purple compound.

The Fourier transform infra-red (FTIR) spectrum of the purple compound is shown in Fig. 4.39. There are major adsorption at 1225 cm^{-1} which may be due to S=O. It also has an adsorption at 1630 cm^{-1} which is probably due to C=C bonds. The adsorption at 3000 cm^{-1} indicates the presence of C-H groups. The excitation and emission spectra (Fig. 4.40) show a high degree of fluorescence confirming a planar multi-ring structure.

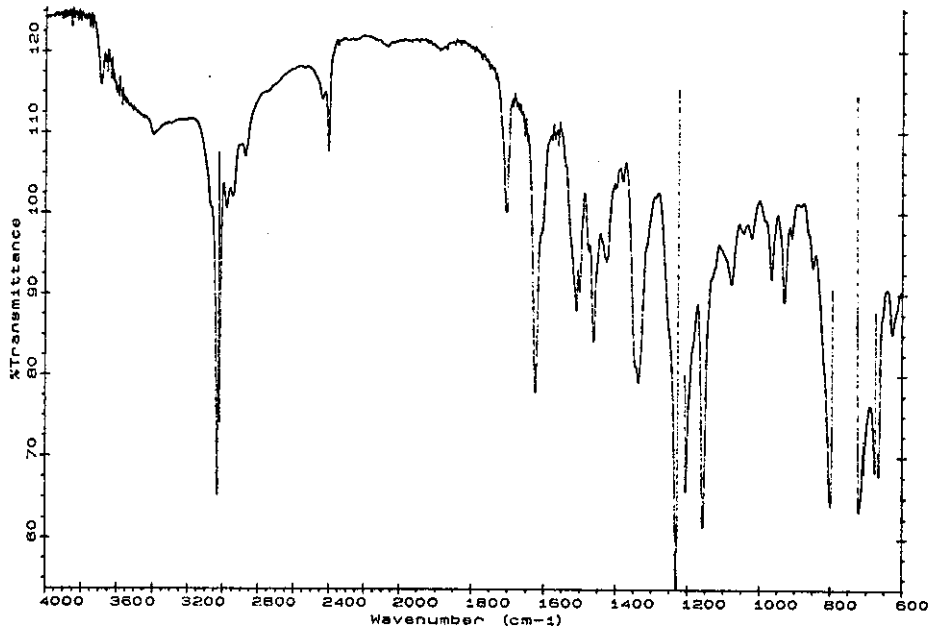


Fig. 4.42 FTIR spectrum of purple CD-3 degradation product

As the UV, NMR, FTIR and mass spectra were inconclusive as to the ring structure of the purple compound, it was hoped that Raman spectroscopy could aid in assignment. Samples of phenazine (Fig. 4.45) and benzo[c]cinnoline (Fig. 4.44)were obtained for comparative studies.

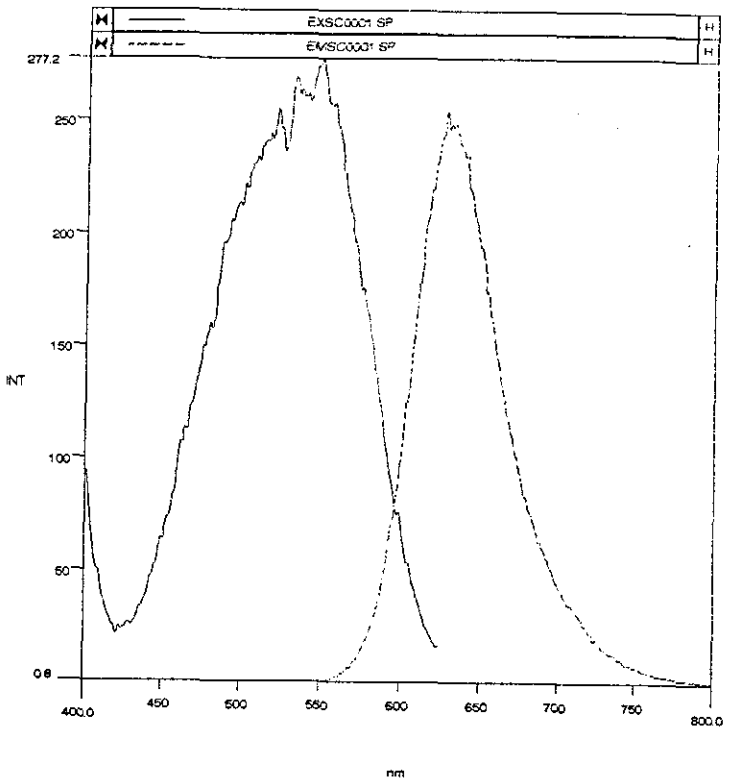


Fig. 4.43 Excitation and emission spectra of purple CD-3 degradation product.

Tabulation of the Raman spectral data is shown in Table 4.8. The Raman spectrum of benzo[c]cinnoline and the Raman spectrum of phenazine appeared to be similar. A typical value for the N=N stretching frequency would be 1578 cm^{-1} [74]. The signal at 1554 cm^{-1} in the phenazine spectrum is typical of a C=N. However, there are many additional symmetries in the unsubstituted compounds and therefore the N=N signals would not be particularly prominent. There are many C=C and C=N signals. There is a signal at 1516 cm^{-1} in the spectrum of the purple CD-3 compound but it is unclear as to whether this is a C=N stretch (as in phenazine) or a N=N stretch (as in benzo[c]cinnoline). Again the results as to the structure of the central ring system were inconclusive. The purple CD-3 compound exhibited a high degree of fluorescence and this obscured the Raman spectrum to some degree.

Phenazine Frequency (cm^{-1})	Benzo[c]cinnoline Frequency (cm^{-1})	Purple CD-3 compound Frequency (cm^{-1})
413	425	
733	712	651
1400	1349	1403
1474	1432	
1554	1551	1516
-	1578	
-	1611	1600
3060	3069	2983

Table 4.8 Tabulation of Raman spectral data for benzo[c]cinnoline, phenazine and the purple CD-3 compound.

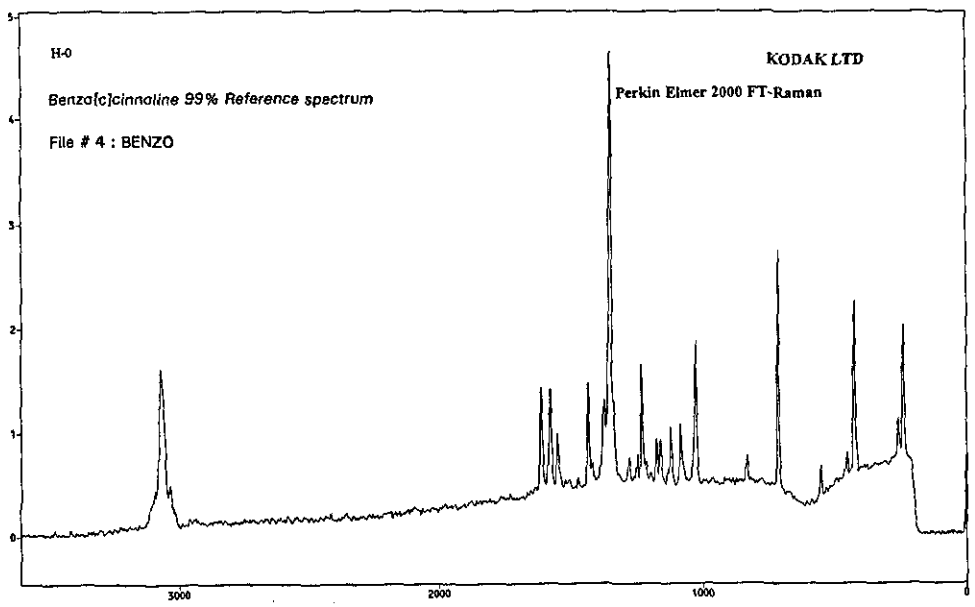


Fig. 4.44 Raman spectrum of benzo[c]cinnoline.

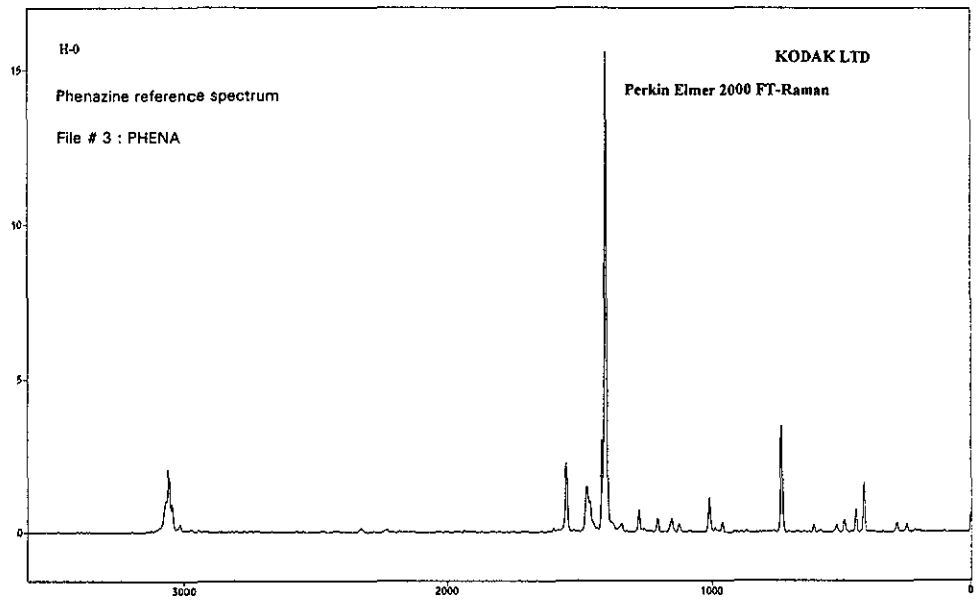


Fig. 4.45 Raman spectrum of phenazine.

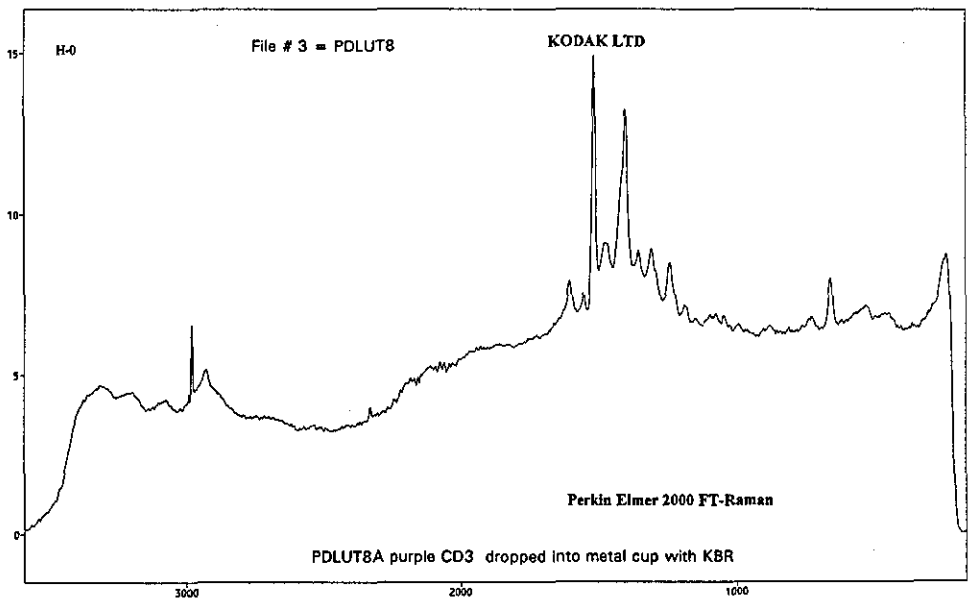


Fig. 4.46 Raman spectrum of purple CD-3 degradation compound

4.8.6 Postulated mechanism for the formation of purple CD-3 degradation product

When a solution of the yellow CD-3 degradation product (Fig. 4. 21) was illuminated by sunlight and ultraviolet radiation at a high pH, it did not cyclise to give the purple compound. It was difficult to postulate a mechanism for the formation of the purple compound from the azo compound without cyclisation of the azo occurring. This would seem to indicate that the benzo[c]cinnoline (Fig. 4.37) was not the purple compound. It was easier to formulate the phenazine structure (Fig. 4.38). A mechanism was formulated for the formation of the phenazine compound (Fig. 4.44). However no intermediate compounds were available to substantiate this mechanism.

The postulated mechanism for the phenazine compound involves attack on the quinonediimine by a neutral p-phenylenediamine at the position show (*a*). Attack on the tertiary amine would lead, eventually, to the blue compound. There then follows three other oxidation steps (*c* to *d*, *g* to *h*, *i* to *j*)and two cyclisation steps (*e* and *h*) to give the eventual phenazine product.

4.9 Summary

Non-polar degradation products from CD-3 containing developer solutions have been isolated by TLC and identified by mass spectrometry, NMR and other spectroscopic means. A yellow azo dimeric compound and blue quinoneimine compound were initially isolated and identified. Mechanisms for their formation could be postulated. The identification of the purple compound proved more difficult. The central ring structure was complex to assign and the only method for positive identification may be X-ray crystallography. However a mechanism for the formation of a phenazine type compound could be postulated.

Looking at the proposed reaction schemes, it can be seen that many other reactions can occur to form trimers and polymeric degradation products. The TLC of the precipitates showed a multitude of non-polar degradation products and it may have been possible to isolate more but this was not thought feasible because of the difficulty and small sample sizes involved.

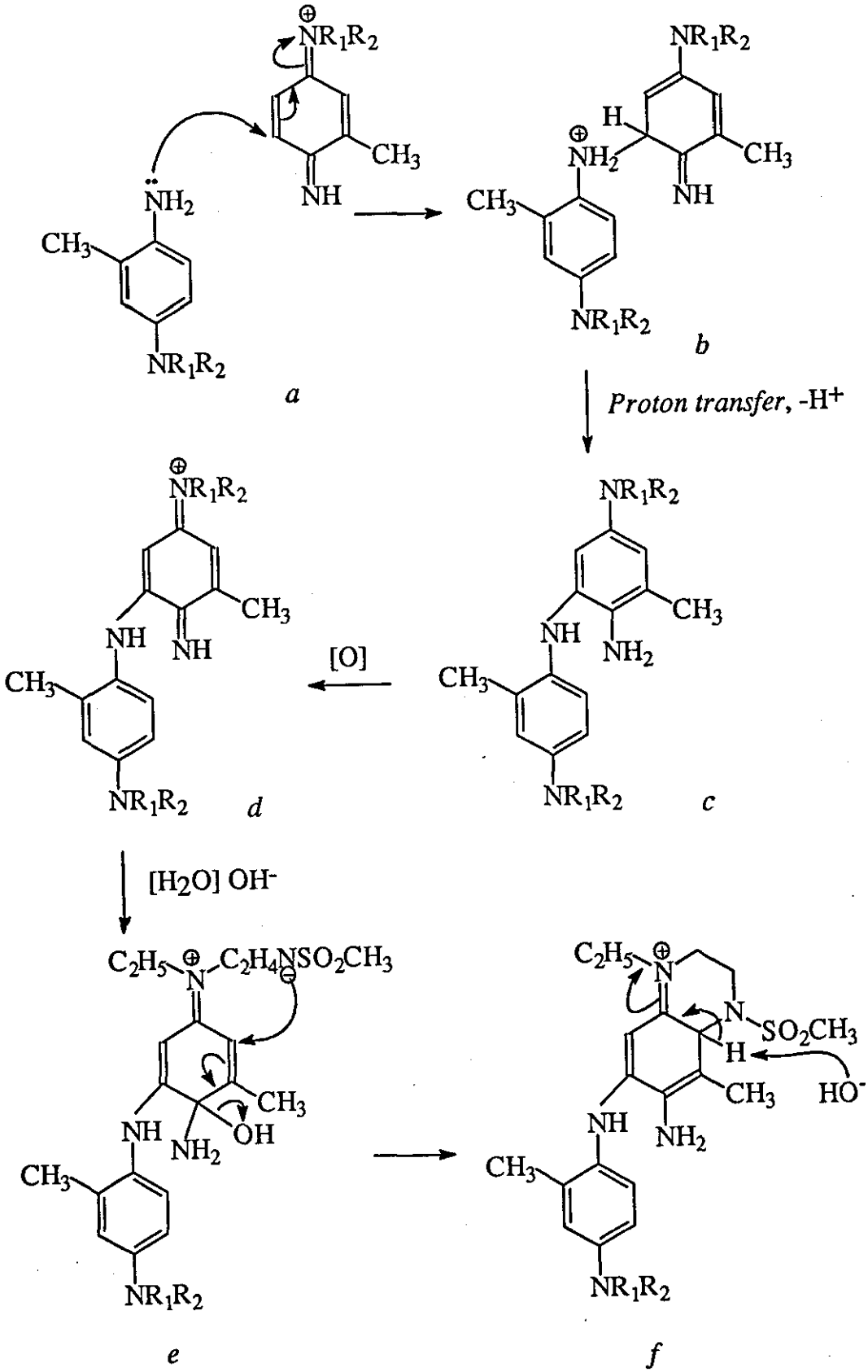


Fig. 4.47 Proposed mechanism for formation of purple CD-3 degradation product.

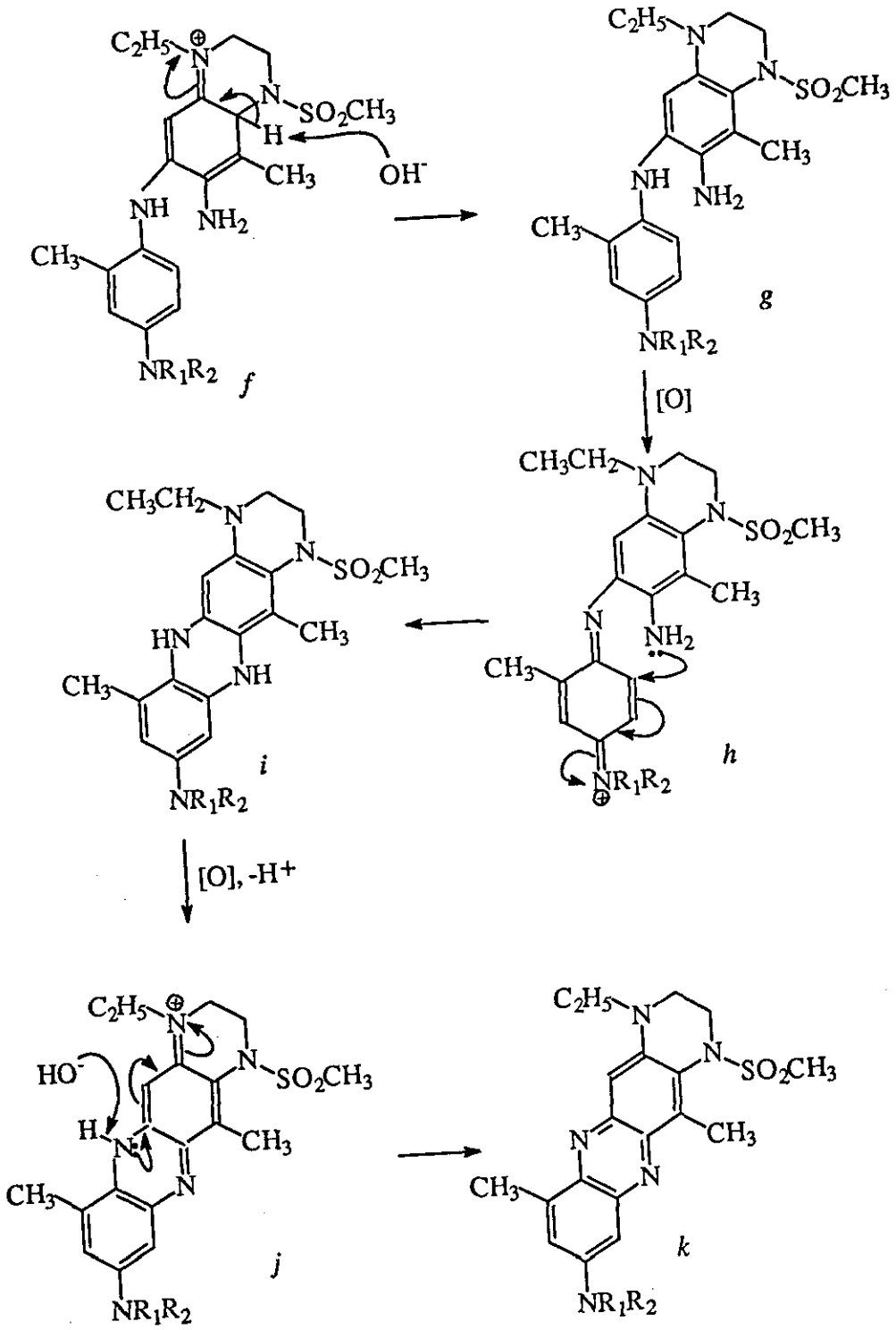


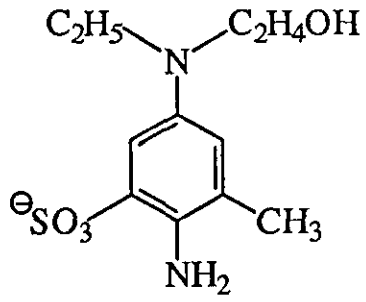
Fig. 4.47a Postulated mechanism for formation of purple CD-3 degradation product (continued).

Chapter 5

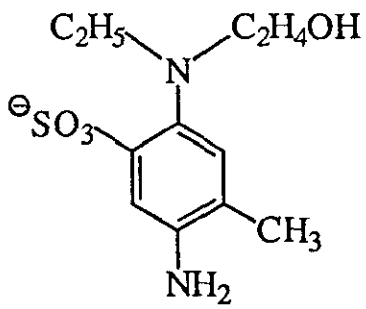
HPLC analysis of CD-4 developing agent and C-41 developing solutions

5.1 Introduction

CD-4 (Fig. 1.2) is the developing agent used in C-41 colour developer solutions to reduce the exposed silver ions in photographic film to metallic silver (see Chap.1). The object of the investigations involving CD-4 developing agent and C-41 developer solution was to develop methods of analysis for CD-4 and its degradation products, apply these methods to C-41 developer solution and monitor its degradation with time. A C-41 solution must be versatile as it must be capable of developing other manufacturers' film as well as Kodak film. Therefore the scope for improving and optimising the developing qualities of C-41 solutions is limited. C-41 developer solutions contain a high proportion of sodium sulphite (5.0 g/l) and lower levels of hydroxylamine sulphate preservative (3.4 g/l). When a C-41 solution was allowed to degrade only a small amount of CD-4 non-polar degradation products were formed in C-41 solutions. A litre of C-41 solution yielded 0.1484 g of precipitate from 4.5 g/l of CD-4, a yield of 3.3 % in contrast to RA4 where the yield was 54%. The majority of oxidised CD-4 developing agent reacts with the sodium sulphite in the developer solution to form CD-4 sulphonates. Previous work [13] had indicated that at pH 10.5, the pH of C-41 developer solutions, the major product of the reaction of CD-4 with sulphite, in the presence of air, is the m-sulphonate (Fig. 5.1). However, over time, several other compounds are formed including the o-sulphonate (Fig. 5.1) and possibly the disulphonate [13].



CD-4 meta-sulphonate



CD-4 ortho-sulphonate

Fig. 5.1 Structures of CD-4 sulphonates

5.2 HPLC analysis of CD-4

Because CD-4 is a less polar compound than CD-3 (See Fig. 1.2) it was postulated that CD-4 could be easily separated using the pH 5.6 acetate buffer, ion-pair reagent, acetonitrile organic modifier and conditions already developed for CD-3 and RA4 solutions. However, CD-4 eluted with a very short retention time under these conditions and it was found necessary to drop the initial organic modifier percentage to 5% in the gradient HPLC analysis run. This gave an adequate separation of CD-4 but peak tailing was still a problem, see Fig. 5.2. for a chromatogram of fresh CD-5.

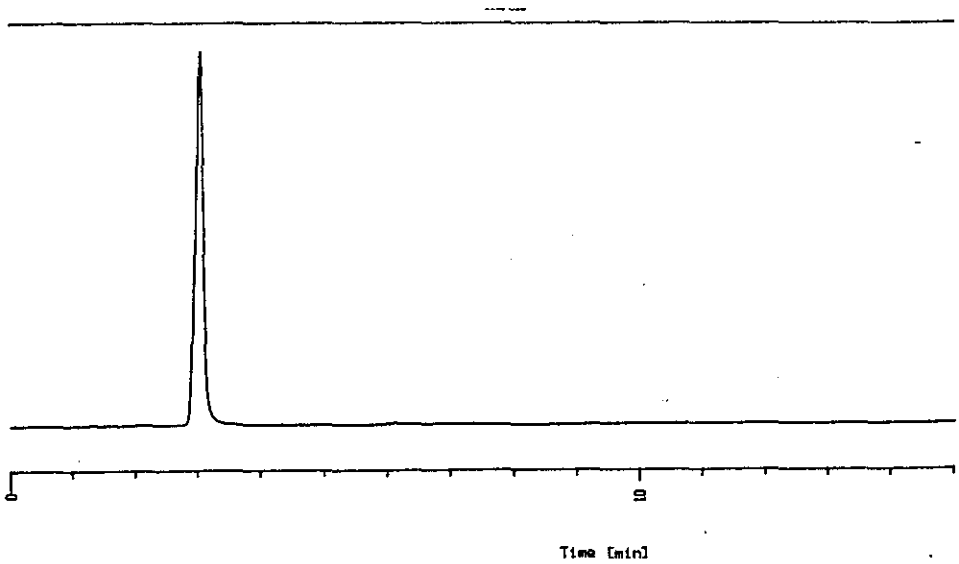


Fig 5.2 Chromatogram of CD-4 (method D, detection at 243 nm, Shandon column)

Retention times and peak shape were not reproducible, the retention time of CD-4 varied from 2 mins to 3 mins, the separation proved to be quantitative. A calibration plot for CD-4 is shown in Fig. 5.3. The plot gave a good correlation coefficient (r^2 of 0.9988).

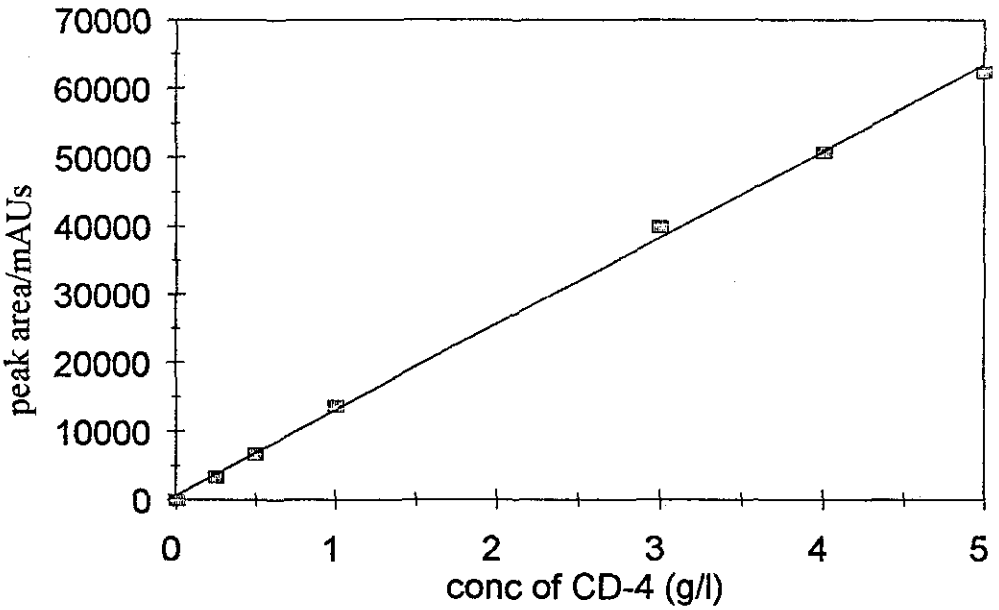


Fig. 5.3 Calibration plot for CD-4 (method D, detection at 243 nm, 20 µl injection, Shandon column).

5.3 HPLC analysis of C-41 developer solutions

The conditions described for the analysis of CD-4 were varied to improve the separation but there was no appreciable improvement in the resolution and reproducibility of CD-4, so the HPLC method developed for CD-4 was applied to C-41 developer solution without any modifications. As a C-41 developer solution degrades CD-4 sulphonates are formed as the oxidised developer reacts with the sulphite present in solution. Even in fresh solutions of C-41 some CD-4 sulphonates were found to be present (see Fig. 5.4.). The gradient conditions used for the separation of CD-4 and sulphonates are shown in Fig. 5.5. The gradient conditions allowed for a good separation of CD-4, then the organic modifier concentration was increased, allowing for the separation of the CD-4 sulphonates which had ion-paired with the t-butylammonium bromide. After separation of the CD-4 sulphonates the organic modifier concentration was increased still further and

separation of the CD-4 non-polar products, which will be described later, was possible.

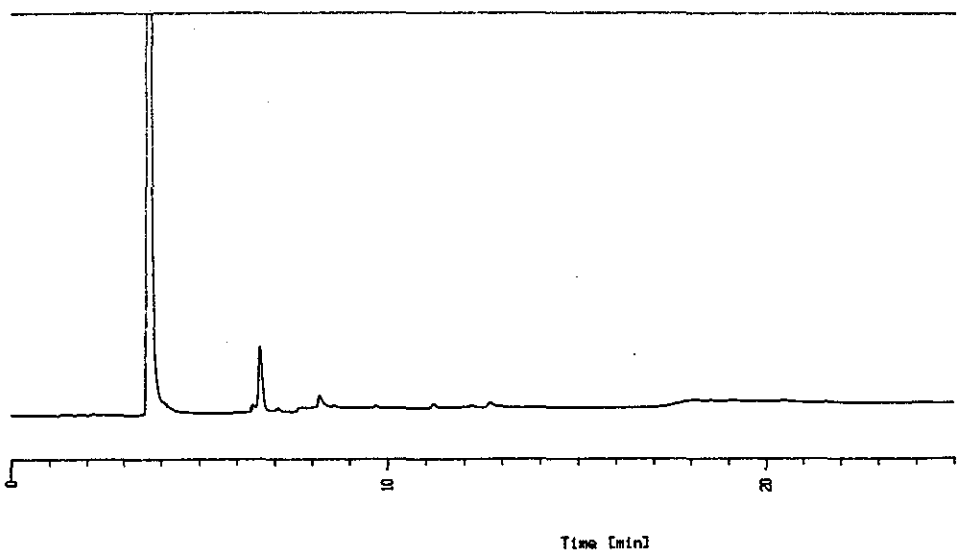
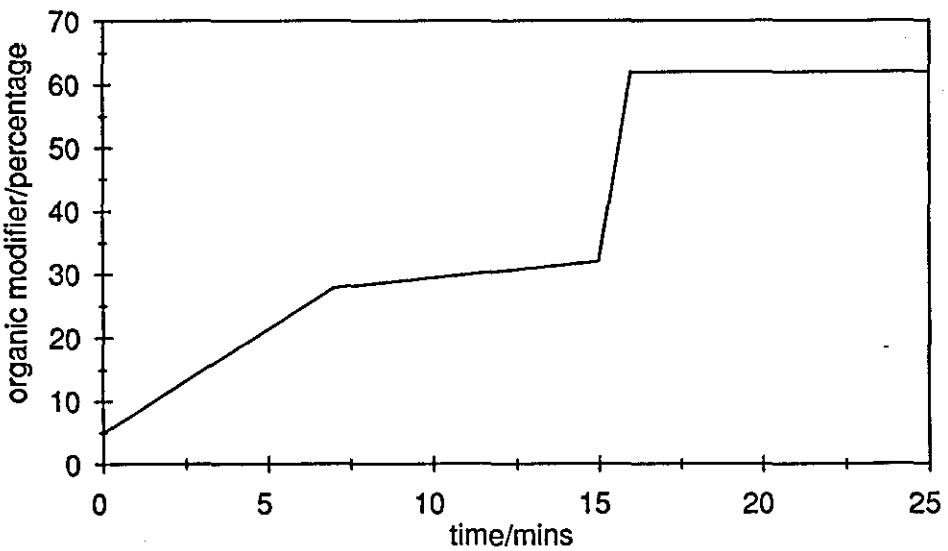


Fig. 5.4 Chromatogram of fresh C-41 (method D, detection at 243 nm, Shandon column)



Organic modifier:- acetonitrile

Fig. 5.5 Gradient conditions for C-41 separation (method D)

CD-4 elutes with a retention time of 3.7 mins which is slightly longer than that already seen in Fig. 5.2. The m-sulphonate has a retention time of 6.5 mins.

5.4 Degradation of C-41 developer solution

Solutions of C-41 were allowed to degrade with time and analysed daily. A chromatogram of a five day old C-41 standard which had been stored in a sealed clear glass container in sunlight is shown in Fig. 5.6.

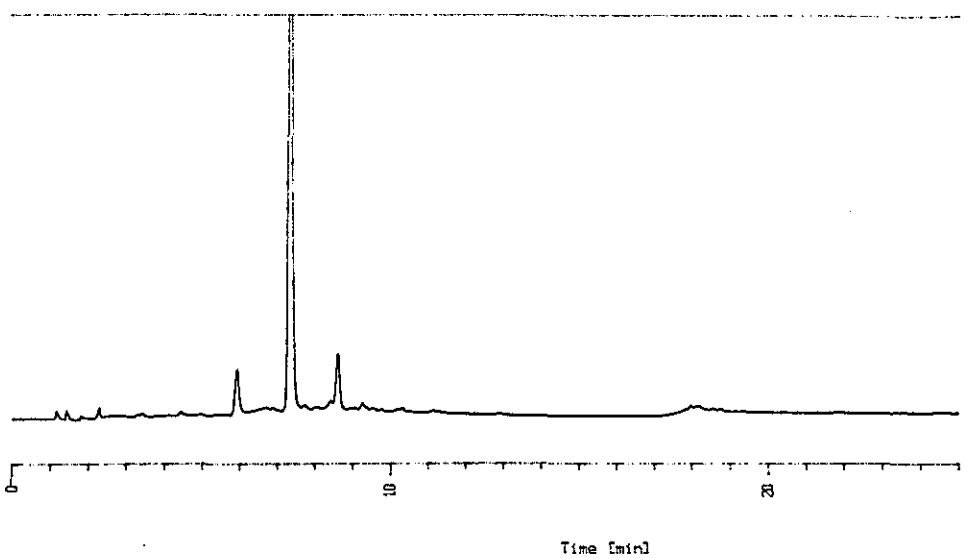


Fig. 5.6 Chromatogram of five day old C-41 solution (method D, detection at 243 nm, Shandon column).

A C-41 developer solution contains 5.0 g/l of Na_2SO_3 and 5.5 g/l of CD-4, in a 1.6:1 molar ratio. The CD-4 sulphonates could be identified from their UV spectra [15] and the m-sulphonate had a λ_{max} at 305 nm and the o-sulphonate had a λ_{max} at 296 nm. High levels of the m-sulphonate (retention time 6.5 mins) had formed whereas the o-sulphonate (retention time 5.8 mins) and an unidentified compound with a similar UV spectrum to the o-, m-sulphonates (retention time 8.8 mins) had formed to a much lesser degree. Several other unidentified products at longer retention times, had formed in lower concentrations. It is possible that these were other sulphonates and disulphonates as they had similar UV spectra to the sulphonates already seen.

As C-41 degraded still further (Fig. 5.7) several non-polar CD-4 degradation products were formed in addition to CD-4 sulphonates including a yellow compound and blue compound which are explained in Chapter 6. These had retention times of 18 to 20 mins.

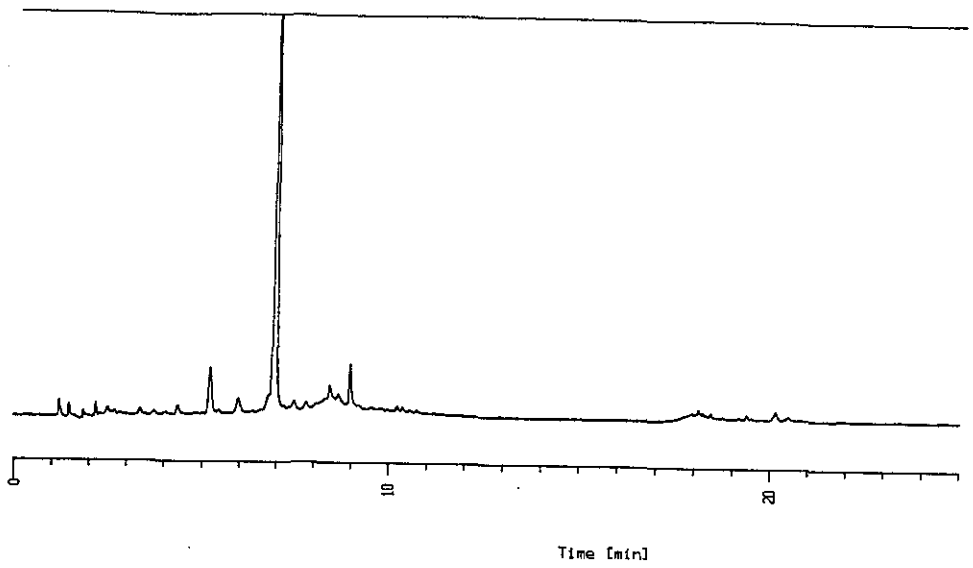


Fig. 5.7 Chromatogram of degraded C-41 (method D, detection at 243 nm, Shandon column).

A graph of CD-4 degradation in C-41 with time is shown in Fig. 5.8. It is not known if the degradation eventually reaches a steady state. However, it can be seen that the concentration of the m-sulphonate begins to decrease after reaching a maximum after five days. Although other sulphonates form they are not formed in sufficient quantity to account for the loss of m-sulphonate so it may be that m-sulphonate forms polymeric non-polar products on further oxidation. Again, as was the case with CD-3, the formation of individual non-polar degradation products was slow but a proliferation of non-polar products was formed (see Fig. 6.3 for the TLC of CD-4/C-41). Random errors in the peak areas are evident in Fig. 5.9. These errors were caused by sampling errors and by the magnitude of the peak areas involved, the small magnitude of the peaks made calculation of the peak areas more difficult. As the sulphonates were not isolated and were not readily available, it was not possible to calculate the mass balance of the developing agent and the resulting degradation products to see if the developing agent had predominantly formed the m-and o-sulphonates.

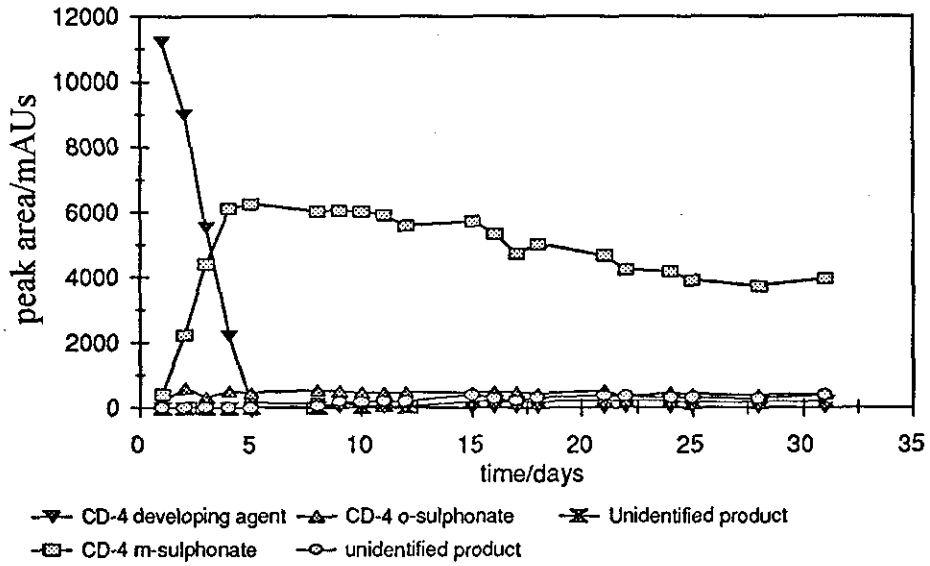


Fig. 5.8 Graph of C-41 degradation with time.

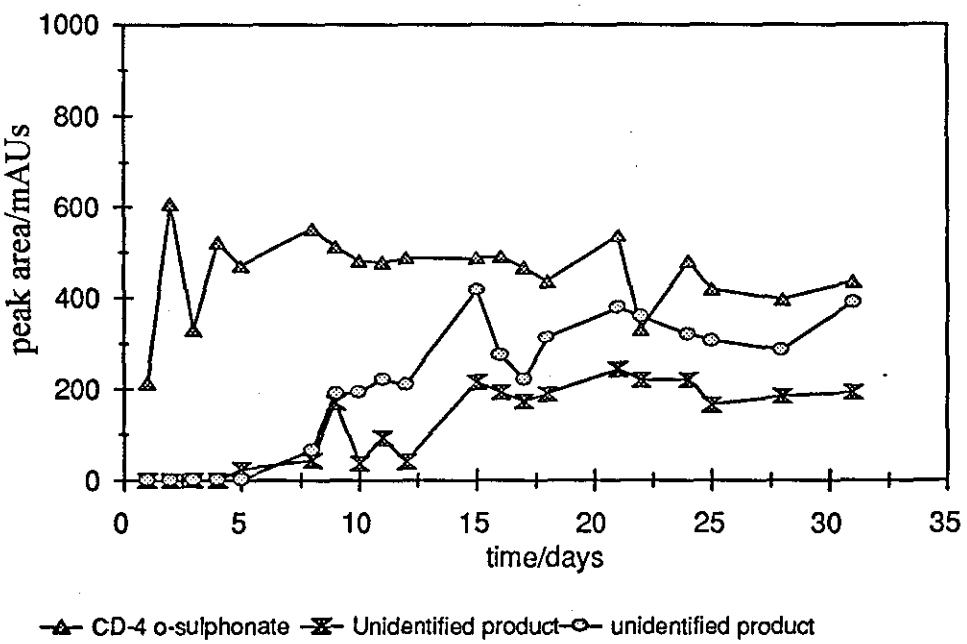


Fig. 5.9 Expansion of graph of C-41 degradation.

The apparent zero order degradation of CD-4 can be seen in Fig. 5.9, as the rate of decrease in the concentration of CD-4 is independent of concentration. The increase and gradual decrease in the m-sulphonate

concentration and the gradual increase in the concentration of other sulphonates can also be seen. All sulphonates had similar UV spectra.

5.5 HPLC and degradation studies of CD-4/sulphite

Similar chromatographic and degradation studies were undertaken for a solution of CD-4 (5.5 g/l) to which a two-fold molar excess of sulphite had been added, the results were compared to those seen in C-41 (1.6:1 molar ratio of CD-4 to sulphite). The solution was buffered with potassium carbonate at pH 10.5 to simulate an authentic developer solution. Samples were taken from a 250 ml stoppered solution daily rather than from individual 2 ml filled vials. The solution was less susceptible to aerial oxidation due to a smaller relative surface area and this meant that degradation proceeded at a slower rate and allowed for a more detailed study of the appearance and disappearance of the various sulphonates.

A chromatogram of the fresh CD-4/sulphite solution is shown in Fig. 5.10. CD-4 elutes with a retention time of three mins and sulphonates began to form at 6 mins (o-sulphonate), 7.5 mins (m-sulphonate) and another unidentified sulphonate at 8.7 mins. The compound at 8.7 mins had a similar UV spectrum to the other two sulphonates. Over the next twenty-two days the concentration of the sulphonates increased (see Fig. 5.13). The m-sulphonate is predominately formed, while the o-sulphonate is formed to a lesser degree, this was a similar pattern to that seen for C-41 developer solution. After about twenty-two days the concentration of the sulphonates start to decrease, slowly in the case of the m-sulphonate and more quickly in the case of the o-sulphonate. A chromatogram of twenty one day old CD-4/sulphite is shown in Fig. 5.11.

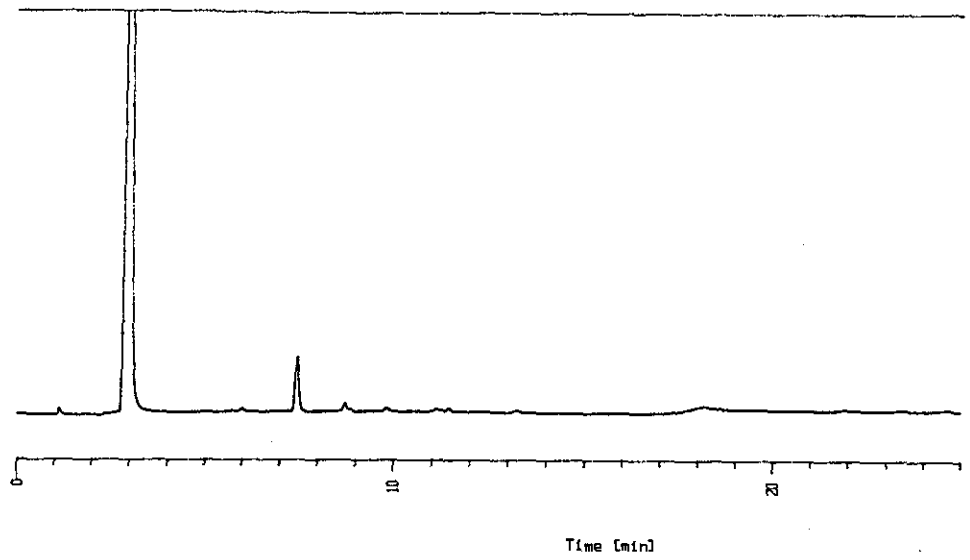


Fig. 5.10 Chromatogram of fresh CD-4/sulphite solution (method D, detection at 243 nm, Shandon column).

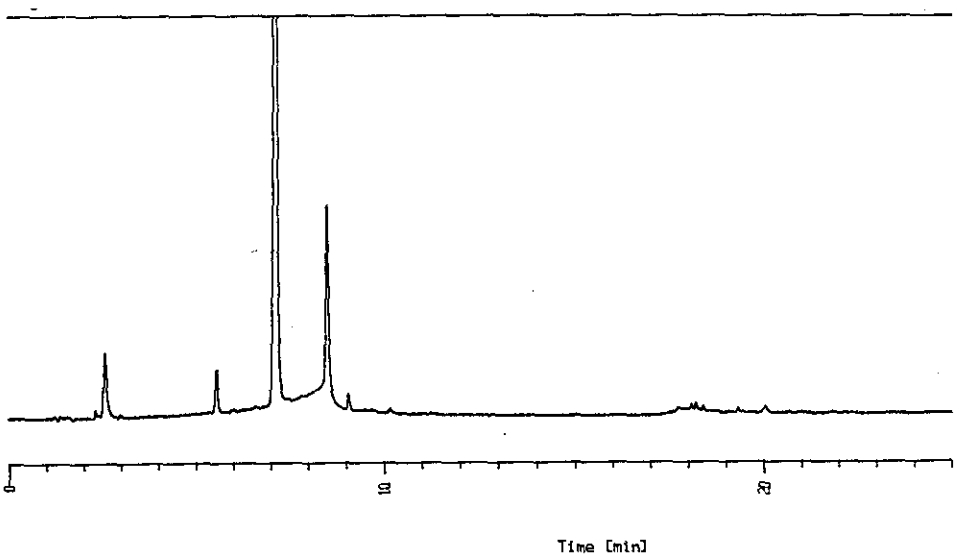


Fig. 5.11 Chromatogram of twenty one day old CD-4/sulphite (method D, detection at 243 nm, Shandon column).

At this stage another compound, with a similar spectrum to the sulphonates already seen, began to form with a retention time of nine mins. This is more apparent in Fig. 5.12., a chromatogram of a twenty-five day old sample. This compound seemed to be stable and its absorbance did not decrease over the rest of the study. Several other smaller peaks were seen but

none were identified. It may be possible that they were disulphonates, trisulphonates or polymeric species.

Graphs of CD-4/sulphite degradation are shown in Fig. 5.13 and Fig. 5.15. The degradation of CD-4 can be clearly seen and appears to be a zero order degradation. The rise and fall in the peak areas of the m- and o-sulphonates can also be seen. The m- and o-sulphonates appear to form simultaneously, albeit with different peak areas. The o-sulphonate decreases in peak area more quickly than the m-sulphonate. Again, sampling errors led to random scattering of the data in Figs 5.13 and 5.14 but the overall profile of the degradation of the CD-4 developing agent and formation of the CD-4 sulphonates can be seen. The spectra of the CD-4 sulphonates are shown in Fig. 5.15.

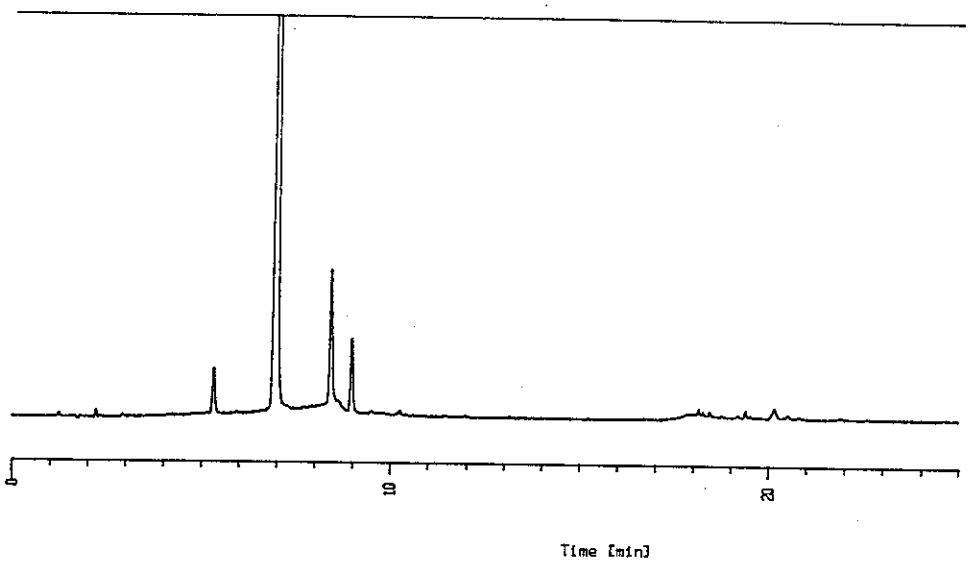


Fig. 5.12 Chromatogram of twenty five day old CD-4/sulphite (method D. detection at 243 nm, Shandon column).

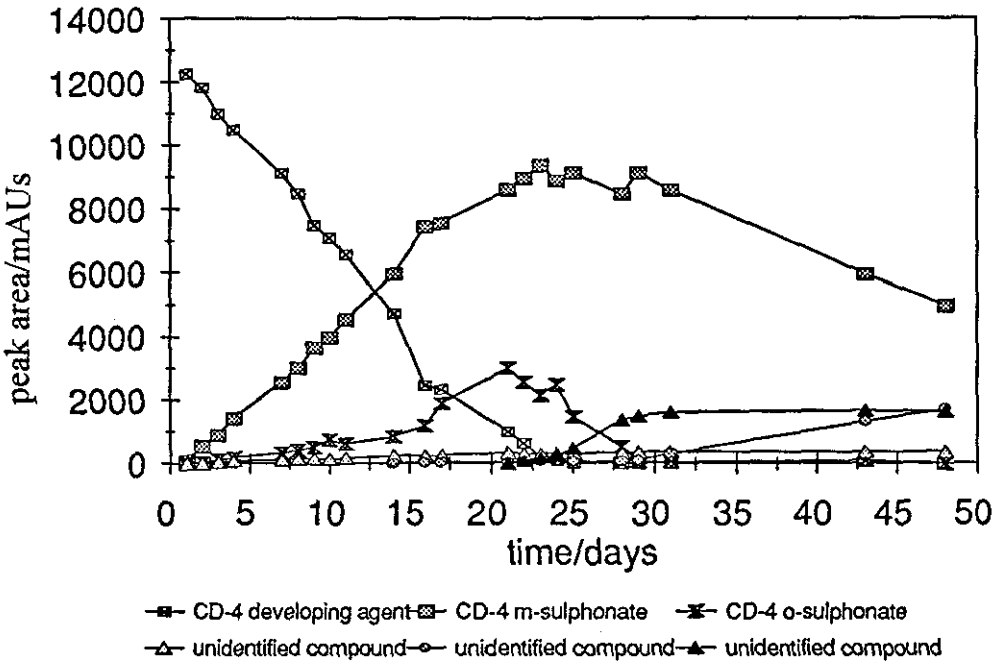


Fig. 5.13 Graph of CD-4/ sulphite degradation with time.

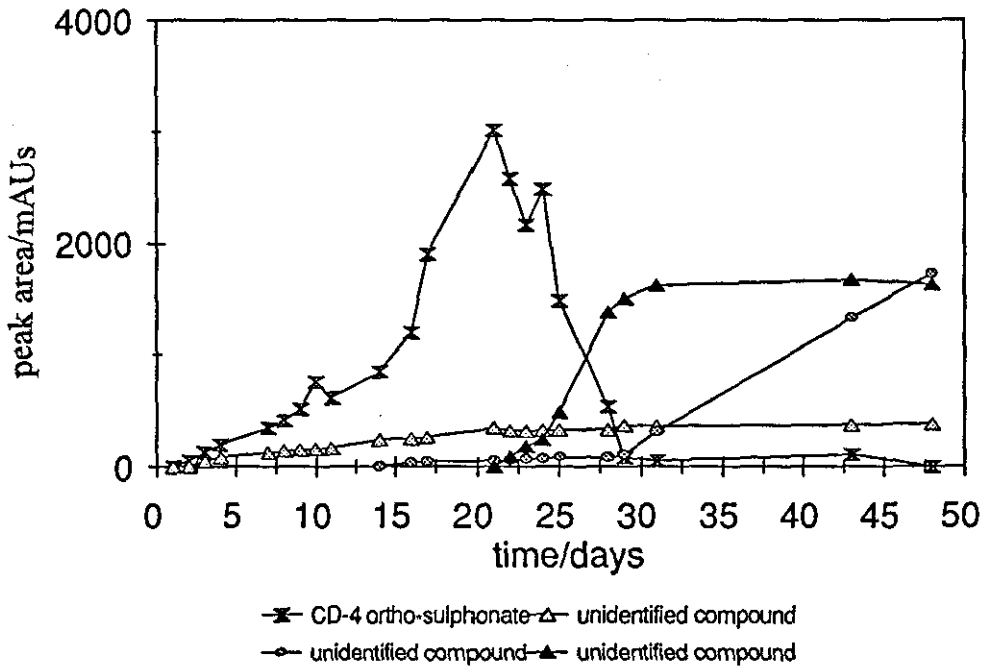


Fig. 5.14 Expanded graph of CD-4/sulphite degradation with time.

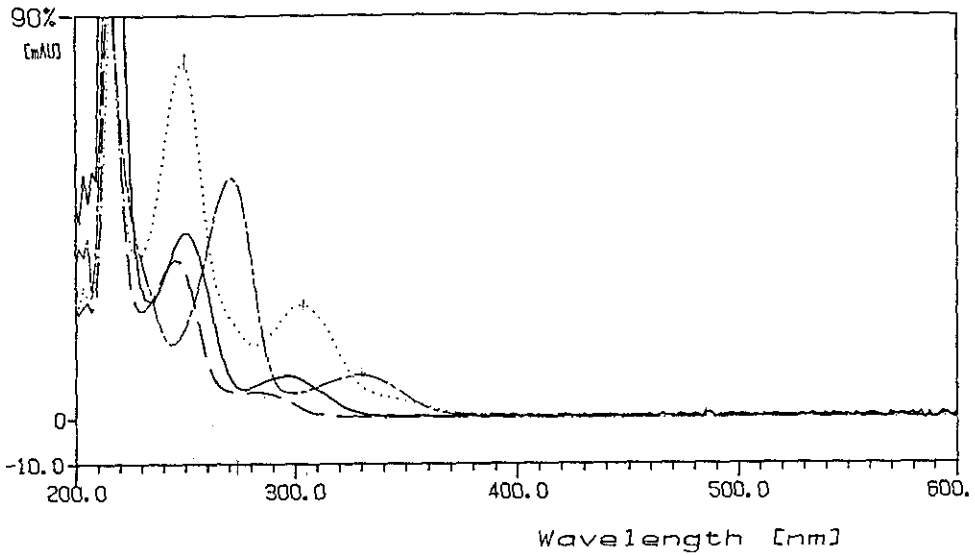


Fig. 5.15 Spectra of CD-4 sulphonates.

The o-sulphonate (retention time 5.3 mins) shifted to a slightly shorter wavelength (λ_{max} at 296 nm) compared to the m-sulphonate (retention time 6.9 mins) which had a λ_{max} at 305 nm [15]. The sulphonate which had a retention time of 9.0 mins had a λ_{max} at 285 nm, but its structure was unknown.

5.6 Comparison of CD-4/sulphite and C-41

As would be expected, there are many similarities in the degradation of C-41 and CD-4/sulphite. The levels of CD-4 developing agent in fresh solutions of each were comparable at 4.5 g/l. But there is a two-fold molar excess of sulphite in the CD-4/sulphite solution and a 1.6:1 molar ration in C-41. This is reflected in the degradation graphs where the level of the m-sulphonate peak area rises to a maximum of 9700 absorbance units in the case of the CD-4/sulphite and to 6000 absorbance units in the case of the C-41 solution. Each solution predominately forms the CD-4 m-sulphonate. The o-sulphonate is formed to a lesser degree. The excess of sulphite in the CD-4/sulphite solution meant that non-polar products were not formed. This is in contrast to C-41 where a small amount of non-polar compounds (3.3 %) are formed.

5.7 Used developer solution samples

A sample of C-41 (PS-003912) which had been used in a commercial laboratory to develop film was obtained from Kodak Ltd, Harrow for evaluation (Fig. 5.16). There are three main signals in the chromatogram at 5.2 mins, 6.8 mins and 8.4 mins. The signal at 6.8 mins has a retention time and spectrum similar to that seen for the CD-4 m-sulphonate in the degraded CD-4/sulphite and C-41 developer solutions. Likewise the signal at 8.4 mins has a retention time and spectrum similar to the o-sulphonate seen earlier. Several other unidentified signals were apparent and there are no non-polar products.

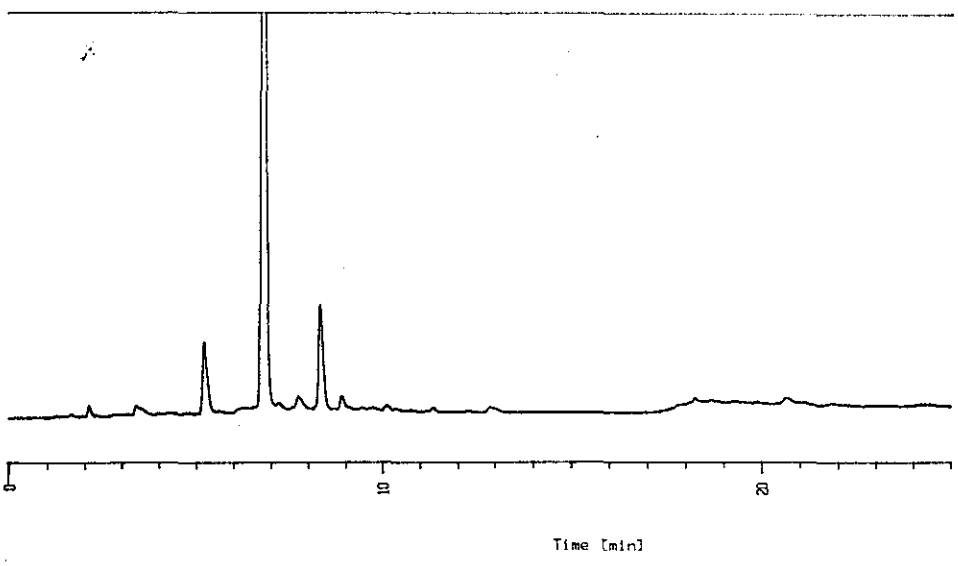


Fig. 5.16 Chromatogram of commercial C-41 developer solution (method D, detection at 243 nm, Shandon column).

5.8 Summary

The degradation of CD-4, C-41 and CD-4/sulphite solutions was different to that seen for CD-3, RA4 and RA100. In the case of CD-4, C-41 and CD-4/sulphite, the major degradation products are polar developing agent sulphonates whereas in the case of CD-3, RA4 and RA100 the major degradation products are non-polar relatively high molecular weight compounds.

CD-4 appeared to degrade with a zero order reaction rate in the developing agent. However under identical conditions, CD-4 developing agent appears to degrade more quickly than CD-3 developing agent. This makes C-41 developer solutions more prone to aerial oxidation. C-41 developer solutions and CD-4/sulphite solutions appear to degrade in a similar manner.

Chapter 6

Isolation and identification of CD-4 developing agent degradation products.

6.1 Introduction

As shown earlier (Chapter 5) C-41 developer solutions contain a higher concentration of sulphite than RA4 or RA100 and the main degradation products formed are the soluble CD-4 sulphonates. As CD-3 sulphonates are formed predominantly, C-41 developer solutions contain only small amounts of non-polar degradation products, however, these products give the developer solutions a turbid, brown coloured appearance. As only a small percentage (3.4 %) of the original CD-4 developing agent forms non-polar degradation products, it was found difficult to isolate sufficient precipitate to separate individual degradation products. It was necessary to prepare a buffered CD-4 solution without sulphite (4.0 g/l, pH 10) and allow this to degrade with time. This produced similar non-polar degradation products to C-41 in sufficient quantity to allow for isolation and identification.

6.2 HPLC of CD-4 and C-41 precipitates

Chromatograms for the precipitates from CD-4 without sulphite and C-41 are shown in Figs 6.1 and 6.2. The precipitates are very similar in nature.

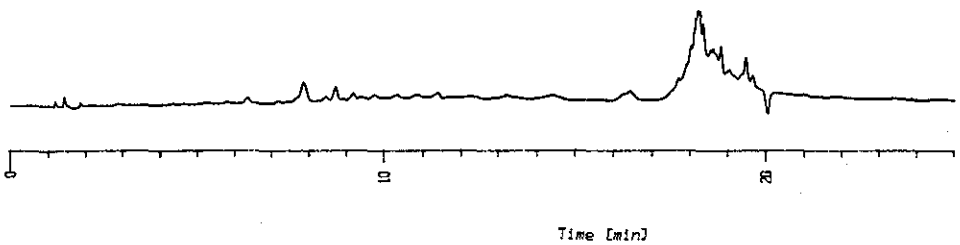


Fig. 6.1 Chromatogram of CD-4 precipitate (method C, detection at 243 nm, Shandon column)

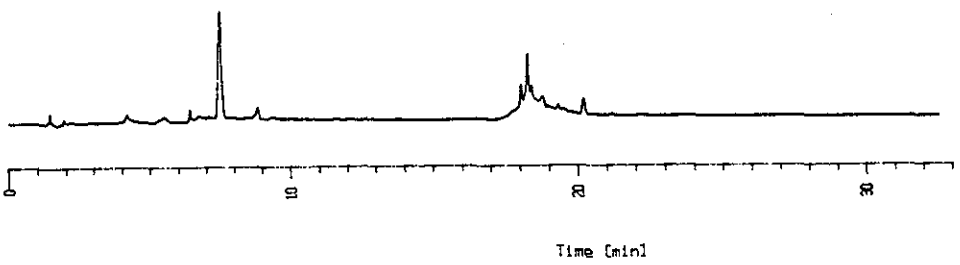


Fig. 6.2 Chromatogram of C-41 precipitate (method C, detection at 243 nm, Shandon column)

6.3 Isolation of CD-4 and C-41 degradation products

The precipitates formed from degraded CD-4 and C-41 solutions were isolated by filtering through a sintered glass crucible. As TLC proved to be the suitable technique for the separation of degradation products from CD-

3/RA100/RA4 solutions, it was also used for the separation of precipitates from CD-4/C-41 solutions. The non-polar degradation products were coloured and easily distinguishable on the plates. Precipitated polymeric material tended not to elute and stayed at the bottom of the plate, not interfering with the separation of the other degradation products. A dichloromethane/methanol eluent (90:10 v/v) was found to be the most appropriate and addition of a small amount of potassium carbonate (1 g/l) to the eluent enhanced resolution of the degradation product bands probably by deprotonating the degradation products. The TLC of degraded CD-4 and degraded C-41 precipitates is shown in Fig. 6.3.

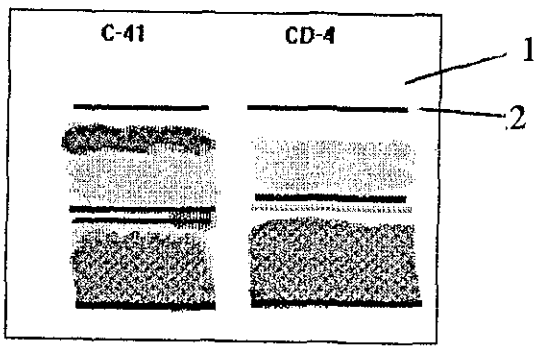


Fig. 6.3 Typical TLC elution of CD-4/C-41 precipitates.

Several compounds were removed from the preparative TLC plates, eluted from the TLC silica and evaporated to dryness under nitrogen. Yellow (1) and blue (2) compounds were further purified using standard TLC plates. No obvious purple compound, similar to that seen in CD-3 was observed.

6.4 Proton NMR of CD-4 developing agent

The ^1H NMR of CD-4 was recorded to aid in the interpretation of the ^1H NMR spectra of CD-4 degradation products. As CD-4 is supplied as an acid salt it was not soluble in deuterated chloroform, the solvent used for the ^1H NMR spectra of the degradation products. It was necessary to prepare a solution of CD-4, raise the pH to deprotonate the primary amine and liquid-liquid extract the neutral CD-4 into dichloromethane. However as CD-4 oxidises quite easily at high pH it was unavoidable that the deprotonated CD-4 contained some degradation products.

The ^1H NMR spectrum of CD-4 is shown in Fig. 6.5, along with the tabulation of the main peaks in Table 6.1. The ^1H spectrum was not very good and it was difficult to calculate coupling constants. A CD-4 molecule is shown in Fig. 6.4.

Chemical shift **δ/ ppm**	Description	Integration	Assignment
1.05	triplet	3H	CH_3, *E*
2.15	singlet	3H	CH_3, *J*
3.25	broad singlet	incomplete	CH_2, *B*
3.60	multiplet	6H	CH_2, *C, D*
6.65	triplet	3H	ArH, *F, G, H*

Table.6.1 Tabulation of ^1H NMR data for CD-4.

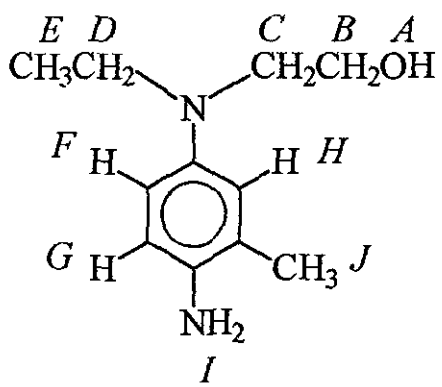


Fig. 6.4 Structure of CD-4 molecule

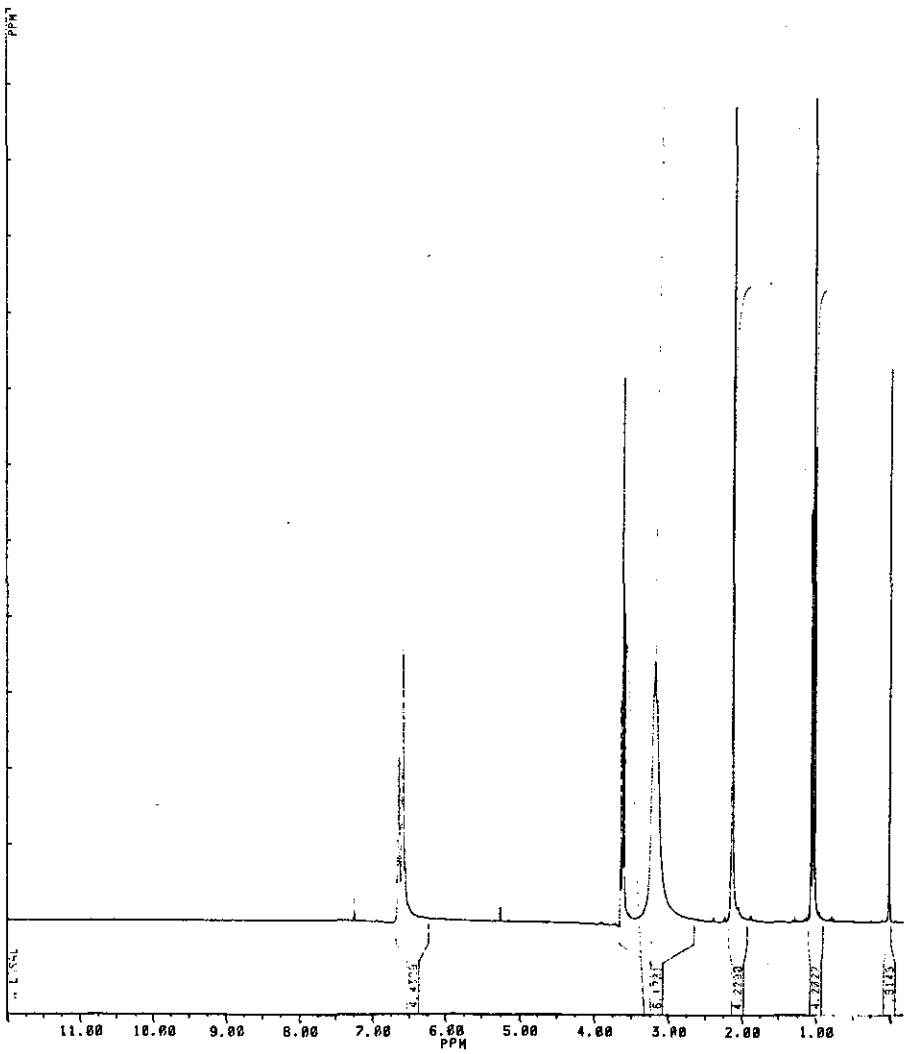


Fig. 6.5 ^{1}H NMR spectrum of CD-4, run in deuterated chloroform (250 MHz).

The sharp singlet at 1.05 ppm integrated to three protons but this chemical shift and multiplicity were typical of a methyl group coupled to a methylene, which corresponded to *E* (cf. ^{1}H NMR of CD-3). There was a sharp singlet at 2.15 ppm which integrated to three protons. This chemical shift was characteristic of a more deshielded methyl group which did not appear to be coupled to any other protons. This corresponded to the methyl group *J* which was attached to the aromatic ring and is similar to the signal seen in CD-3 (2.15 ppm). Again the integration did not help.

There was a broad singlet centred on 3.25 ppm, however integration of this signal did not seem to be complete. It was quite possible that this signal was due to a methylene group (*B)* whose signal had been distorted by its proximity to the alcoholic proton. The multiplet centred on 3.60 ppm was not clearly resolved but its position was typical of methylene groups such as *C* and *D*. The signal due to *D* should have appeared as a quartet and the signal due to *C* should be a triplet. The signals centred on 6.65 ppm were not clearly resolved but their position indicated that they are the protons on the aromatic ring (*F, G, H*).

6.5 Identification of yellow CD-4 degradation product

6.5.1 HPLC of yellow CD-4 degradation product

A typical chromatogram for the yellow degradation compound is shown in Fig. 6.6. The yellow compound eluted with a retention time of 20.0 mins. The peak is negative due to the reference wavelength (550 nm). The yellow compound gave a higher absorbance at the reference wavelength than at the detection wavelength.

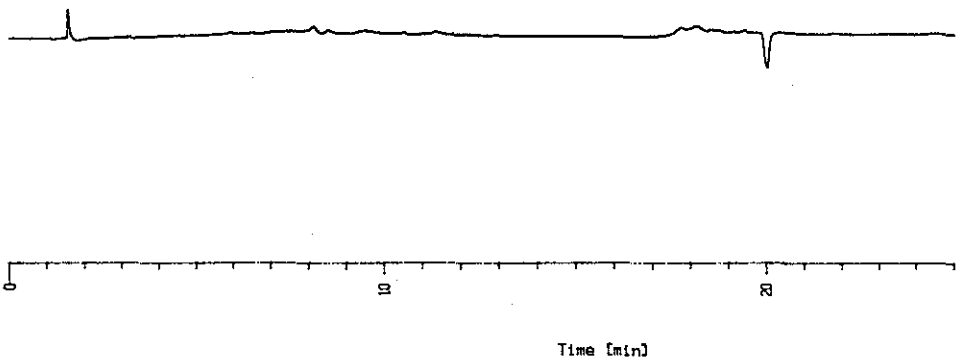


Fig. 6.6 Chromatogram of yellow CD-4 degradation product (method D, detection at 243 nm, Shandon column)

6.5.2 UV spectra of yellow CD-4 degradation product

The UV spectrum of an ethanolic solution of the yellow CD-4 compound, is shown in Fig. 6.7. The compound was also run in acidified ethanol and basic ethanol. The absorption maxima values are given in Table. 6.2. Acidification led to protonation of the tertiary amine, leading to reduced conjugation and shorter absorbance maxima. Raising the pH led to deprotonation of the nitrogen groups, enabling the lone pairs to donate electrons into the ring system, increasing conjugation and leading to longer absorbance maxima. The absorbance maxima are typical of an azo compound (see chapter 4.6.2).

Absorption maxima ethanol λ_{max} *(nm)*	**Absorption maxima** ethanol + two drops of dilute HCl λ_{max} *(nm)*	**Absorbance maxima** ethanol + two drops of dilute NaOH λ_{max} *(nm)*
204.2	205.5	208.3
266.0	282.9	266.4
312.0	313.2	312.0
459.0	452.0	460.8

Table. 6.2 UV spectral data for yellow CD-4 degradation product.

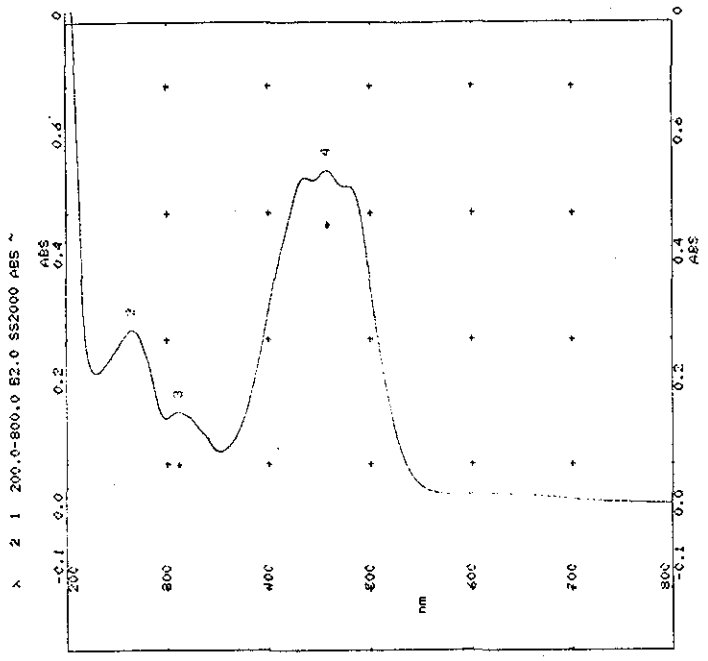


Fig. 6.7 UV spectrum of an ethanolic solution of the yellow CD-4 degradation product

6.5.3 Mass spectrometry of yellow CD-4 degradation product

The yellow CD-4 degradation product was analysed using mass spectrometry. The EI mass spectrum is shown in Fig. 6.8.

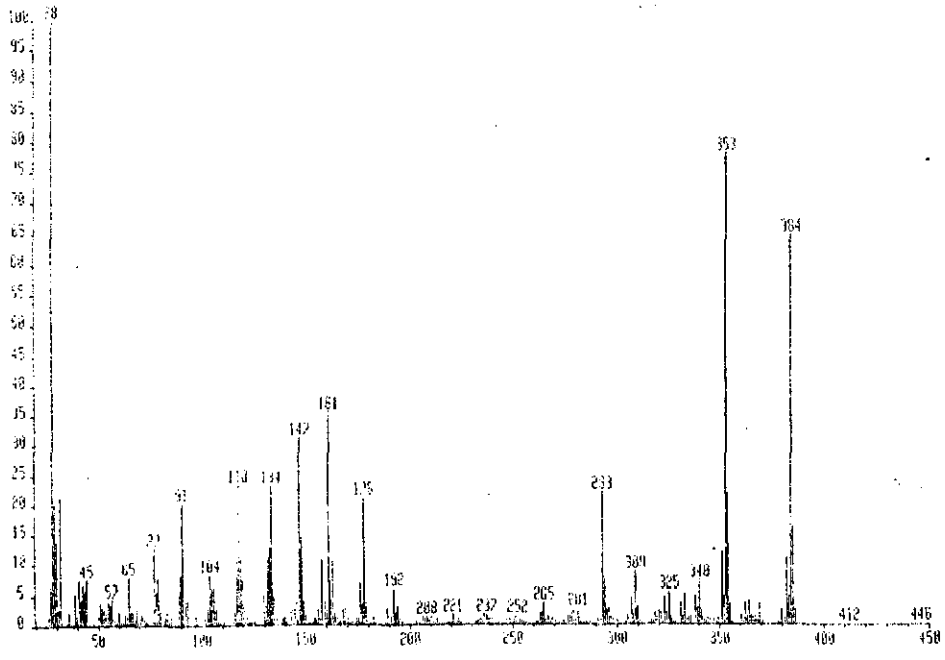


Fig. 6.8 EI mass spectrum of yellow CD-4 degradation product.

Major fragments appear at m/z 353, m/z 293 and a multitude of fragments at m/z lower than 200. The molecular ion appears at m/z 384. The fragment at m/z 353 could be due to a loss of CH_2OH (31 mass units) from a sidechain (Fig. 6.9). The fragment at m/z 293 involves the loss of 91 mass units from the molecular species at m/z 384. This may be related to the loss of a complete sidechain, which would be 88 mass units, but the fragmentation pattern is not obvious. The NH_3 DCI mass spectrum has an MH+ peak at m/z 385 which confirms the molecular weight as 384. Looking at the structure of CD-4 the possibility of forming an azo compound between the primary amines can be seen and is similar to the pattern seen for CD-3 and the CD-3 derived dimeric azo compound. The mechanism of formation has been speculated on by Tong *et al.* [52]. This azo compound (Fig. 6.10) would have a molecular weight of 384 and this molecular weight is confirmed by the mass spectral data.

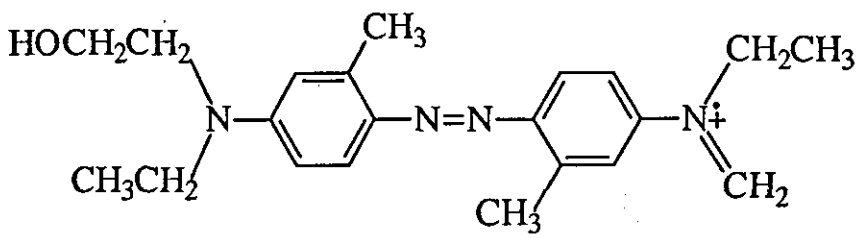


Fig. 6.9 Structure of fragment at m/z 353.

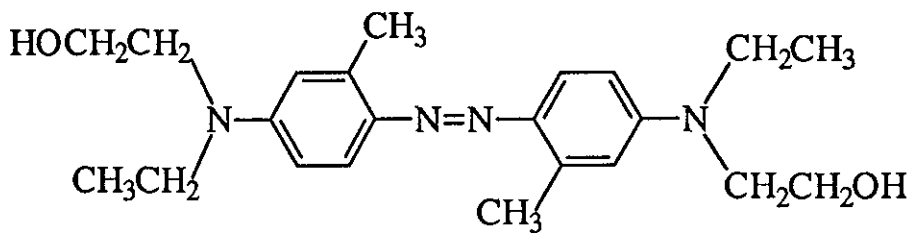


Fig. 6.10 Structure of yellow compound isolated from CD-4 precipitate.

6.5.4 Proton NMR of yellow CD-4 degradation product

Proton NMR analysis was carried out on the yellow compound isolated from the precipitate of degraded CD-4 solution. The ^1H NMR is

shown in Fig. 6.12, together with the chemical shifts in Table. 6.3. Expansion of the ^{1}H NMR spectrum from 1.0 ppm to 4 ppm is shown in Fig. 6.13 and an expansion of the ^{1}H NMR spectrum from 6.2 ppm to 7.6 ppm is shown in Fig. 6.14.

Chemical shift δ/ ppm	Description	Integration	Assignments
1.20	triplet, 6.7 Hz	3H	CH_3, *I, I'*
1.50	singlet	impurity	H_2O
2.65	singlet	3H	CH_3, *E, E'*
3.47	quartet, 6.7 Hz	2H	CH_2, *H, H'*
3.53	triplet, 6.7 Hz	2H	CH_2, *C, C'*
3.82	possible quartet	2H	CH_2, *B, B'*
6.60	multiplet	2H	ArH, *G, G',D, D'*
7.30	singlet	impurity	$CHCl_3$
7.65	doublet, 9 Hz	1H	ArH, *F, F'*

Table. 6.3 Tabulation of ^{1}H NMR data for yellow CD-4 compound.

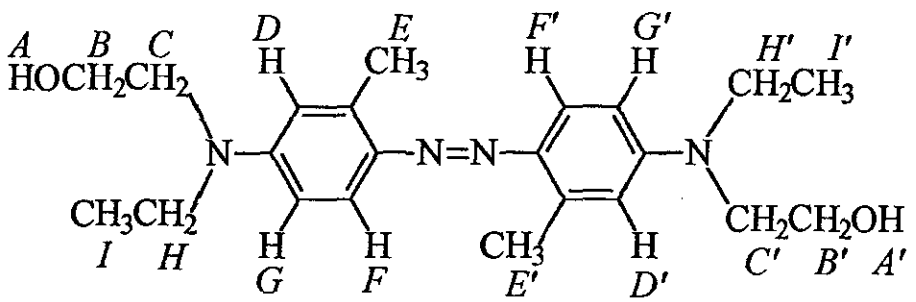


Fig. 6.11 Postulated structure of yellow CD-4 compound.

A structure for the yellow CD-4 compound was postulated using mass spectrometry (see Chapter 6.3.3). A structure for this is shown in Fig. 6.11.

An expansion of the 1.0 ppm to 4.0 ppm region showed the triplet at 1.2 ppm. The multiplicity and chemical shift were typical of a methyl group (*I, I'*) attached to a methylene on the tertiary sidechain. The signal at 1.5 ppm was due to water and is an impurity in the solvent system used. At 2.65 ppm

there was a sharp singlet (6.7 Hz). Comparison with the CD-4 ^{1}H NMR and the CD-3 azo compound NMR indicated that this was due to the methyl groups (*E, E'*) attached to the aromatic ring, as the integration, multiplicity and chemical shift had been altered by proximity to the azo bond.

There was a triplet at 3.53 ppm which overlapped slightly with a quartet at 3.47 ppm. The multiplicity and integration of the signal at 3.47 ppm (quartet, 2H) indicated that this was a methylene group coupled to a methyl group. Comparison with the original CD-3 molecule showed that this must be due to the CH_2 (*H, H'*) attached to the ethyl group of the sidechain. The triplet at 1.20 ppm and the quartet at 3.47 ppm have the same coupling constant (6.7 Hz). A signal at 3.53 ppm was a triplet and integrates to 2H. This signal was due to one of the methylene groups of the NCH_2CH_2OH group as seen in the ^{1}H NMR spectrum of CD-4. It was most likely the methylene attached to the tertiary group (*C, C'*) as the methylene group attached to the alcohol would have been more deshielded and probably broadened. A signal at 3.82 ppm may have been a quartet which had been broadened. It integrated to 2H and was probably the methylene (*B, B'*) closest to the alcohol group, which would account for its broadness and deshielding.

An expansion of the 6.4 ppm to 7.8 ppm region showed a multiplet at 6.60 ppm which was not clearly resolved. The chemical shift was typical of aromatic protons. There was another aromatic signal at 7.65 ppm. Comparison of the relative integrations showed that the signals were in a 2:1 ratio (6.60 ppm: 7.65 ppm). The doublet at 7.65 ppm was in a different chemical environment to the others and may have been the aromatic proton *(F, F')* closest to an azo group. This proton was coupled to one other proton and appeared as a doublet (coupling constant 9 Hz). The remaining two protons were in similar chemical environments and should show up as a doublet of doublets *(G, G')* and a singlet *(G, G')* which may be split into a doublet by *F, F'*. However these signals were not resolved enough to see coupling. The signal at 7.25 ppm was an impurity due to $CHCl_3$. The effect on the ortho aromatic protons of going from an electron donating amino group to an electron withdrawing azo group have been reported by Smith [79] and are explained in detail for the yellow CD-3 degradation product in Chapter 4.6.4. The same criteria also hold for the CD-4 degradation product.

The interpretation of the ^{1}H NMR spectrum points to the structure in Fig. 6.11 as being the correct structure.

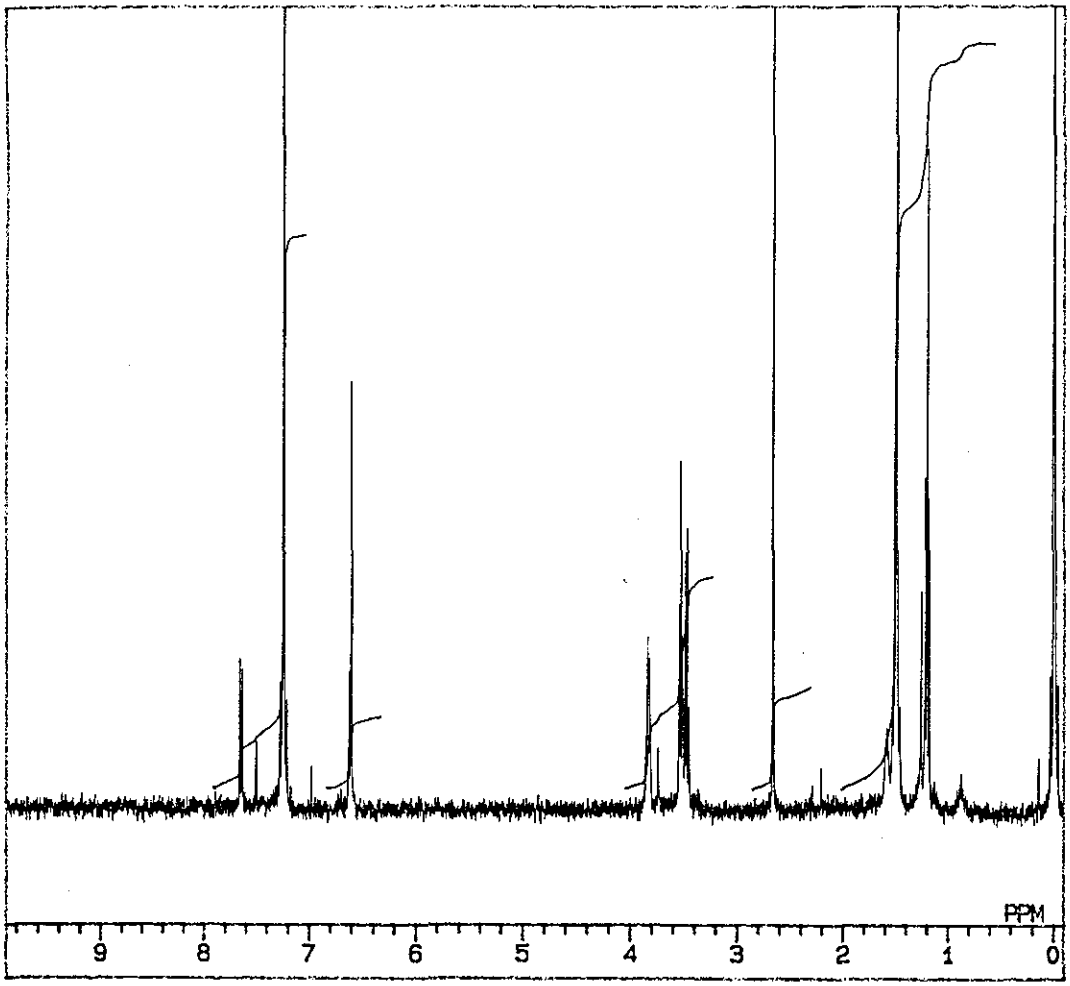


Fig. 6.12 ^{1}H NMR spectrum of yellow CD-4 degradation product, run in deuterated chloroform (400 MHz).

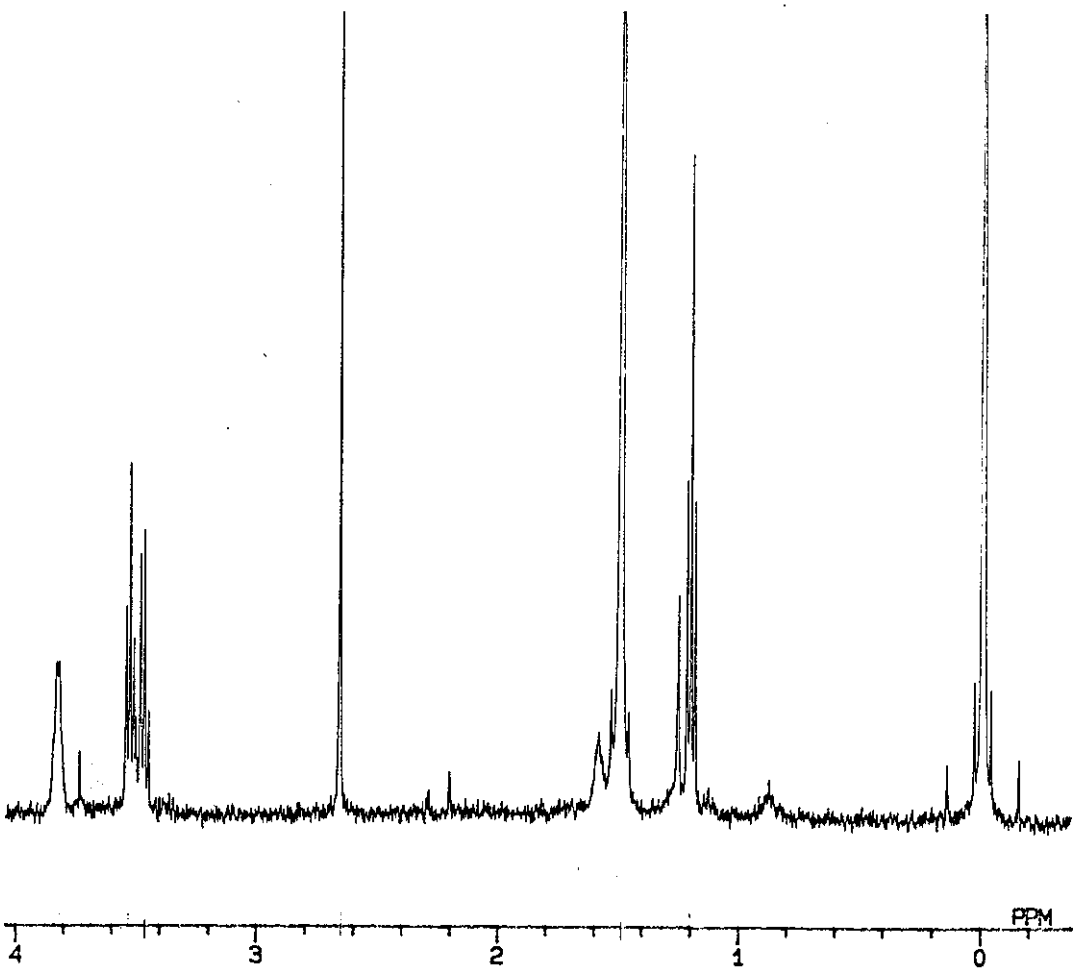


Fig. 6.13 Expansion of 0.0 ppm to 4.0 ppm region of ^1H NMR spectrum of yellow CD-4 degradation product, run in deuterated chloroform.

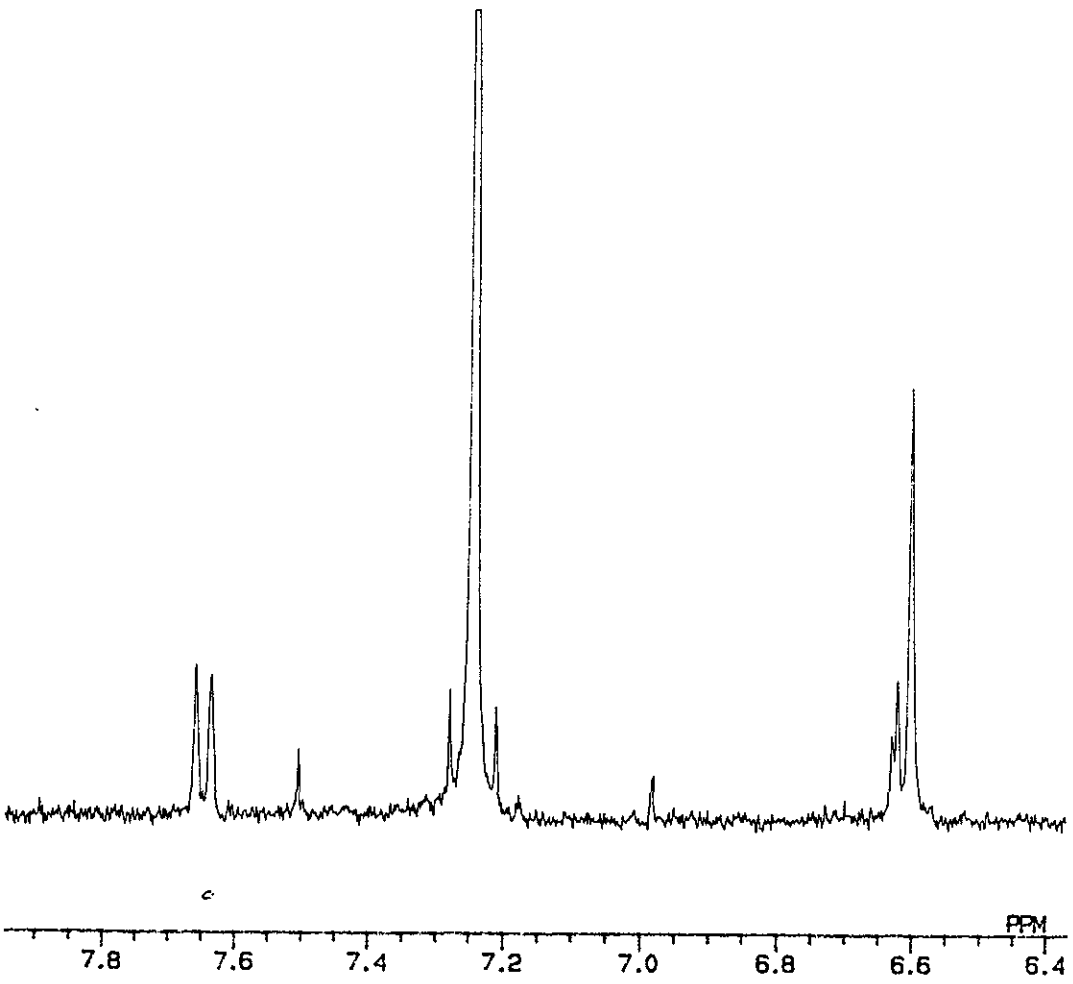


Fig. 6.14 Expansion of 6.4 ppm to 6.8 ppm region of ^1H NMR spectrum of yellow CD-4 degradation product, run in deuterated chloroform.

6.6 Identification of blue CD-4 degradation product

6.6.1 HPLC of blue CD-4 degradation product

A chromatogram of the blue compound isolated from CD-4 is shown in Fig. 6.15. The blue compound eluted with a retention time of 18.8 mins.

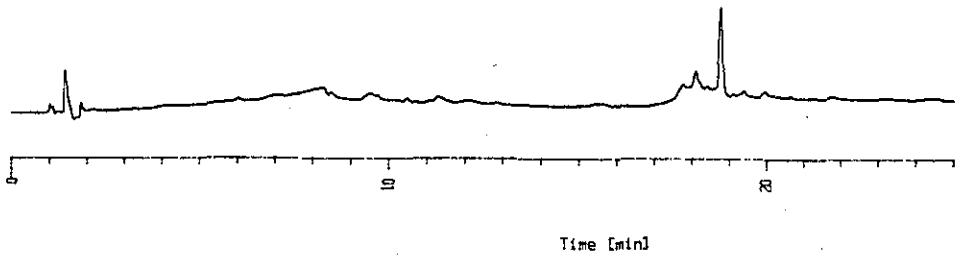


Fig. 6.15 Chromatogram of blue CD-4 degradation product (method D, detection at 243 nm, Shandon column).

6.5.2 UV spectra of blue CD-4 degradation product

The UV spectrum of an ethanolic solution of the blue CD-4 degradation product, run in ethanol is shown in Fig. 6.16. The absorbance maxima values are shown in Table. 6.4. The effect of pH on absorbance maxima was not completely clear but protonation and deprotonation of the tertiary amine (see Fig. 6.18) may occur leading to shorter absorbance maxima at low pH and slightly longer absorbance maxima at high pH. See section 4.7.2 for a more detailed study of the blue CD-3 degradation product.

Absorbance maxima ethanol λ_{max} *(nm)*	**Absorbance maxima** ethanol + two drops of dilute HCl λ_{max} *(nm)*	**Absorbance maxima** ethanol + two drops of dilute NaOH λ_{max} *(nm)*
200.9	204.8	208.5
276.2	282.9	276.0
-	376.0	-
638.4	624.0	624.4

Table 6.4 UV spectral data for blue CD-4 degradation product.

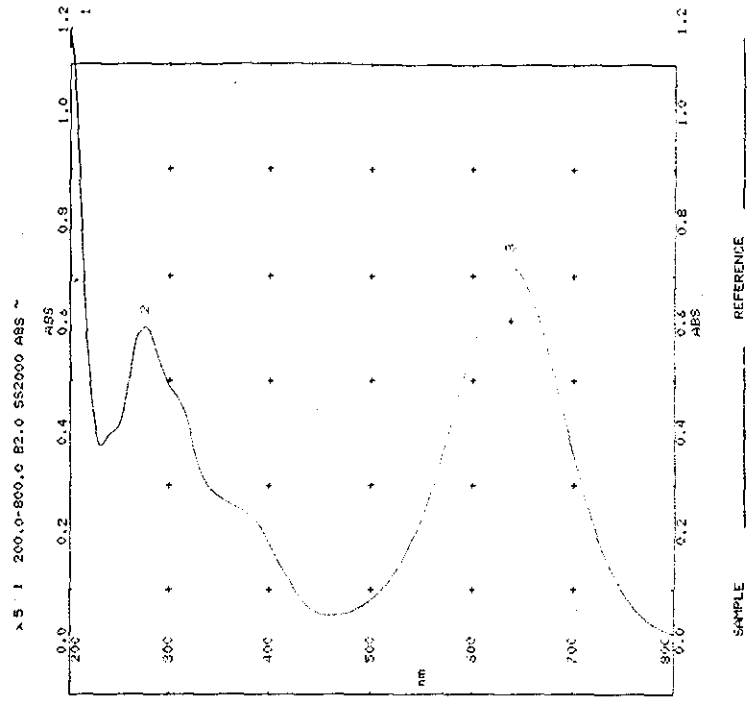


Fig. 6.16 UV spectrum of an ethanolic solution of the blue CD-4 degradation product

6.6.3 Mass spectrometry of blue CD-4 degradation product

The blue compound was analysed using mass spectrometry. The EI mass spectrum of this compound is shown in Fig. 6.17.

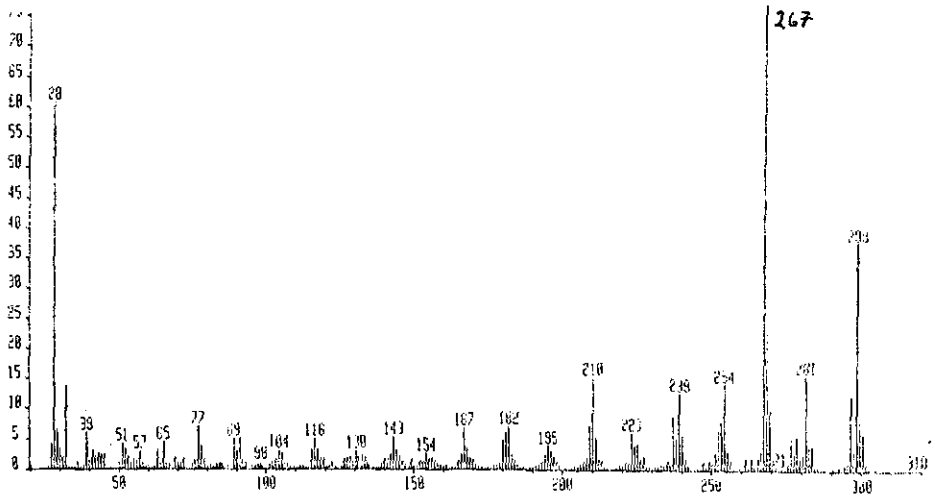


Fig. 6.17 EI mass spectrum of blue CD-4 degradation product.

The molecular species signal was present at m/z 298 and there were fragments at m/z 281, 267, 254, 239 and 210. Comparison with the blue CD-3 compound would lead to the structure shown in Fig. 6.18.

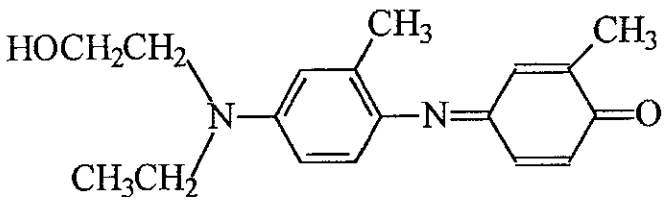


Fig. 6.18 Proposed structure of blue CD-4 compound.

The fragment at m/z 281 involved the loss of an OH group from the sidechain to form the fragment shown in Fig. 6.19.

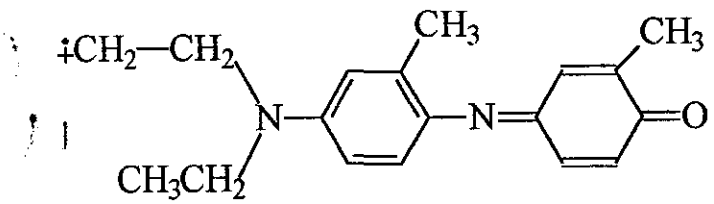


Fig. 6.19 Structure of fragment at m/z 281.

The fragment at m/z 267 is due to the loss of a further methylene group. The fragment at m/z 210 involves the loss of 88 mass units and is due to the loss of a sidechain from the parent molecule (Fig. 6.20).

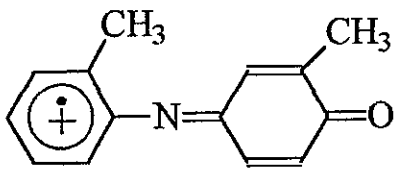


Fig. 6.20 Structure of fragment at m/z 210.

6.6.4 Proton NMR of blue CD-4 degradation product

The ^1H NMR of the blue compound isolated from CD-4 is shown in Fig. 6.21. An expansion of the 1.0 ppm to 4.0 ppm region is shown in Fig. 7.22. An expansion of the 6.2 ppm to 7.6 ppm region is shown Fig. 6.23. A table of chemical shifts, description and integrations for the signals are given in Table. 6.5.

Chemical shift δ/ ppm	Description	Integration	Assignments
1.20	triplet, 6.8 Hz	3H	CH_3, *I*
1.55	singlet	-impurity	H_2O
2.30	singlet	3H	CH_3, *B*
2.31	singlet	3H	CH_3, D
3.45	quartet, 6.8 Hz	-	
3.53	triplet, 5.9 Hz	6H	CH_2's, *J, L, K*
3.85	unresolved quartet	-	
6.45	doublet of doublets, 10.7 Hz, 2.3 Hz	1H	ArH, *E*
6.53	unresolved singlet	1H	ArH, *A*
6.58	singlet	2H	C=CH's, *G, H*
6.73	singlet	1H	C=CH, *C*
7.21	doublet, 10.7 Hz	1H	ArH, *F*
7.28	sharp singlet	impurity	$CHCl_3$

Table.6.5 Tabulation of ^1H NMR data for blue CD-4 degradation product, run in deuterated chloroform

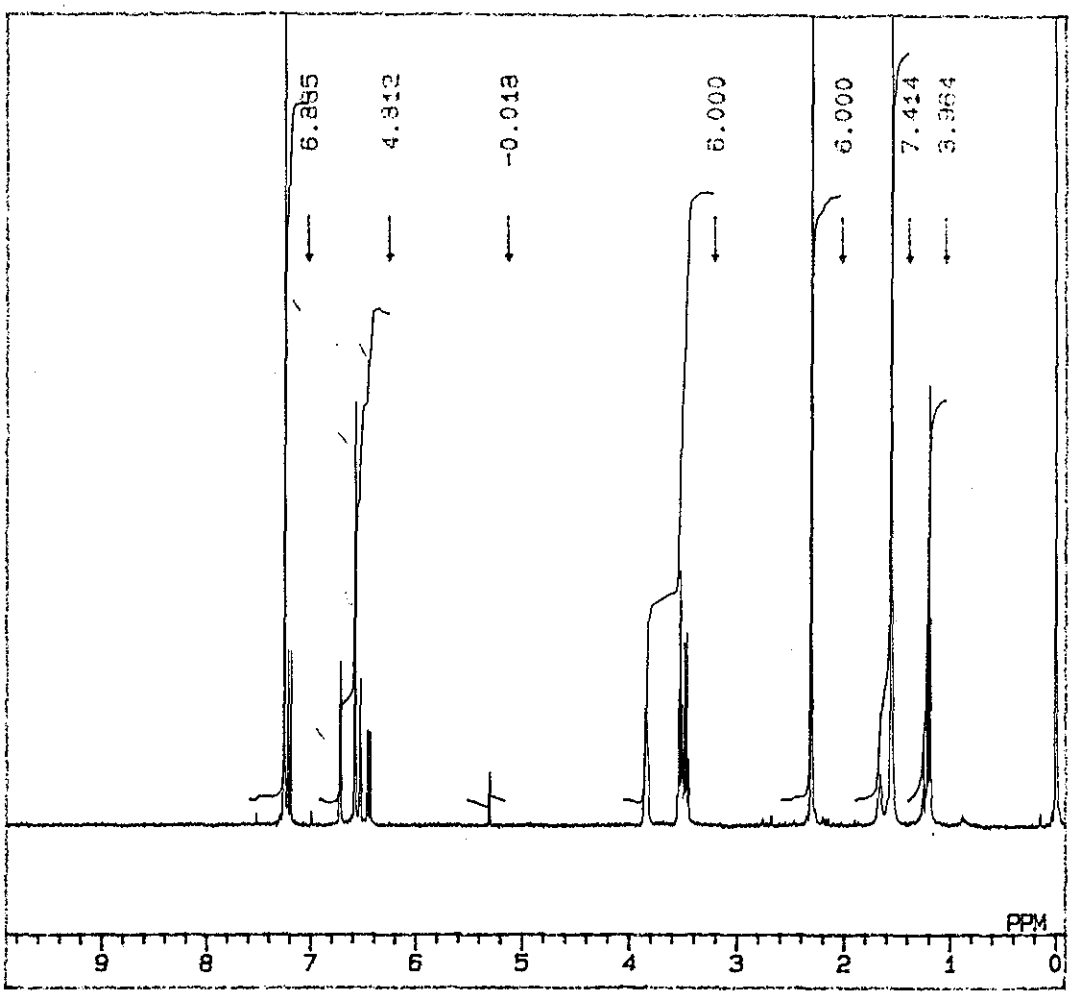


Fig. 6.21 ^{1}H NMR of blue CD-4 degradation product, run in deuterated chloroform (400 MHz).

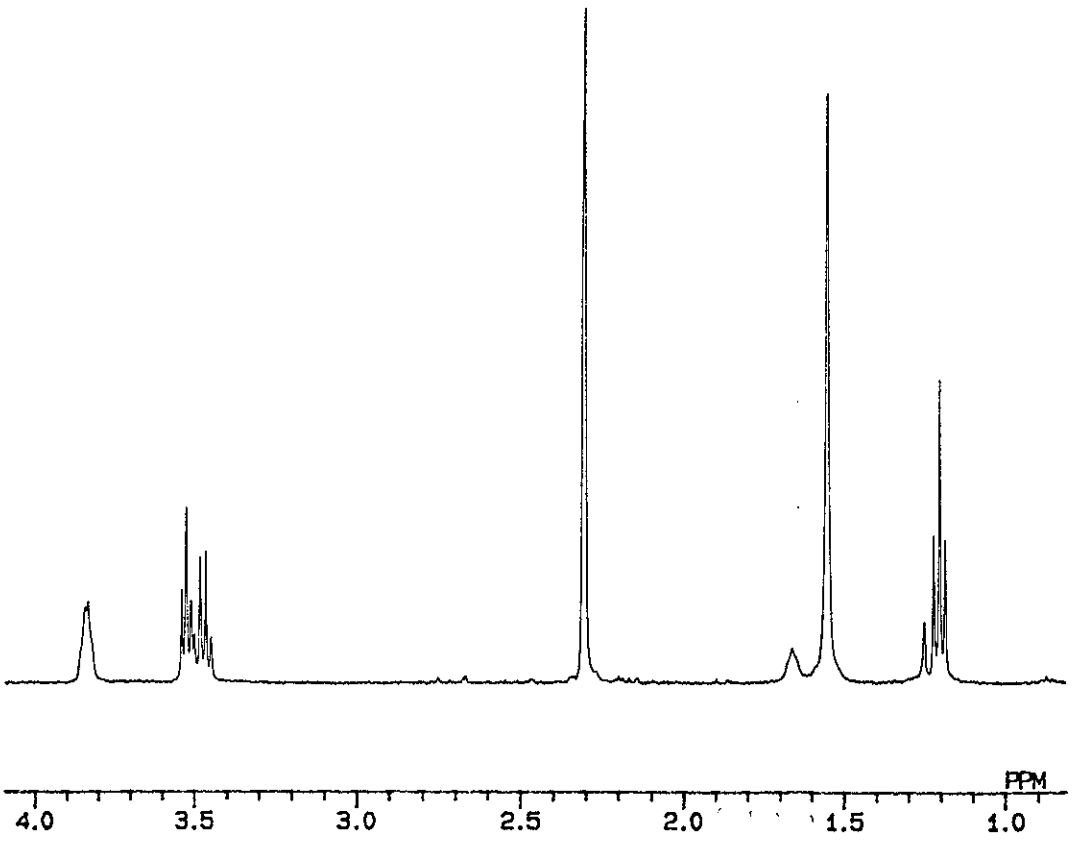


Fig. 6.22 Expansion of 1.0 ppm to 4.0 ppm region of ^{1}H NMR of blue CD-4 degradation product, run in deuterated chloroform

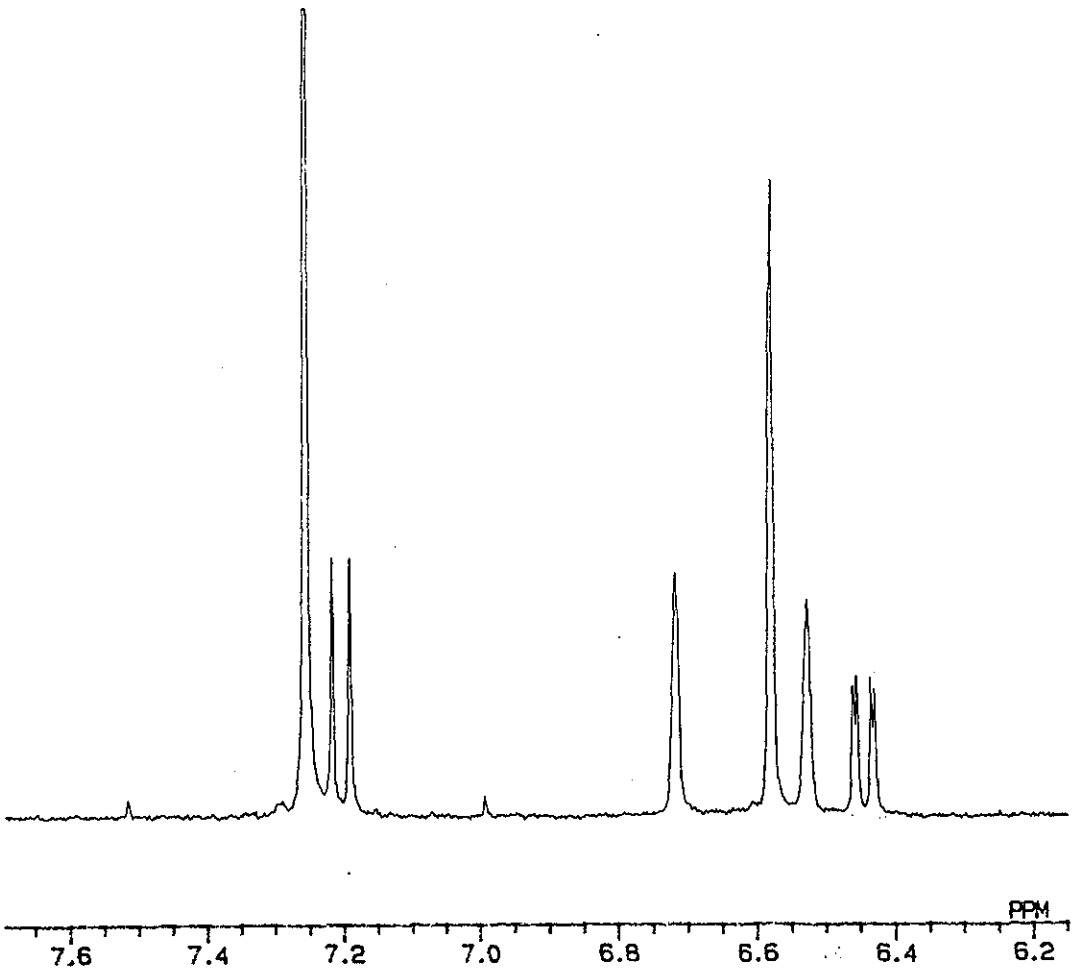


Fig. 6.23 Expansion of 6.2 ppm to 7.6 ppm region of ^{1}H NMR of blue CD-4 degradation product, run in deuterated chloroform

Overall the ^1H NMR spectrum of the blue CD-4 compound was more complex than that of the yellow azo compound seen previously but similar to the blue CD-3 degradation product. The triplet (6.8 Hz) at 1.20 ppm integrated to a methyl group and was probably due to the CH_3 of an ethyl group (*I*). The singlet at 1.55 ppm was an impurity, due to water. There were two singlets at 2.30 ppm and 2.31 ppm which can be seen in Fig. 7.30. These singlets integrated to 6H overall and were probably two unsaturated methyl groups in slightly different chemical environments. Comparision with the proton NMR of CD-4, where the methyl group had a chemical shift of 2.15 ppm, showed that the corresponding group *I* (2.30 ppm) in the blue degradation product was deshielded by its proximity to the C=N group. The chemical shifts and comparison with the ^1H NMR spectra of the blue CD-3 molecule indicated that one of the methyl groups (*B*) was attached to an aromatic ring whilst the other (*D*) was part of an olefin system and was deshielded by an electron withdrawing group such as a carbonyl group.

There was a series of signals from 3.45 ppm to 3.85 ppm which together integrated as 6H. The signal at 3.45 ppm was a quartet and was probably due to CH_2 (*J*) of an ethyl group, as it had a similar coupling constant (6.8 Hz). This signal overlapped slightly with the triplet at 3.53 ppm. This triplet (5.9 Hz) was attached to two other protons. Looking at the structure of the CD-4 tertiary amine sidechain, this signal was probably to a CH_2 (*L*) of the CH_2CH_2OH and was attached to the amine as the CH_2 closest to the alcohol would be more deshielded. The signal at 3.85 ppm is unresolved but appeared to be a quartet. This was the CH_2 (*K*) closest to the alcohol as it was more deshielded by the electron withdrawing effect of the oxygen and its signal was broadened, which is a typical effect of alcohols. The signal should have been a doublet of triplets as it was attached to a CH_2 giving a doublet and was also attached to an alcoholic proton which was in a different chemical environment, thus splitting the triplet. However this coupling was unresolved. If deuterated water was added to the solution, proton exchange should occur and a resolved triplet should be seen for the CH_2 closest to the alcohol.

There was a large number of signals in the region of 6.45 ppm to 6.73 ppm. Together these integrate as 6H and a postulated structure is shown in Fig. 6.24.

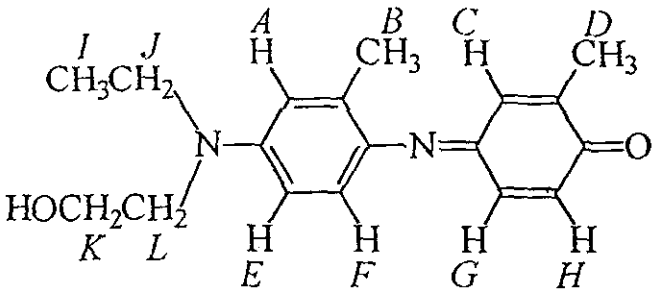


Fig. 6.24 Proposed structure of blue compound isolated from CD-4 precipitate

Looking at the integration, it can be seen that the signal at 6.45 ppm integrated to 1H and was a doublet of doublets. This signal was split by two adjacent protons which were in different chemical environments. Looking at the proposed structure this signal was due to the proton *E* or the proton *G*. However proton *G* was more deshielded as it was attached to an olefin and adjacent to a C=N group. So the doublet of doublets was assigned to proton *E*, which was split by ortho coupling to *F* (Hz 10.7 Hz) and this doublet was further split by long range coupling with *A*, with a much smaller coupling constant of 2.3 Hz. There was an unresolved broadened singlet at 6.53 ppm which integrated as 1H and this may have been due to *A*, as it may be split by long range coupling with *E*. It would not be as deshielded as *C* which is adjacent to the C=N.

There is a singlet at 6.58 ppm which integrated as 2H. It is possible that this was due to *G* and *H*. The signal was not fully resolved and may have been a doublet as *G* and *H* may have been coupled to each other. The singlet at 6.73 ppm integrated to 1H and could have been *C* as it is deshielded by its proximity to the C=N. This left the doublet (10.7 Hz) at 7.21 ppm which integrated to 1H. This signal was deshielded and split by an adjacent proton. The proton *F* was the most likely as it was split through vicinal coupling with *E* and deshielded by its proximity to the C=N. This assignment is supported by the fact that both the doublet of doublets at 6.45 ppm and the doublet at 7.21 had the same coupling constants. Looking at the yellow CD-4 azo compound, a similar doublet was seen at 7.65 ppm.

The proton NMR of substituted indoanilines similar to the blue CD-3 and blue CD-4 compounds have been reported by Corbett [78]. A comparison of the chemical shifts reported by Corbett and the chemical

shifts observed in the proton NMR of the blue CD-4 degradation product is shown in Table. 6.7.

proton (see Fig. 6.24)	chemical shift in blue CD-4 product δ/ ppm	chemical shift from literature [78] δ/ ppm
A	6.53	6.97
C	6.73	7.24
E	6.45	6.97
F	7.21	6.73
G	6.58	7.32
H	6.58	6.61

Table 6.7 Tabulation of chemical shifts for ring protons in blue CD-4 degradation product and o-methyl-indoaniline.

The shifts reported in the literature did not include coupling constants and the frequency of the NMR spectrometer was not reported so it was difficult to determine how accurate the literature values for the various chemical shifts were. The trend of the chemical shifts for the blue CD-4 product and the o-methyl-indoaniline varied slightly but the overall trend could be seen. The ring protons gave chemical shifts in the 6.45ppm to 6.70 ppm region except for protons adjacent to electron withdrawing groups such as *F* (Fig. 6.24), which gave signals in the 7.20 ppm region.

6.7 Mass spectra of minor CD-4 degradation products

A second minor yellow CD-4 degradation compound, which appeared above the azo compound already identified, in the TLC (see Fig. 6.3) was isolated, albeit in small quantities. An EI mass spectrum is shown in Fig. 6.25.

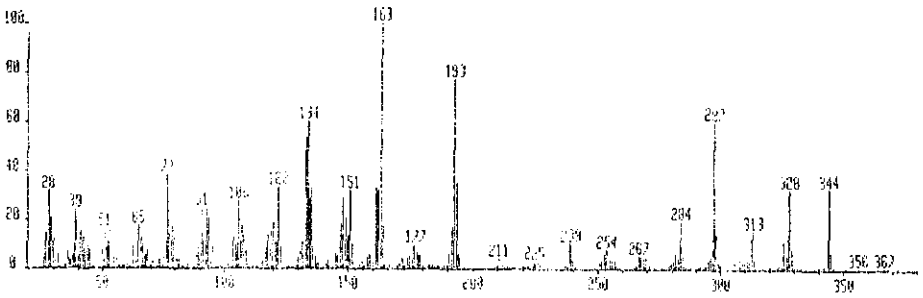


Fig. 6.25 EI mass spectrum of minor yellow CD-4 compound.

There are major fragments at m/z 328, 313, 297, 193, 163 and 134. The molecular species is at m/z 344. The exact nature of this compound is unclear but the compound shown in Fig. 6.26 would require a molecular ion at m/z 342. The molecular species may be reduced in the mass spectrometer to give m/z 344.

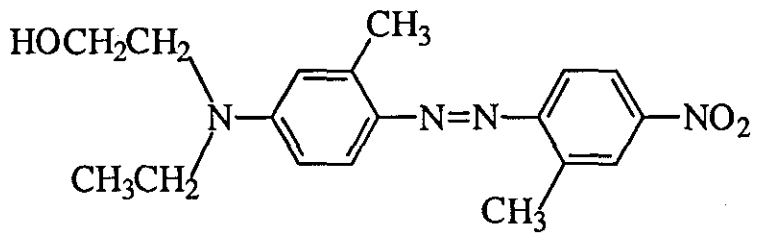


Fig. 6.26 Speculated structure for minor yellow CD-4 degradation product.

The fragment at m/z 328 may involve the loss of a CH_2 group from the tertiary amino sidechain. The fragment at m/z 313 could be due to the loss of $HOCH_2$- from the sidechain, likewise with m/z 297 which could be due to the loss of $HOCH_2CH_2$. The origins of the other fragments are unclear.

Several other compounds were isolated from the precipitates of CD-4. The FAB mass spectrum of a brown compound is shown in Fig. 6.27 and shows an intense peak at m/z 563 probably due to a protonated molecular ion corresponding to a molecular weight of 562. A structure could not be easily postulated for this compound but it may be a trimeric species.

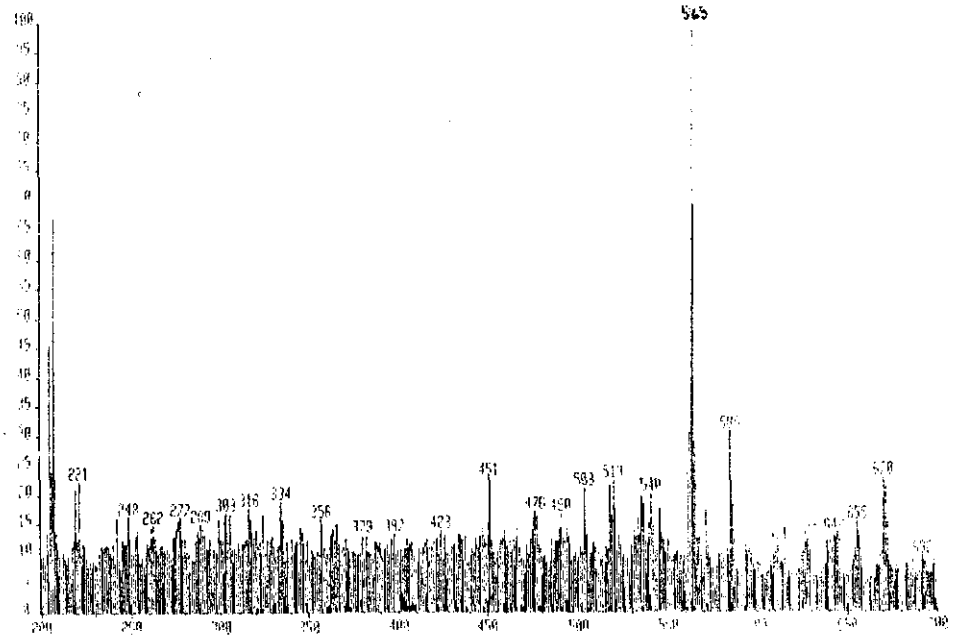


Fig. 6.27 FAB mass spectrum of brown CD-4 compound.

6.8 Summary

The developing agent CD-4 is present in C-41 developer solutions, along with a relatively high concentration of sulphite. Over a period of time and exposure to air, most of the developing agent was converted into developing agent sulphonates and, to a lesser degree, an insoluble highly coloured tar which precipitated from solution. This was different to CD-3 developing agent solutions where the degradation products were predominately non-polar in nature.

A number of significant compounds were isolated by TLC and identified spectroscopically. The main compounds were a yellow azo-dimer and a blue quinoneimine. Similar mechanisms to those seen for the corresponding CD-3 degradation products could be postulated for the formation of the CD-4 degradation products. No purple compound, similar to that seen in CD-3 developing agent solutions, was identified.

Chapter 7

Other manufacturers solutions

7.1 Introduction

It was proposed that the HPLC methods developed for the analysis of Kodak RA4, RA100 and C-41 developer solutions could be applied to other similar solutions from other manufacturers. For this purpose several other manufacturers' photographic developer solutions were obtained. These included Fotospeed CKRA4 which is analogous to Kodak RA4, Agfa AP-70 which is analogous to Kodak C-41 and Photocolor FP, which is analogous to RA4/C-41, as it is designed to develop both paper and film using the same solution. Similar studies to those already described for RA4 and C-41 were conducted. The appropriate methods of analysis were applied and degradation studies were conducted. The solutions were degraded under identical conditions to the analogous Kodak solutions in that they were stored in sealed glass containers in sunlight. Comparisons were made between the various solutions.

7.2 Afga AP-70 solution

7.2.1 HPLC of Agfa AP-70 developer solution

The Agfa AP-70 working solution was prepared as described previously (see Chapter 2.1.2). The HPLC method used for the analysis of the Agfa solution was same as that used for the analyses of C-41 and CD-4/sulphite, as it was found that the Agfa solution contained a similar developing agent to Kodak C-41 developer solution (CD-4) because it had a similar retention time and an identical UV spectrum. Again problems were encountered in the retention time of the developing agent and it tended to vary from two mins to three mins. A chromatogram of fresh Agfa AP-70 is shown in Fig. 7.1. The concentration of developing agent is comparable to that in Kodak C41 developer and this is reflected in the graph of degradation (7.4) where the initial concentration of developing agent gave a detector response of 11000 mAu (cf. C-41 12000 mAu).

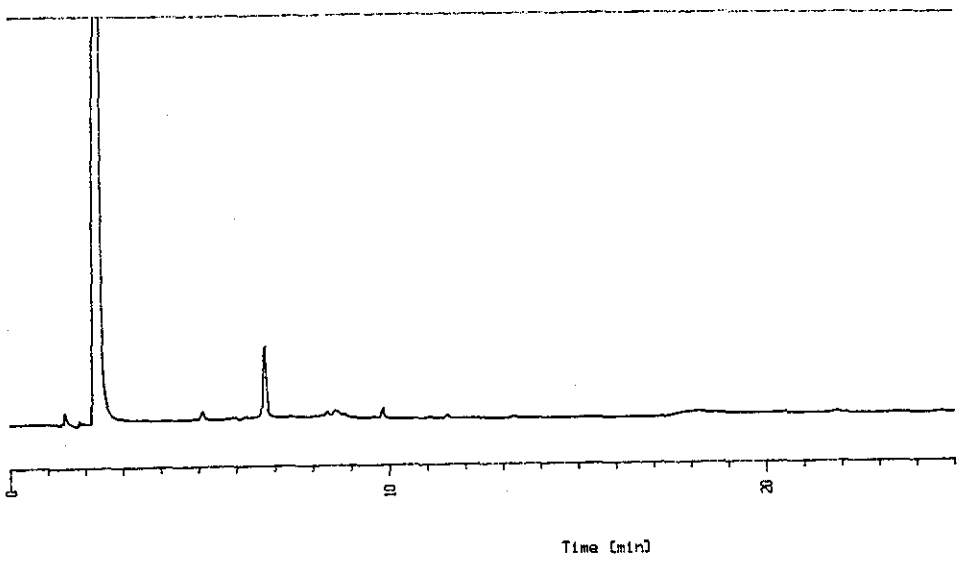


Fig. 7.1. Chromatogram of fresh Agfa AP-70 developer solution (method D, detection at 243 nm, Shandon column)

7.2.2 Degradation of Agfa AP-70 developer solution

As seen later (Fig. 7.4) Agfa AP-70 appeared to contain a higher level of sulphite than the corresponding Kodak C-41 solution and the meta sulphonate can be seen forming in fresh developer solution in higher concentrations to that seen in the corresponding Kodak C-41 solution. The Agfa AP-70 solution degraded over the next few days with a similar pattern to Kodak C-41 in that the m-sulphonate was predominantly formed and the o-sulphonate to a lesser degree. A twelve day old sample is shown in Fig. 7.2.

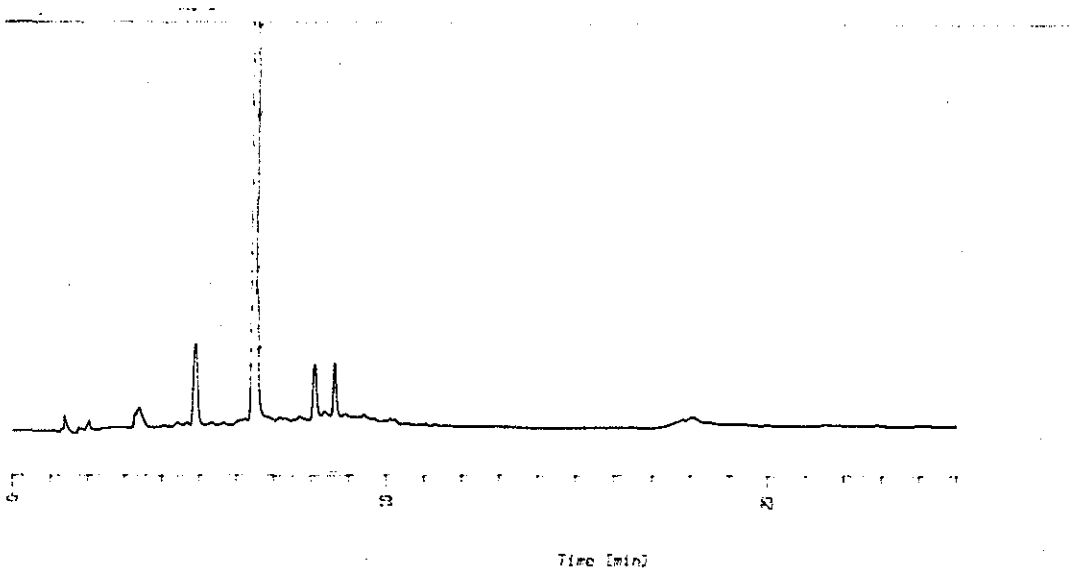


Fig. 7.2 Chromatogram of twelve day old Agfa AP-70 (method D, detection at 243 nm, Shandon column)

Various sulphonates can be clearly seen at 5 mins, 6.8 mins (m-sulphonate), 8.0 mins (o-sulphonate) and 8.6 mins. The sulphonates were identified by their UV spectra and compared to those already seen in Kodak C-41 developer solutions and the λ_{max} values taken from the literature [15]. However, after eight days the level of m-sulphonate started to fall off and another unidentified sulphonate at 8.6 mins started to form. The higher level of sulphite can be seen in the formation of developing agent m-sulphonate. In the case of Agfa AP-70 the maximum level is 7000 mAu whereas in the case of Kodak C-41 the maximum level of CD-4 m-sulphonate is 6000 mAu. The initial concentrations of the developing agents were similar at 11000 mAu. As the developing agent is similar to that used in Kodak solutions (from the retention time and UV spectra) it was assumed that the sulphonates were formed in a similar way also. The absorbance of this sulphonate at 8.6 mins continued to increase and the levels of the ortho and m-sulphonates continued to fall. In the chromatogram of the twelve day old sample (Fig. 7.2) no absorptions due to degradation products in the 18 to 20 min region can be seen. As the level of sulphite in the Agfa AP-70 solution was higher than the level in Kodak C-41 no non-polar degradation products could be visibly seen in the degraded solutions.

As the sample degraded further the o-sulphonate at 8 mins disappeared completely and the absorbance of the sulphonate at 8.6 mins continued to increase. A chromatogram of a twenty four day old AP-70 sample is shown in Fig. 7.3.

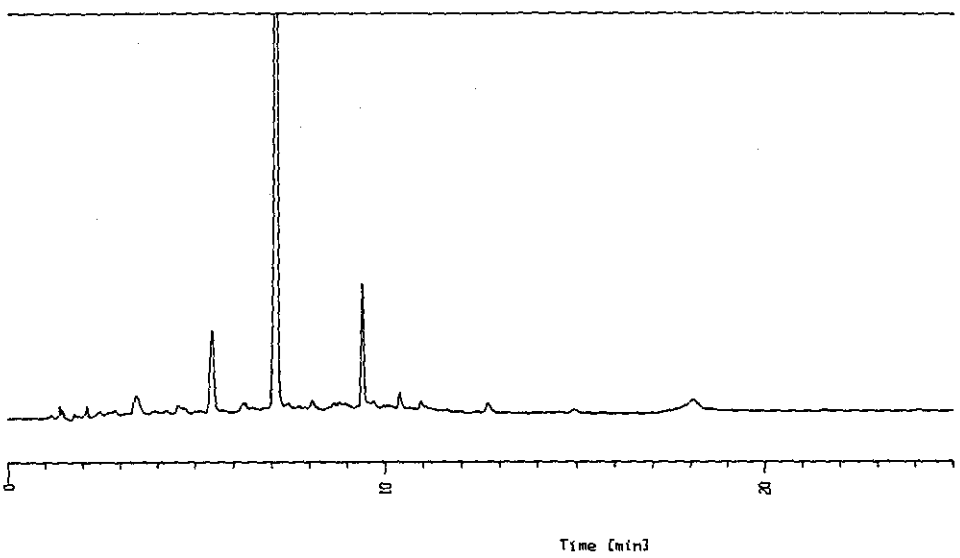


Fig. 7.3 Chromatogram of twenty four day old Agfa CD-70. (method D, detection at 243 nm, Shandon column)

A graph of Agfa AP-70 degradation with time is shown in Fig. 7.4. The decrease in the concentration of the developing agent together with the rise and fall in the concentration of the m-sulphonate is clearly seen. The other sulphonates form to a much lesser degree. There is some random scattering of the peak areas but this was due to sampling errors and did not reflect a random oscillation in the concentrations of the individual components.

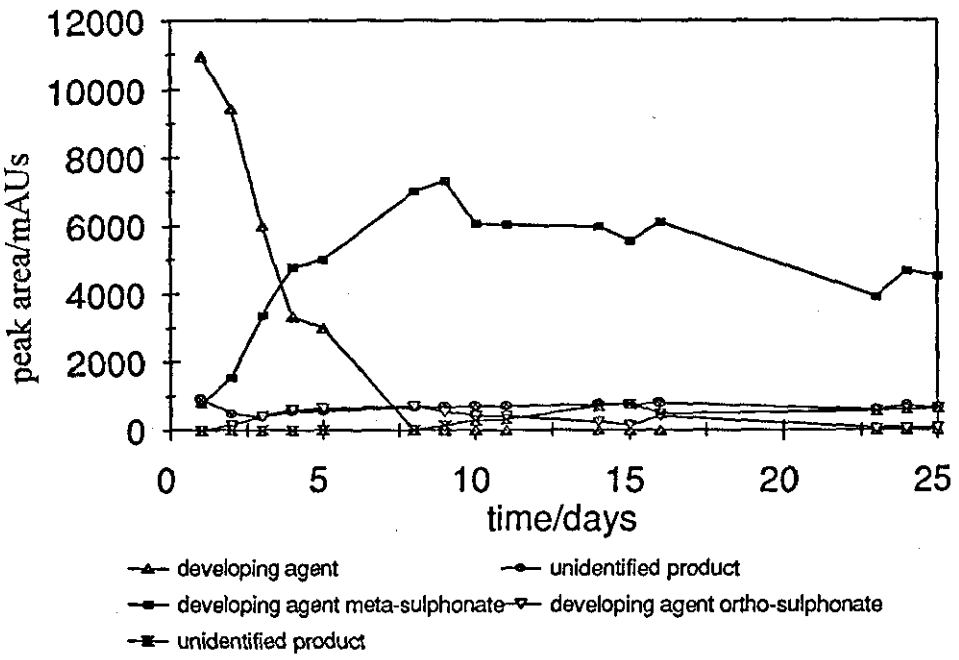


Fig. 7.4 Graph of Agfa AP-70 degradation with time (analysis according to method D, detection at 243 nm, Shandon column).

7.3. Fotospeed CKRA4

7.3.1 HPLC of Fotospeed CKRA4

The HPLC method of analysis applied to Kodak RA4 developer solution was also applied to Fotospeed CKRA4. The Fotospeed working solution was prepared as described in Chapter 2.1.2. The developing agent was found to be similar to Kodak CD-3 (from the retention time and spectrum) and the concentration of developing agent was found to be slightly higher than the Kodak solution at 4.85 g/l using the CD-3 calibration graph (Fig. 3.6)(cf. RA4 4.5 g/l). A group of peaks, similar to those found for Phorwite stain reducing agent was also seen from 10 to 14 mins albeit in a lower concentration to that seen in Kodak RA4. Comparison of the spectra and retention times for the peaks in Fotospeed to those in Kodak RA4 showed that the compounds were very similar.

A chromatogram of fresh Fotospeed CKRA4 is shown in Fig. 7.4. Although the exact composition of the Fotospeed solution was unavailable the chromatogram did show that the basic components are similar to those found in Kodak RA4/RA100 developer solutions.

7.3.2 Degradation of Fotospeed CKRA4

Solutions of Fotospeed CKRA4 were allowed to degrade in a similar way to Kodak RA4 and seemed to follow a comparable degradation pattern. Nevertheless the level of preservatives may be different as the developing agent in Fotospeed begins to degrade immediately on preparation of the working solution whereas the developing agent in RA4 did not degrade until the preservative had been oxidised (see Fig. 3.23). It may be that Fotospeed CKRA4 is marketed at the amateur who may use the solution to develop photographic paper and discard it after one usage. Kodak RA4 is used in professional photographic laboratories and is designed to be replenished and used continuously.

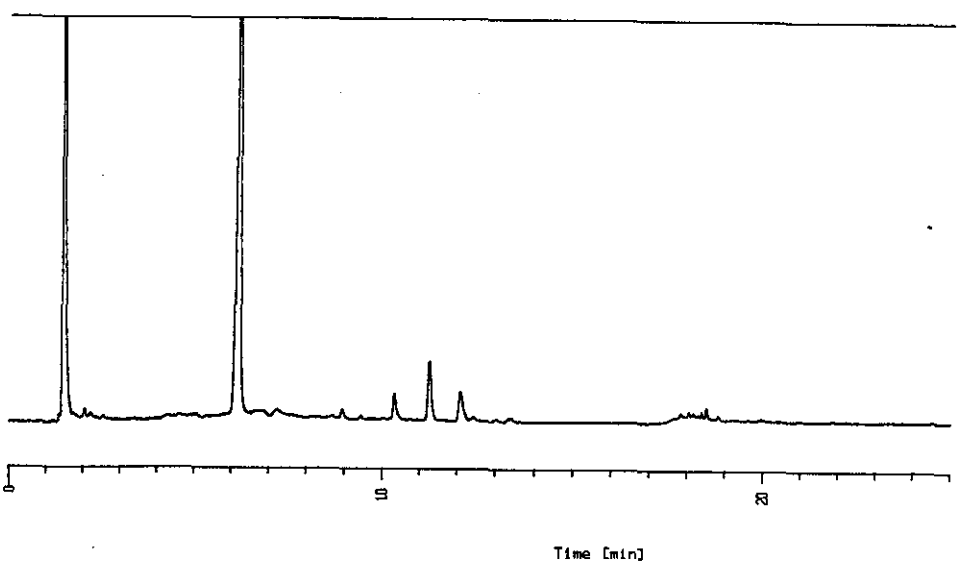


Fig. 7.4 Chromatogram of fresh Fotospeed CKRA4 (method C, detection at 243 nm, Shandon column)

Already unretained polar products can be seen eluting at 1.5 mins in the fresh sample (Fig.7.4). The levels of sulphite and other

antioxidants/preservatives are quite low as non-polar degradation products are starting to form at 18 to 20 mins in this relatively fresh sample.

An eight day old Fotospeed sample is shown in Fig. 7.7. A large number of non-polar degradation products are clearly seen from 18 to 20 mins. Comparison of non-polar degradation products from Fotospeed CKRA4 with those from Kodak RA4, using TLC, showed that the non-polar degradation products formed were identical in that yellow, blue and purple products were formed. The level of CD-3 had dropped and further polar products have formed and eluted unretained at 1.5 mins. The level of Phorwite is unchanged as was the case in the Kodak RA4 solution.

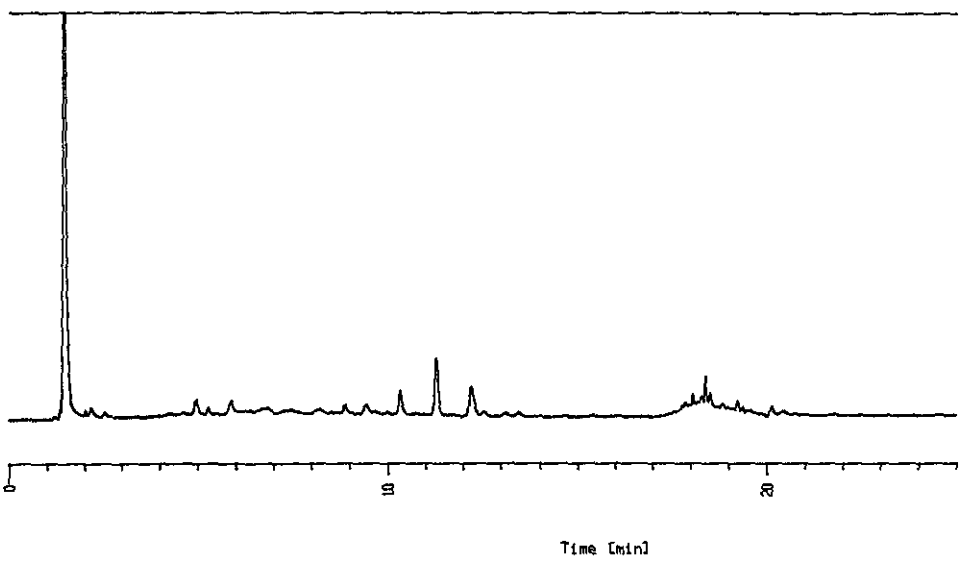


Fig. 7.5 Chromatogram of eight day old Fotospeed CKRA4 (method C, detection at 243 nm, Shandon column)

The Fotospeed sample continued to degrade and an eleven day old sample is shown in Fig. 7.6. All the developing agent had degraded and several additional compounds have formed. A compound with a retention time of two mins and a spectra similar to methyl-1,4-benzoquinone was seen (see Fig. 7.7). The spectrum of methyl-1,4-benzoquinone is shown in Fig. 3.21.

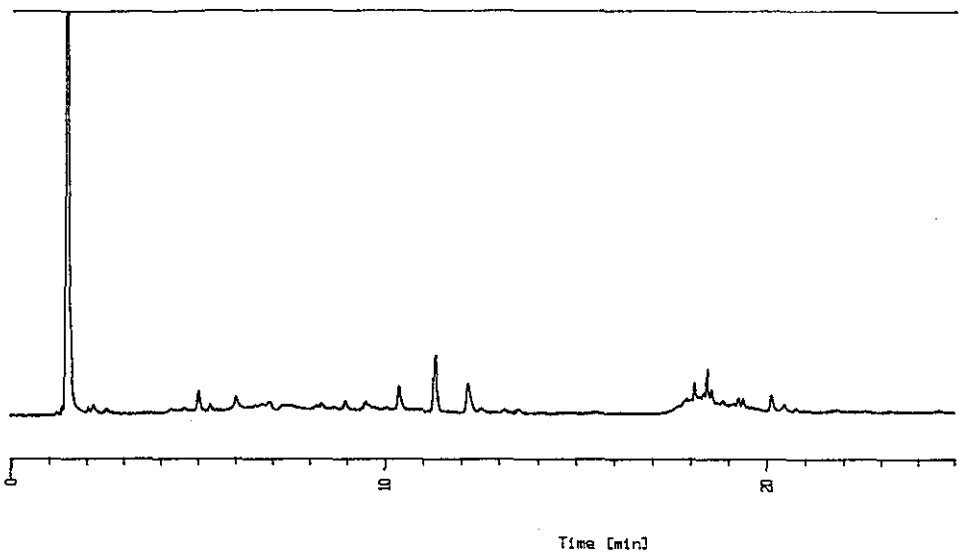


Fig.7.6 Chromatogram of eleven day old Fotospeed CKRA4 (method C, detection at 243 nm, Shandon column)

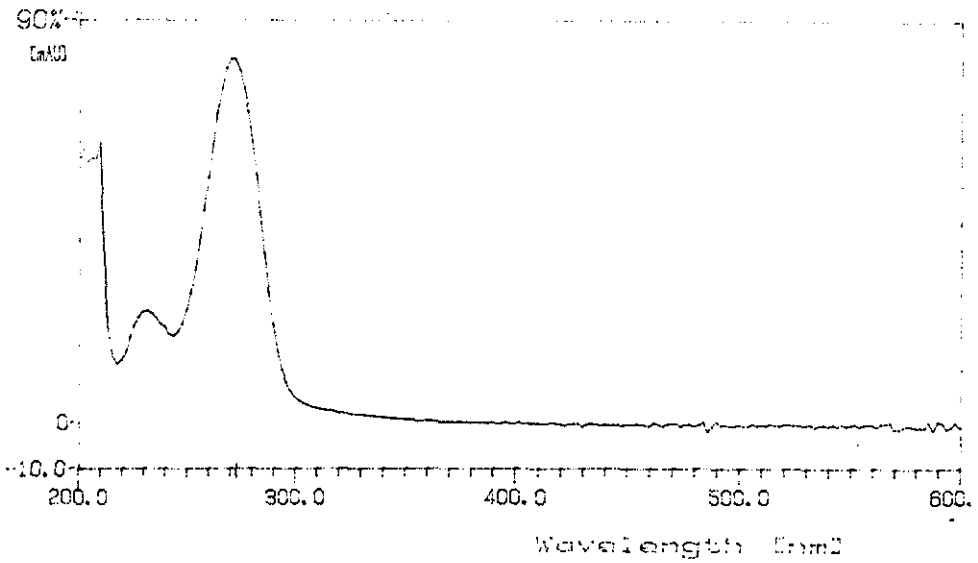


Fig. 7.7. Spectra of peak (retention time 2 mins) in Fotospeed CKRA4 (analysis according to method C, Shandon column)

A graph of Fotospeed degradation with time is shown in Fig. 7.8. Again there is some random scattering of the peak areas, due to sampling errors.

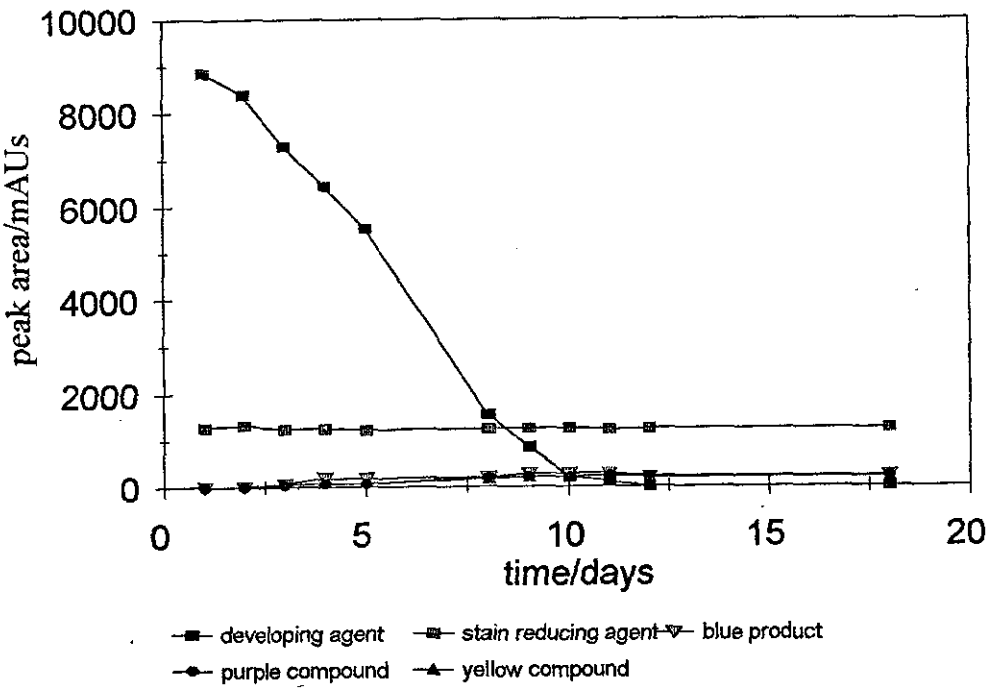


Fig. 7.8. Graph of Fotospeed CKRA4 degradation with time (analysis according to method C, detection at 243 nm, Shandon column)

The graph is very similar to that already seen for Kodak RA4 (see Fig. 3.23). Individual non-polar degradation products are again difficult to resolve as there is such a multitude present. However the blue, purple and yellow degradation products similar to those already seen for CD-3/RA4/RA100 can be seen again.

7.4. Photocolor degradation

Photocolor FP was analysed using the same method as that used for Kodak C-41. Photocolor FP seems to be unlike Kodak C-41, RA4, Agfa AP-70 or Fotospeed CKRA4 as the retention time of the developing agent was different to those already seen in the other developer solutions. It was also apparent that there was other unidentified compounds in the fresh Photocolor solution. In a one day old sample the developing agent in Photocolor was seen eluting with a retention time of 3.9 mins. A degradation product with an elution time of 7.8 mins was also apparent along with a compound at 18.2 mins (Fig. 7.9).

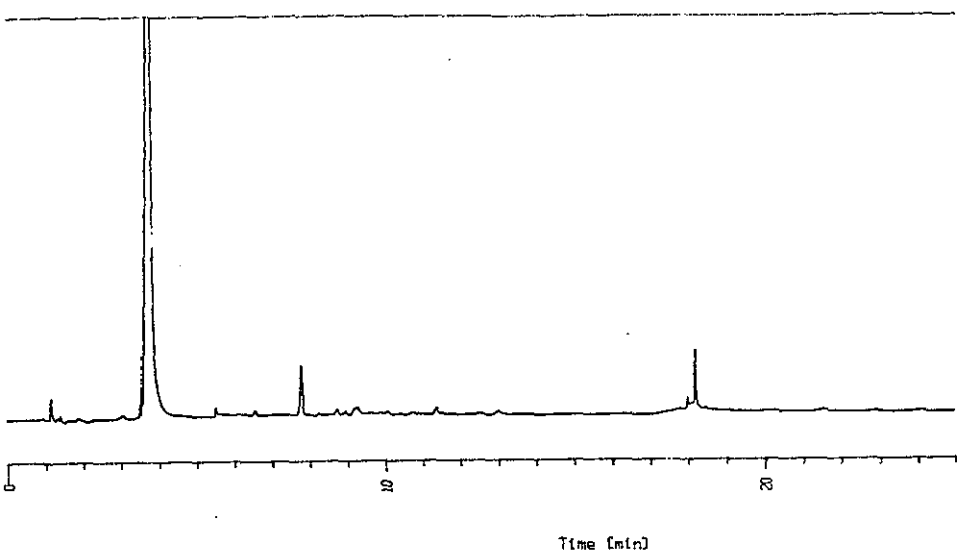


Fig. 7.9 Chromatogram of one day old Photocolor FP developer solution (method D, detection at 243 nm, Shandon column)

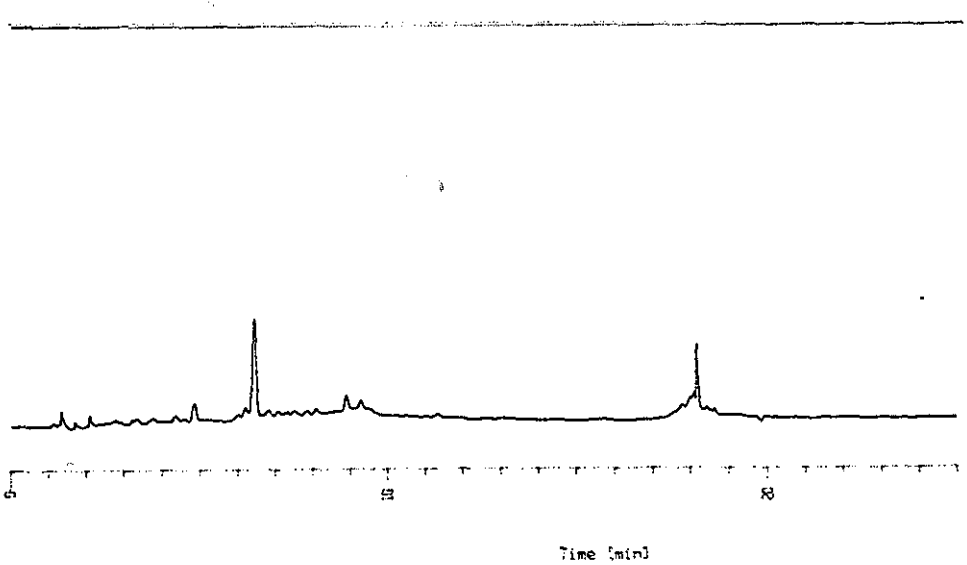


Fig. 7.10 Chromatogram of nine day old Photocolor FP developer solution (method D, detection at 243 nm, Shandon column.

In a nine day old sample the developing agent had totally degraded and the absorption of the degradation product at 7.8 mins had increased. The compound at 18.2 mins had not increased in absorption, at 243 nm, but by looking at the diode array spectrum it was possible to see that the absorption in the 500 nm to 600 nm region had increased dramatically.

Several other absorptions could be seen for unidentified compounds between 6 mins and 10 mins. Some non-polar degradation products were seen in the 18.5 mins to 20 mins region.

Because the Photocolor developer solution was very different to the Kodak, Agfa and Fotospeed solutions already investigated, it was decided not to study the degradation any further as it was outside the scope of the project.

7.5 Summary

It was shown that the methods developed for the analysis of Kodak RA4/RA100 and C-41 developer solutions could be applied to other similar solutions from different manufacturers. The Fotospeed CKRA4 developer solution was found, by chromatographic analysis, to be similar in composition to Kodak RA4/RA100 solutions and the degradation studies showed that the Fotospeed solution degraded in a similar way to the Kodak solutions. The Agfa AP70 developer solution was found, by chromatographic analysis, to be similar in composition to Kodak C-41 developer solution. Degradation studies showed that the Agfa solution contained a higher concentration of sulphite and non-polar degradation products were not seen, unlike the Kodak C-41 solution. The Photocolor developer solution was unlike any of the other developer solutions and so was considered outside the scope of this study. However, with the experience of the chromatography of developer solutions now gained, it would have been possible to develop chromatographic methods for this developer solution also.

Chapter 8

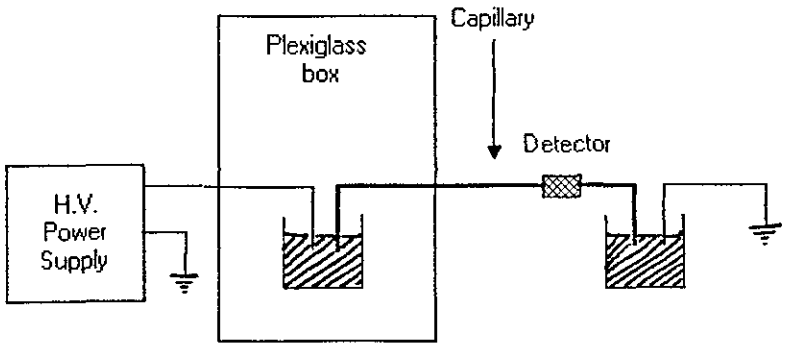
Capillary electrophoresis

8.1 Introduction

Capillary electrophoresis (CE) has emerged, over the past few years, as a powerful separation technique, complementary to HPLC. It has many advantages over HPLC, such as short analysis time, high efficiency ($N > 10^5$ to 10^6), wide application range, simple method development, and low consumption of buffers [68]. One of the main disadvantages of CE is that due to the small sample volume it is necessary to detect the sample on-capillary which can lead to poor sample detection limits. Also, as the sample is detected on-line, it is not usually possible to derivatise the analyte, post-separation, for detection.

Capillary electrophoresis is ideally suited to the analysis of developer solutions. As developing agents are charged polar species, they can be easily separated by an applied electric field as in CE. The developing agent sulphonates are also charged species and are easily separated by CE. Other components of the developer solution such, as Phorwite, are partially ionised polar species and can be separated before or after the electroosmotic flow, depending on the ionic charge, pH and buffer type. However, non-polar degradation products such as the CD-3 and CD-4 azo compounds (Chapters 4 and 6) are not charged and they migrate with the electroosmotic flow.

Electroosmotic flow is a fundamental property of CE and is the bulk flow of liquid in the capillary as a consequence of the surface charge on the interior capillary wall. Under aqueous conditions the fused silica capillary has many silanol groups which exist in anionic form (SiO^-). Counter-ions, cations in this case, build up near the surface to maintain a charge balance and create a potential difference very close to the wall. When a potential is applied to the capillary the cations are attracted to the cathode and because they are solvated they drag the bulk solution with them, so even neutral compounds migrate and separate with the electroosmotic flow.



The sample is injected onto the capillary and then an electric field is applied. Different ions move at different speeds in the electric field and thus a separation is achieved.

Fig. 8.1 Basic capillary electrophoretic system

Another type of capillary electrophoresis, called micellar electrokinetic chromatography (MEKC), involves the separation of neutral compounds by the addition of surfactants such as sodium dodecyl sulphate (SDS) to the mobile phase. This system was used for the separation of Phorwite. The hydrophobic tails of the SDS molecules are orientated towards the neutral Phorwite molecule to avoid interaction with the hydrophilic buffer. The negatively charged heads of the SDS molecules are orientated towards the hydrophilic buffer. Thus the micelle formed is repelled by the cathode and migrates against the electroosmotic flow. However, the EOF is generally faster than the migration of the micelles and the net flow is towards the cathode.

Fresh and degraded developer solutions were analysed using CE. The solutions were identical to those analysed by HPLC (see Chapters 3 and 5).

8.2 CE of CD-4 and C-41 developer solutions

CD-4 developing agent was separated using a disodium o-phosphate buffer (pH 6.77, 5.68 g/l). At this pH CD-4 was protonated and separated

before the electroosmotic flow as it was attracted to the negatively charged cathode.

A calibration plot for CD-4 is shown in Fig. 8.2. The CE separation of CD-4 was found to be reproducible and quantitative. Regression analysis on the plot gave a good correlation coefficient (r^2 of 0.9983).

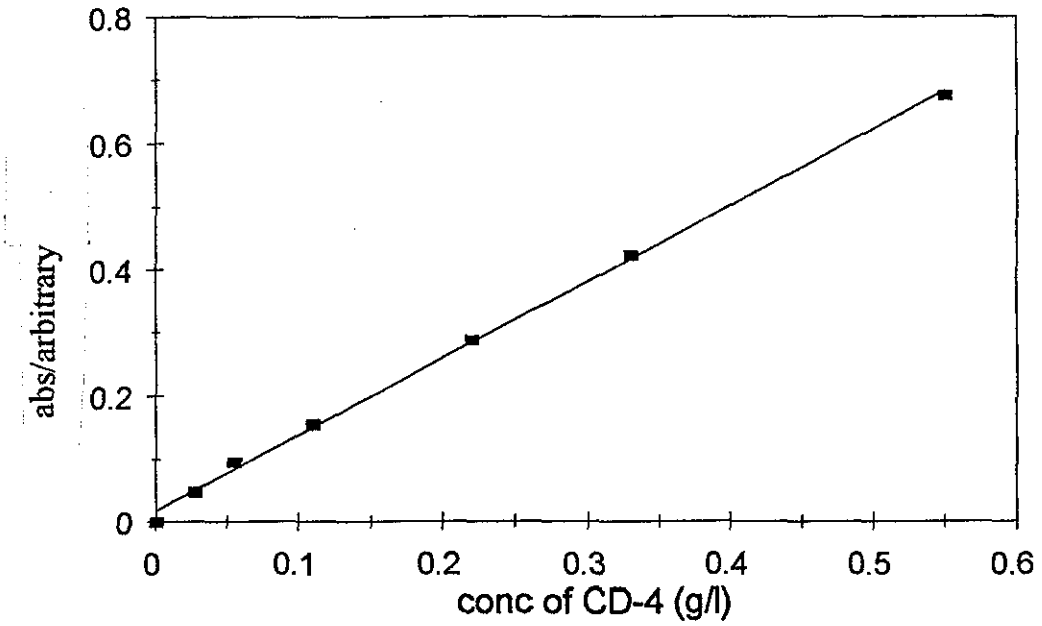


Fig. 8.2 CE calibration plot for CD-4 (pH 6.77 phosphate buffer, .+ 15 kV detection at 254 nm).

C-41 developer solution was separated using the same buffer. Again CD-4 developing agent separated before the electroosmotic flow. The CD-4 sulphonates which are the main degradation products of C-41 solutions are negatively charged and are attracted to the anode but are carried towards the cathode by the electroosmotic flow (Fig. 8.3) and separate after the electroosmotic flow marker. Evans *et al* showed the separation of short chain (C_1-C_5) alkylsulphonates using CE [75]. Analysis of C_4 to C_{12} alkylsulphonic acids was performed by Harrold *et al* [76], using a pH 3.5 pyromellitic acid buffer. The separation of eight aromatic sulphonic acids, including dodecylbenzenesulphonate, toluene-sulphonate and 4-nitrobenzenesulphonate was shown by Brumley [77], using a pH 8.3 boric acid-borate buffer. All methods separated the sulphonates as ionised compounds and so they migrated after the electrosoomotic flow, in a similar fashion to the method developed for the CD-4 sulphonates.

An electropherogram of fresh C-41 is shown in Fig. 8.4. The protonated developing agent CD-4 elutes with a migration time of 5.4 mins whilst the EOF marker (benzamide) elutes with the electroosmotic flow at 7.2 mins. The negatively charged CD-4 sulphonates elute with migration times of 9.5 mins and 10.7 mins. It was postulated that the m-sulphonate was the larger peak at 9.5 mins whereas the lower concentration o-sulphonate was the peak starting to emerge at 8 mins. This assumption was made on the basis of the degradation pattern found using HPLC (see Chapter 3) where the large signal was due to the m-sulphonate and the smaller signal due to the o-sulphonate. The separation in CE is dependent on charge, size and shape of the analyte. For the o- and m-sulphonate, charge and size are very similar, therefore the separation could be due to differences in shape. This phenomena has been explored by Rowe *et al.* [69]. It was anticipated that the sulphonic acid groups would line up towards the anode and that the sulphonates would be dragged along by the electroosmotic flow. The shape of the o-sulphonate and m-sulphonate could be compared to a wedge. In the case of the o-sulphonate, the 'blunt' edge of the wedge points toward the anode and so the molecule migrates faster. In the case of the m-sulphonate the 'blunt' edge of the wedge points towards the cathode and so the molecule migrates more slowly (Fig. 8.3).

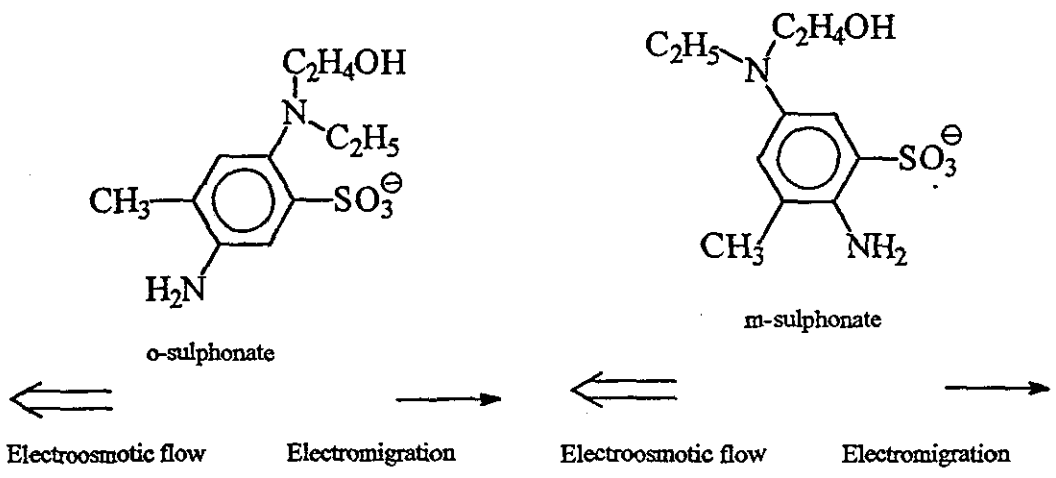


Fig. 8.3 Orientation of sulphonates in CE capillary

Using the calibration plot in Fig. 8.2. the C-41 solution was found to have a concentration of CD-4 of 4.6 g/l. This is slightly higher than the 4.5 g/l which is specified for a C-41 solution but a more detailed study would have to be undertaken to investigate the significance of this figure.

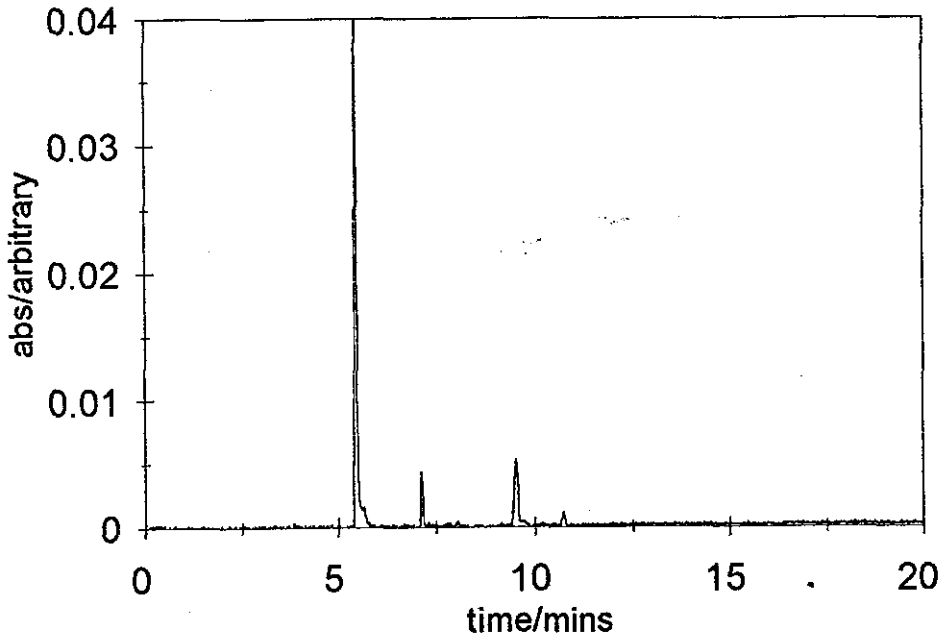


Fig. 8.4 Electropherogram of fresh C-41 developer solution (pH 6.77 phosphate buffer, + 15 kV, detection at 254 nm).

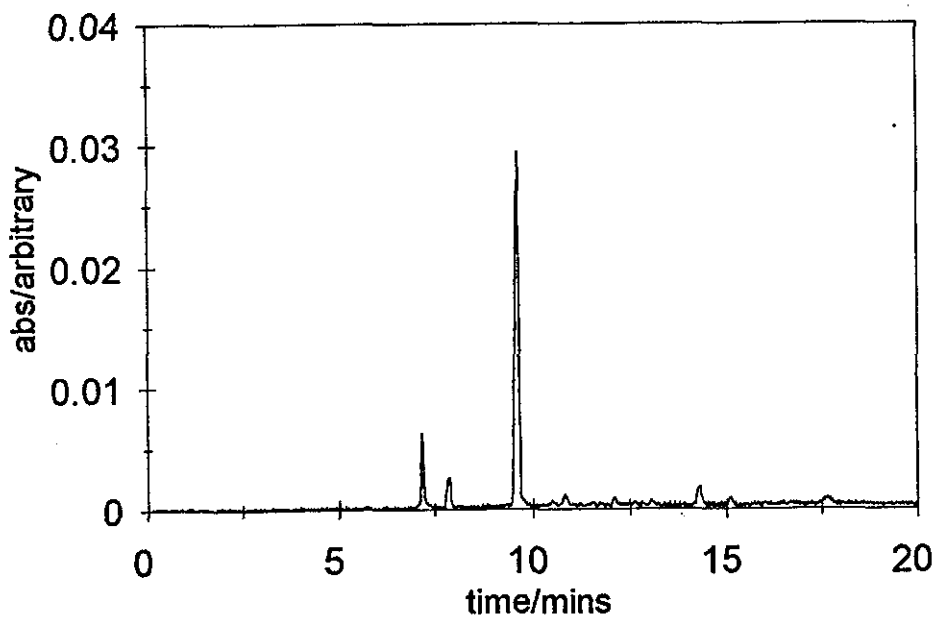


Fig. 8.5 Electropherogram of degraded C-41 developer (pH 6.77 phosphate buffer, + 15 kV, detection at 254 nm).

An electropherogram of a twenty one day old C-41 sample, (see Chapter 2.2.3 for degradation conditions) is shown in Fig. 8.5. The developing agent has completely degraded and the concentration of sulphonates has increased. The m-sulphonate (migration time 9.5 mins) has increased to a much larger level compared to the o-sulphonate (migration time 7.9 mins). Another compound migrates with a retention time of 14.5 mins but its exact structure was unknown. A much smaller signal is seen at 15.3 mins but again the structure of this compound was not known.

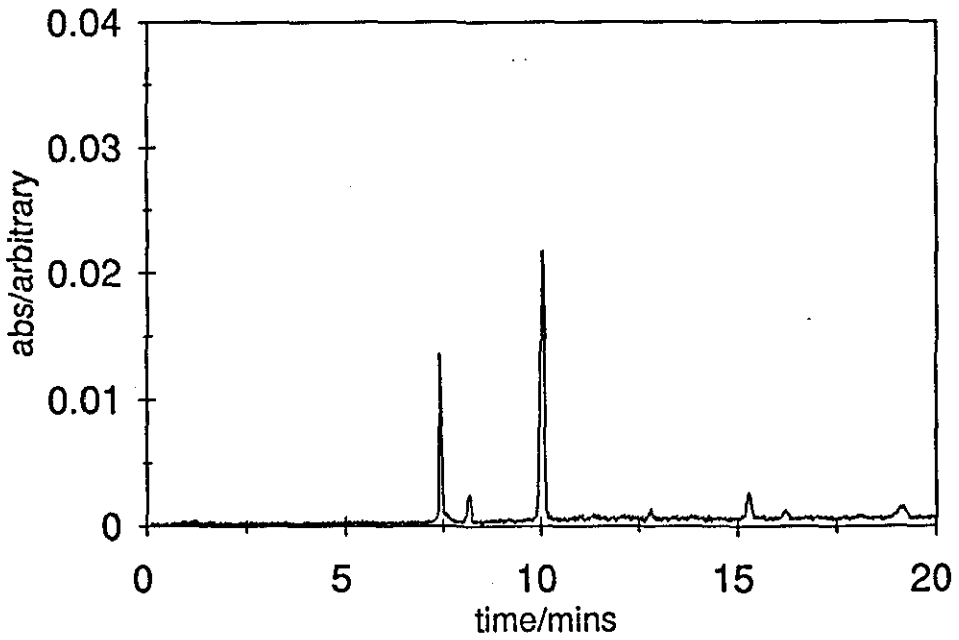


Fig. 8.6 Electropherogram of degraded C-41 which was stored in the dark (pH 6.77 phosphate buffer, + 15 kV, detection at 254 nm)

The electropherogram of a 21 day old C-41 sample which had been stored in the dark is shown in Fig. 8.6 The ratio of sulphonates are not dissimilar to those already seen for a C-41 sample which had been allowed to degrade in sunlight. Again the m-sulphonate is the major product (migration time 10 mins) and the o-sulphonate is formed to a lesser degree (migration time 7.9 mins). Again two smaller signals can be seen at 15.2 mins and 16.2 mins respectively. The similarity of the samples stored in light and dark seems to indicate that the degradation of CD-4 to form sulphonates is not dependent on light.

8.3 CE of degraded CD-4/sulphite

A solution of CD-4 with a two-fold excess of sodium sulphite was buffered at pH 10, using sodium carbonate and allowed to degrade in sunlight and analysed using the same CE method as used for C-41 developer solution (Fig.8.7). Again the o-sulphonate can be seen at 8.3 mins, whereas the higher concentration m-sulphonate can be seen at 10.3 mins. Several other sulphonates with longer migration times can be seen at 12.6 mins, 13.2 mins, 13.5 mins and 14.4 mins. A large peak can be seen at 16 mins and this may be a disulphonate but there was no evidence to support this.

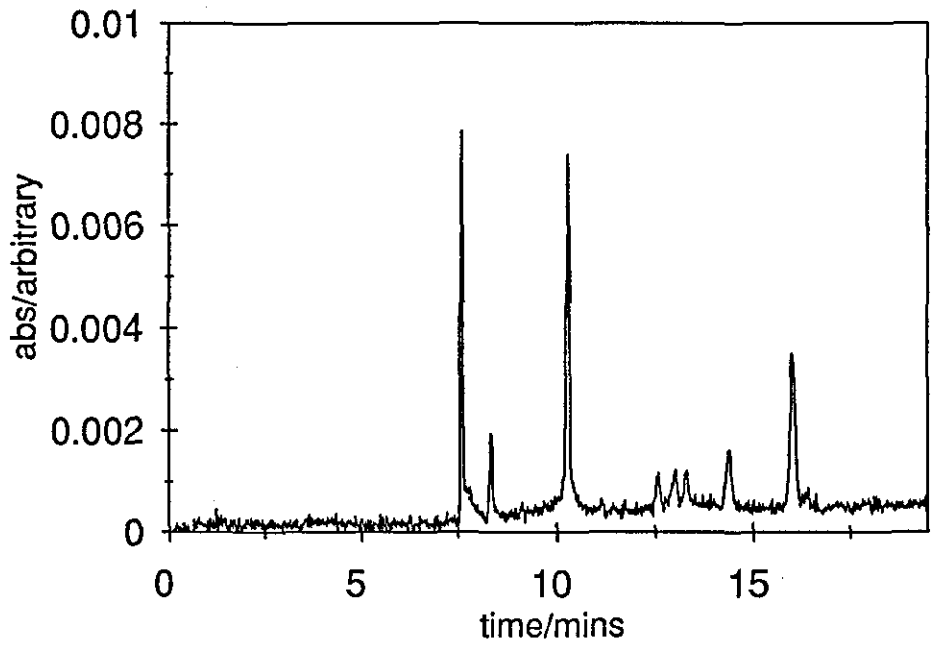


Fig. 8.7 Electropherogram of degraded CD-4/SO_3 solution (pH 6.77 phosphate buffer, + 15 kV, detection at 254 nm)

8.4. CE of Agfa AP-70 developer solution

A solution of one day old Agfa AP-70 developer solution was analysed using the same method as used for the C-41 developer solution. A similar pattern as that seen in C-41 was seen again (Fig. 8.8). The developing agent migrates before the electroosmotic flow at 5.5 mins, the electroosmotic flow has a migration time of 7.3 mins. The o-sulphonate can be seen at 8 mins, whilst the higher concentration m-sulphonate migrates at 9.9 mins. A much smaller signal can be seen at 11.2 mins but its exact structure was not known.

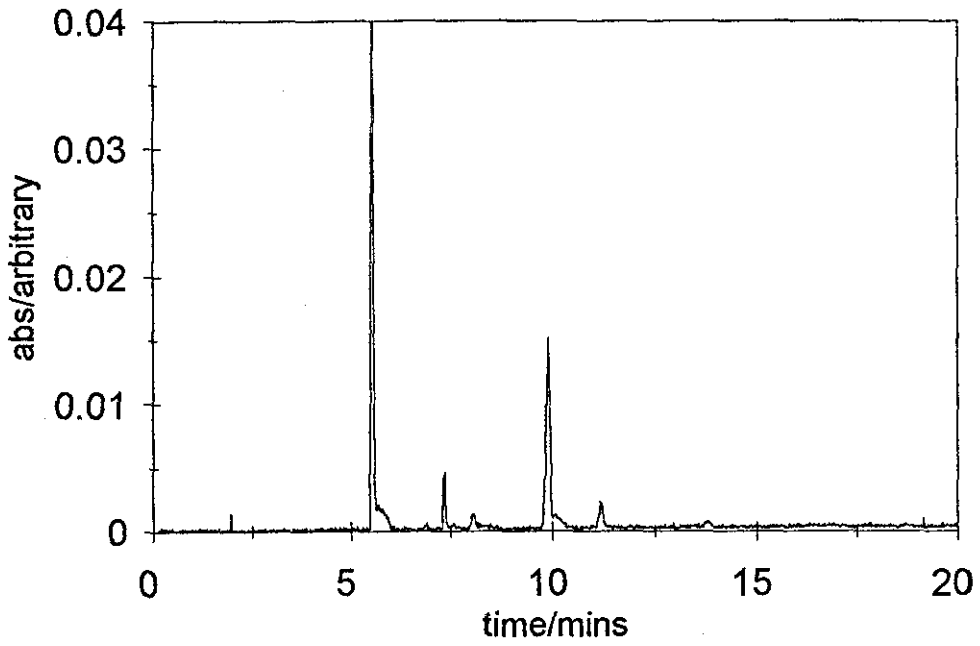


Fig. 8.8 Electropherogram of one day old Agfa AP-70 developer solution (pH 6.77 phosphate buffer, + 15 kV, detection at 254 nm)

8.5 CE of Phorwite stain-reducing agent.

8.5.1 Analysis of Phorwite using carbonate buffer

Phorwite is the stain-reducing agent in RA4 and RA100 developer solutions. Its assigned structure is shown in Fig. 8.9. As is seen it can have either protonated amino or free sulphonic acid groups or both depending on pH. This makes the pH of the separating buffer crucial. Phorwite was analysed using carbonate buffer (pH 10, 0.057 M). At this high pH the sulphonic acid groups were ionised and so Phorwite migrated after the electroosmotic flow. Various other pH's were investigated but none led to an adequate separation of Phorwite. An electropherogram of Phorwite is shown in Fig. 8.10 together with a calibration graph in Fig. 8.11.

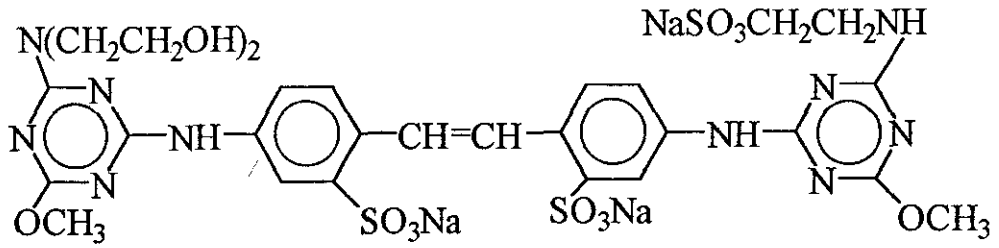


Fig. 8.9 Structure of Phorwite stain reducing agent.

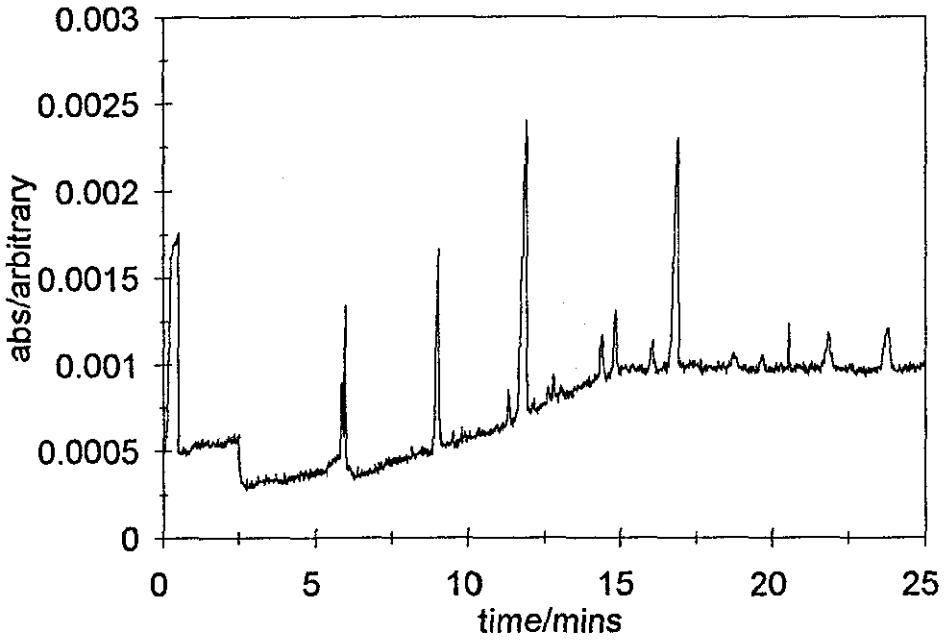


Fig. 8.10 Electropherogram of Phorwite (pH 10 carbonate buffer, + 15 kV, detection at 254 nm)

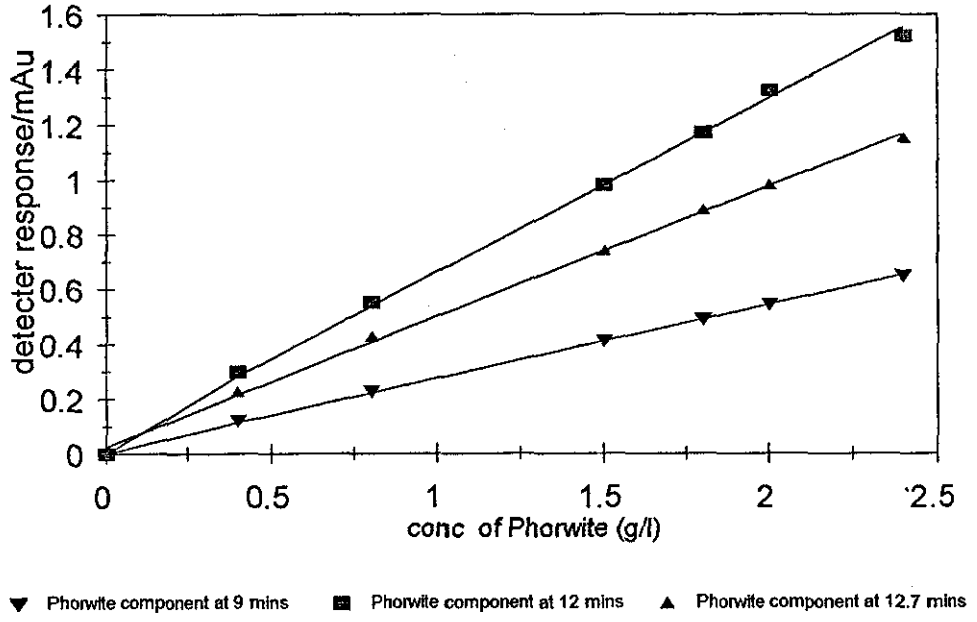


Fig. 8.11 CE calibration graph for Phorwite (pH 10 carbonate buffer., + 15 kV, detection at 254 nm)

The major Phorwite peaks can be seen at 9 mins, 12 mins and 12.7 mins. There is a rising baseline due to heating effects. However although the

signals for Phorwite were quantitative they were not reproducible and the peak shapes and migration times tended to vary. The poor peak shape was caused by differences in the conductivity of the buffer and the analyte whereas migration time differences may have been caused by heating effects and interactions with the silanol groups of the fused silica capillary.

Different approaches were taken in an attempt to improve the separation. An increase in the ionic strength of the buffer lead to heating effects and a rising baseline due to the increased current. A lower concentration buffer led to an improved separation of Phorwite but the developing agent CD-3 (see Chapter 8.6) migrated with the electroosmotic flow. As CD-3 and Phorwite are major components of RA4 and RA100 developer solutions this was not considered ideal.

8.5.2 Analysis of Phorwite using MEKC

A solution to these problems was the use of micellar electrokinetic chromatography (MEKC). MEKC allows for the separation of neutral species by use of surfactants in the separating buffer. At pH 7 Phorwite is a neutral compound or zwitterion which will migrate with the electroosmotic flow in capillary electrophoresis. However, on addition of an anionic surfactant such as sodium dodecyl sulphate (SDS) ($CH_3(CH_2)_{11}OSO_3Na$) micelles are formed and the separation of Phorwite was facilitated. This buffer system worked well but Phorwite had a long migration time and once again peak shapes were not ideal. On addition of 10% acetonitrile the peak shape improved dramatically and migration times were reproducible and reasonably short. Addition of this organic modifier led to less hydrophobic interactions between the micelle and the solute. It also decreased the hydrophobic interactions which maintain micellar structure, allowing more rapid chromatographic kinetics.

A MEKC electropherogram of water is shown in Fig. 8.12. An electropherogram of Phorwite is shown in Fig. 8.13.

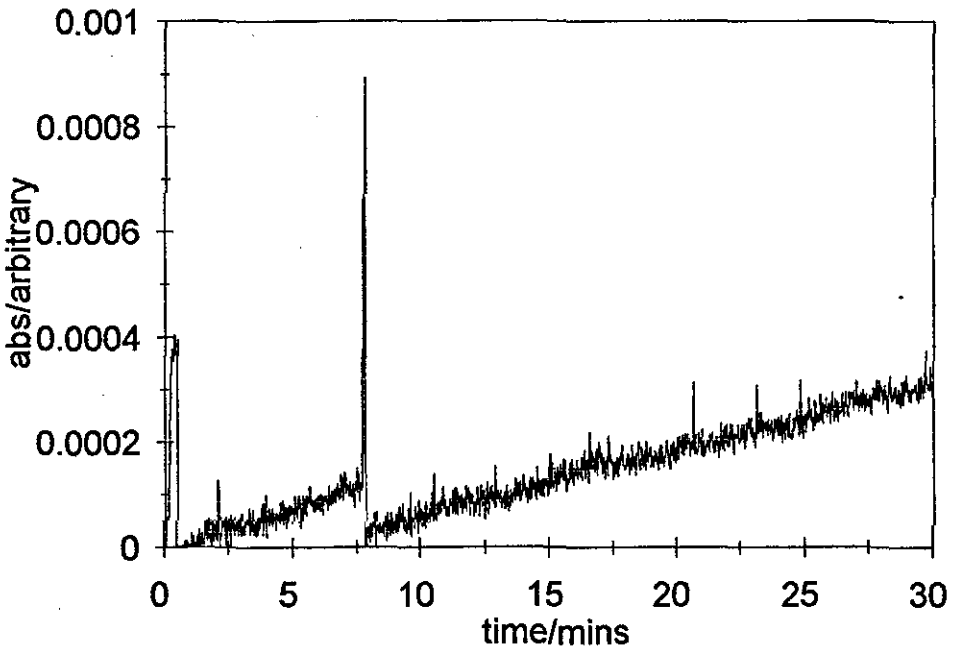


Fig. 8.12 Electropherogram of water using MEKC buffer (pH 7.48 phosphate buffer with SDS (0.0057 M) and acetonitrile (10 % v/v), +15 kV, detection at 254 nm)

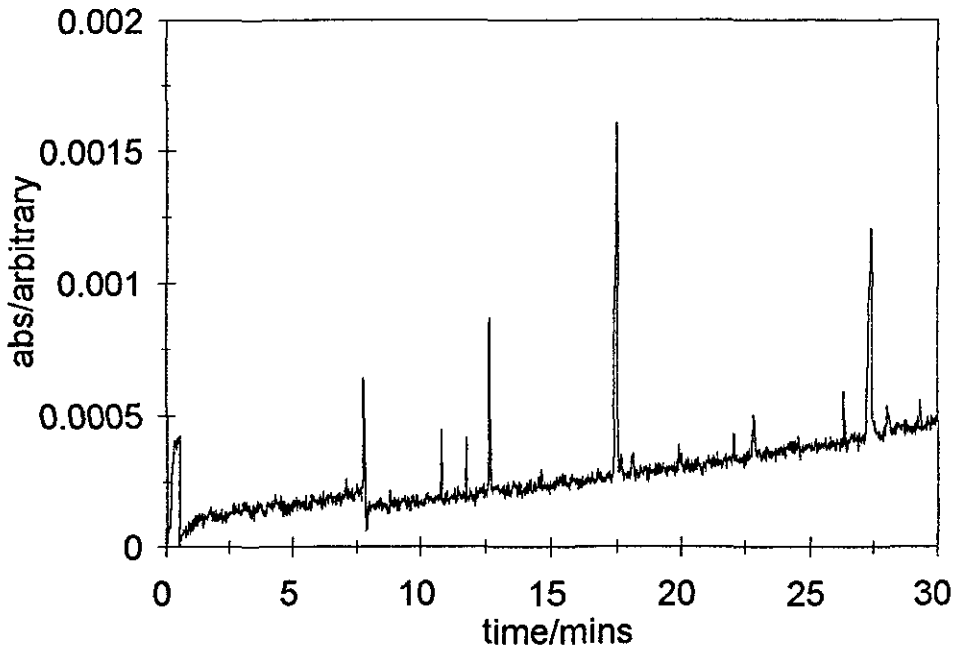


Fig. 8.13 MEKC electropherogram of Phorwite using MEKC buffer (pH 7.48 phosphate buffer with SDS (0.0057 M) and acetonitrile (10 % v/v), +15 kV, detection at 254 nm)

The electroosmotic flow can be seen at 7.5 mins and the main Phorwite peaks can be seen at 12 mins, 16.3 mins and 24.2 mins. Peak shape has improved over the peak shape seen using the carbonate buffer. Further investigations could lead to a better separation.

8.6. CE of CD-3 developing agent and RA4 developer solution

The CE analysis of CD-3 developing agent and RA4 developer solution proved to be more difficult than originally anticipated. As CD-3 has different charges depending on pH (see Fig. 8.14) the pH at which the electropherogram is run is crucial. It is possible to separate CD-3 at low pH by protonating the primary amine and so CD-3 would migrate before the electroosmotic flow. However, the other major component in RA4 developer solutions is Phorwite and the sulphonic acid groups on this molecule are protonated at this low pH and so it will migrate with the electroosmotic flow. By raising the pH these sulphonic acid groups are deprotonated and Phorwite will separate after the electroomotic flow. But CD-3 exists as a zwitterion at

this higher pH and the overall charge on the molecule is neutral so it migrates with the electroosmotic flow. By raising the pH even further (to pH 10), it is possible to deprotonate both CD-3 and Phorwite so that both will separate as anionic species.

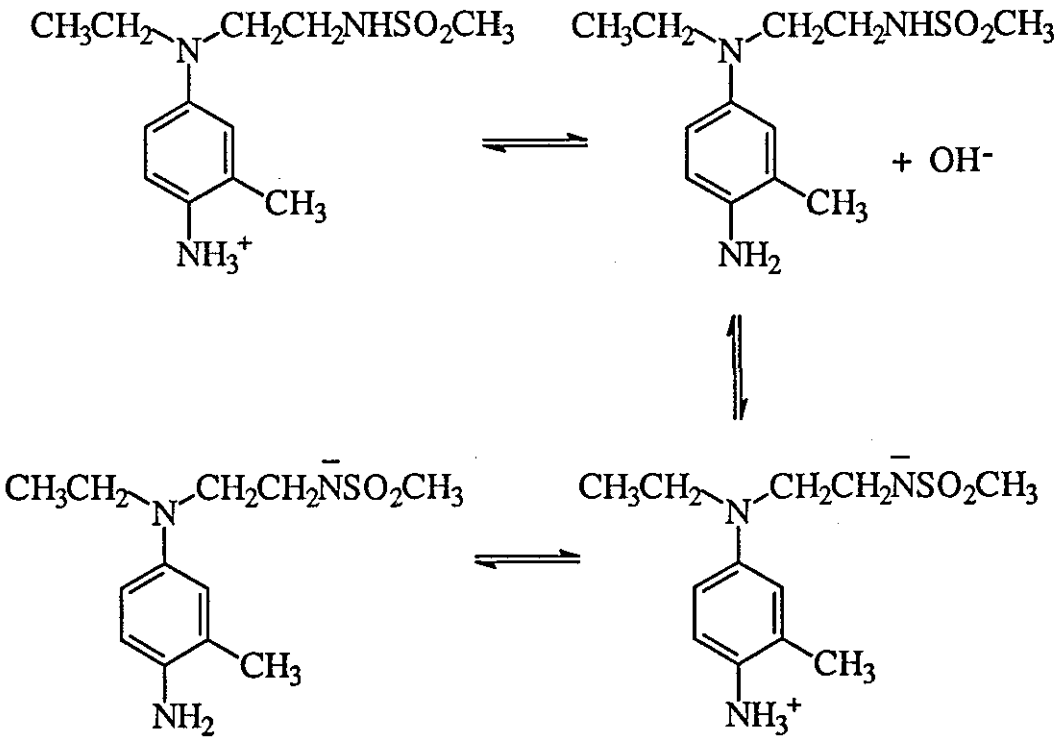


Fig. 8.14 Difference in ionic charge of CD-3 with pH.

8.6.1 Analysis of CD-3 and RA4 using carbonate buffer

A potassium carbonate buffer (pH 10, 0.057M) buffer was used for separations. The method was found to be reproducible and quantitative. A calibration graph for CD-3 is shown in Fig. 8.15. The graph had a good correlation coefficient ($r^2 = 0.9940$).

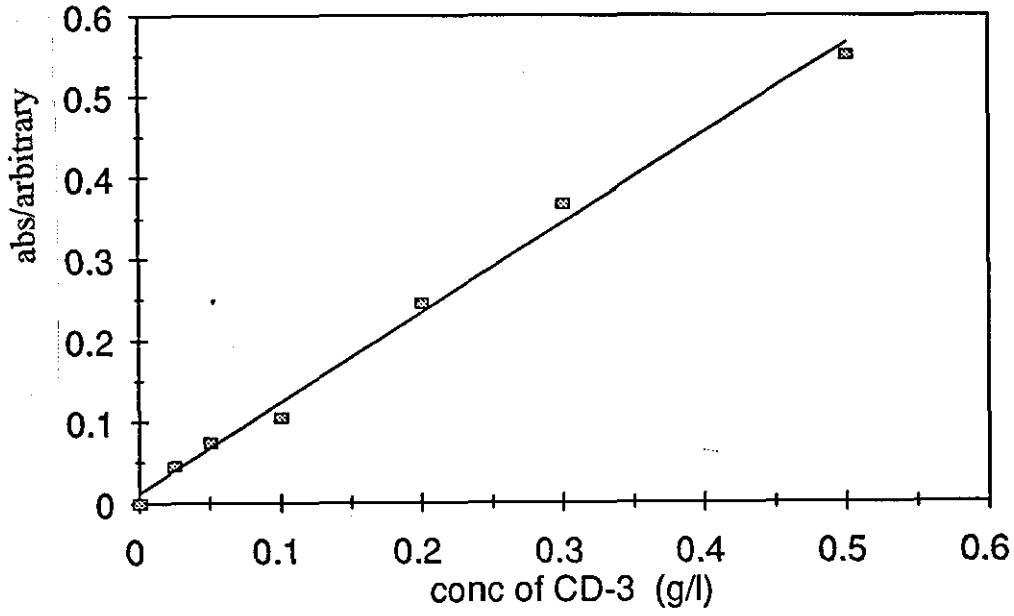


Fig. 8.15 CE calibration plot for CD-3 (pH 10 carbonate buffer, + 15 kV, detection at 254 nm)

A fresh solution of RA4 solution was separated under these conditions and is shown in Fig. 8.16.

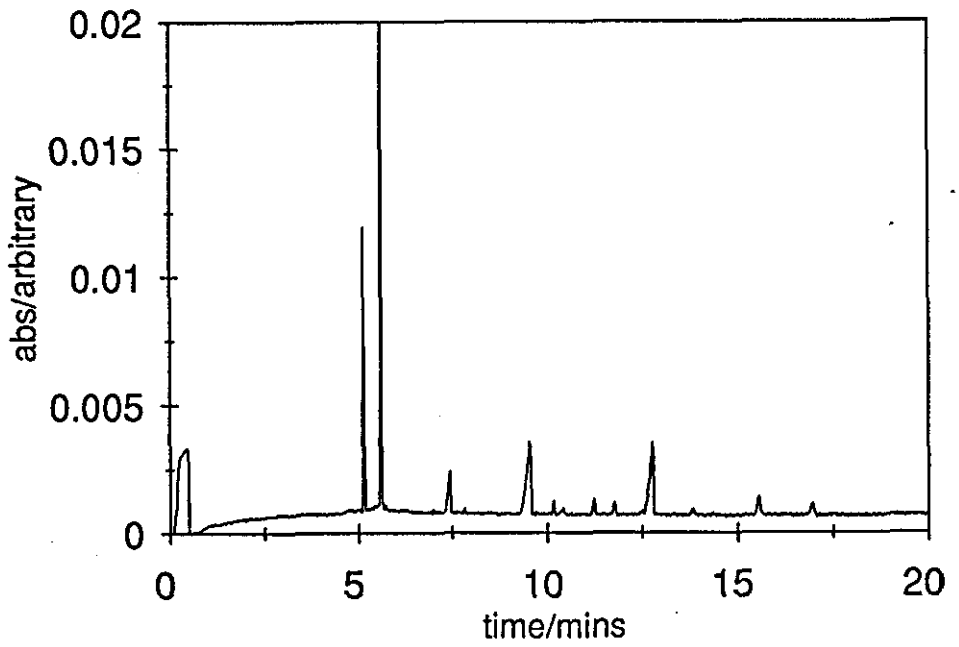


Fig. 8.16 Electropherogram of fresh RA4 solution.(pH 10 carbonate buffer, + 15 kV, detection at 254 nm)

The electroosmotic flow elutes with a migration time of 5.1 mins, followed by CD-3 at 5.6 mins. Then there are a series of peaks for Phorwite with the three main peaks at 7.5 mins, 9.6 mins and 12.8 mins. There are several smaller signals possibly due to Phorwite similar to those seen by HPLC.

An electropherogram of a degraded RA4 sample is shown in Fig. 8.17. The developing agent CD-3 has fully degraded and several minor signals can be seen. It is probable that CD-3 has degraded to non-polar degradation products (see Chapter 6) and these will elute with the electroosmotic flow. Phorwite did not appear to degrade but the signals have sharpened considerably compared with those in fresh RA4. It is possible that Phorwite had not equilibrated in the system in the case of the fresh RA4 sample. This could be due to a number of factors including heating effects and conductivity effects and buffering effects.

An electropherogram of an RA4 sample which had been allowed to degrade in the dark is shown in Fig. 8.18. The electropherogram is similar to that for degraded RA4 which had been stored in sunlight except that, again, Phorwite does not seem to have equilibrated in the system.

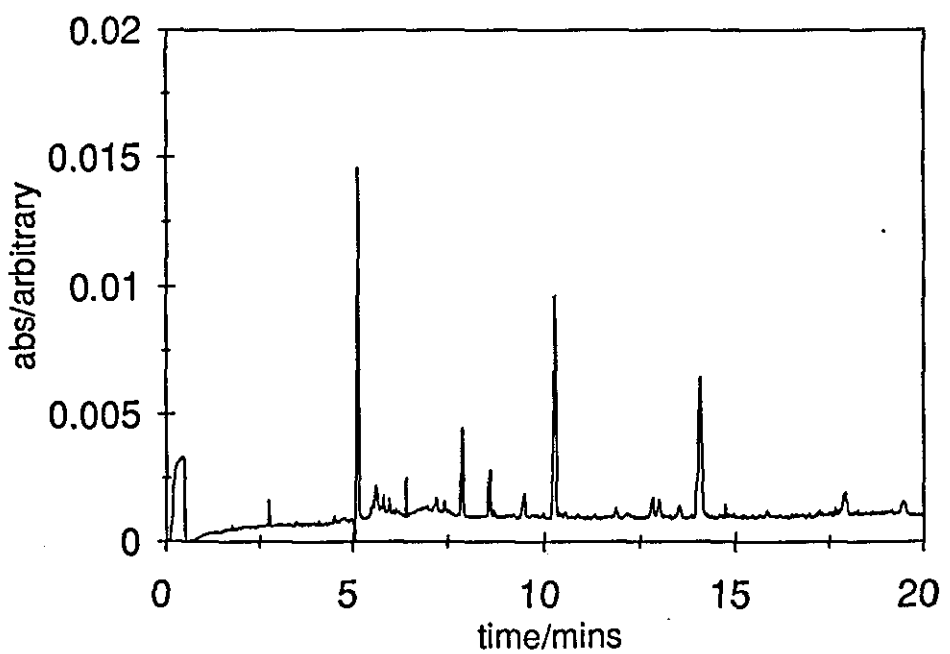


Fig. 8.17 Electropherogram of degraded RA4 developer solution(pH 10 carbonate buffer + 15 kV, detection at 254 nm)

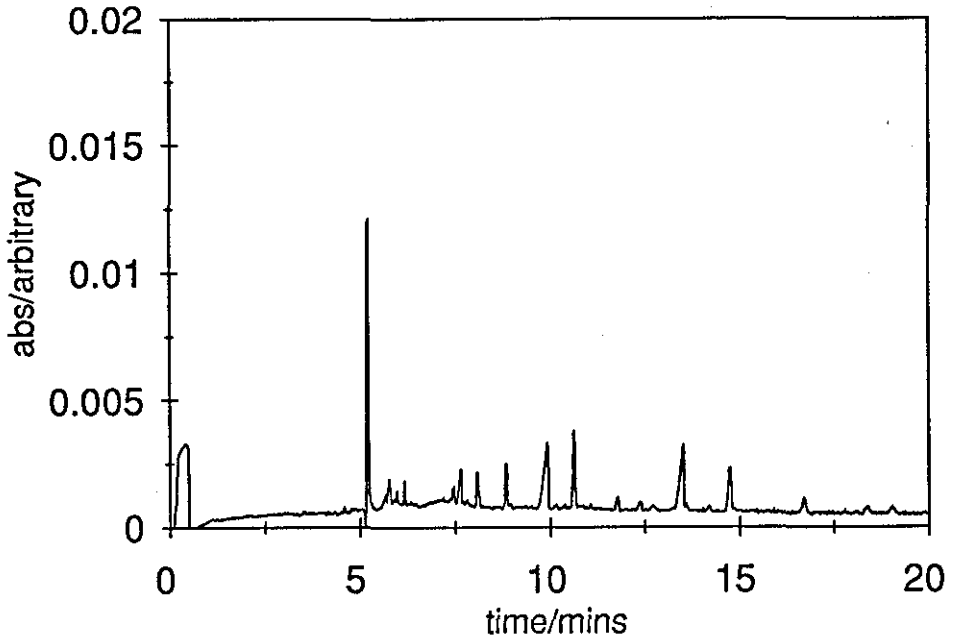


Fig. 8.18 Electropherogram of degraded RA4 developer solution which had been stored in the dark (pH 10 carbonate buffer, + 15 kV, detection at 254 nm).

8.6.2 Analysis of CD-3 and RA4 using MEKC

Although the carbonate buffer was found to be suitable for the separation of CD-3, problems were encountered in the separation of Phorwite. It was therefore preferable to investigate other buffer systems for the separation of Phorwite and CD-3 in the same sample. The MEKC buffer system used for the analysis of Phorwite was used again. A sodium dihydrogen orthophosphate buffer (pH 7.48, 0.04 M) with SDS (0.0057 M) and acetonitrile (90:10 v/v) was again used. An electropherogram of CD-3 is shown in Fig. 8.19. The electroosmotic flow can be seen at 7.5 mins. The developing agent CD-3 migrates after the electroosmotic flow at 8 mins.

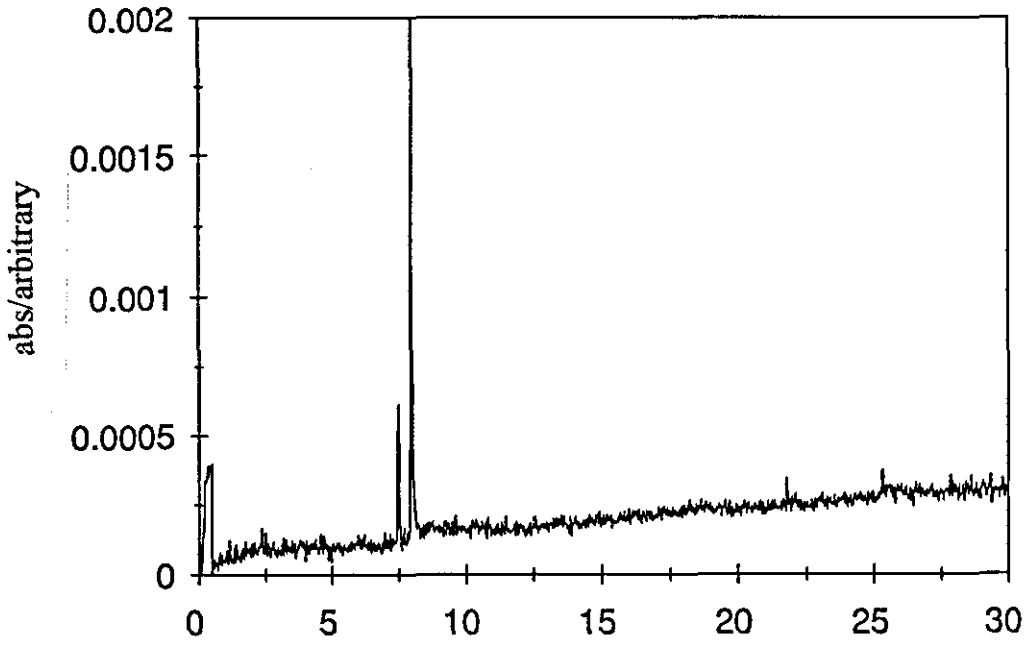


Fig. 8.19 Electropherogram of fresh CD-3 solution, using MEKC buffer (pH 7.48 phosphate buffer with SDS (0.0057 M) and acetonitrile (10 % v/v), +15 kV, detection at 254 nm)

The developing agent CD-3 is present as a zwitterion or neutral at pH 7.48 and forms a micelle with SDS. Phorwite also forms micelles with SDS and these micelles have longer migration times, which could be a size effect or that Phorwite has a greater overall negative charge. The separation of CD-3 under these conditions was found to be reproducible and quantitative. The buffer was applied to the analysis of RA4 developer solution and the electropherogram of fresh RA4 is shown in Fig. 8.20. The electroosmotic flow migrates at 7.5 mins with CD-3 migrating at 8 mins. Phorwite is present as a series of peaks at 12.5 mins, 17.9 mins and 29.1 mins.

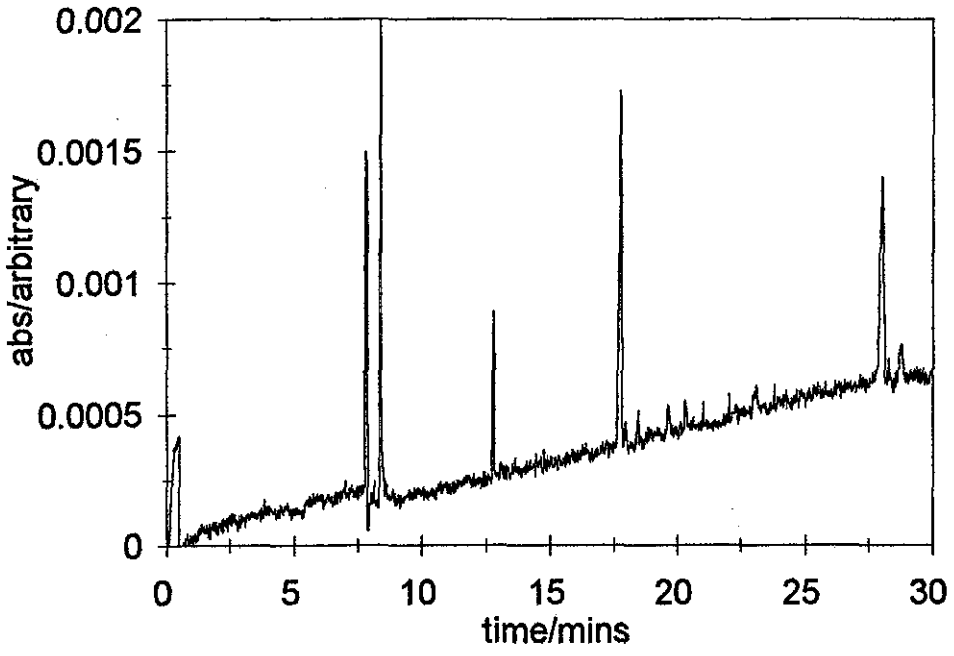


Fig. 8.20 Electropherogram of fresh RA4 developer solution using MEKC buffer (pH 7.48 phosphate buffer with SDS (0.0057 M) and acetonitrile (10 % v/v), +15 kV, detection at 254 nm)

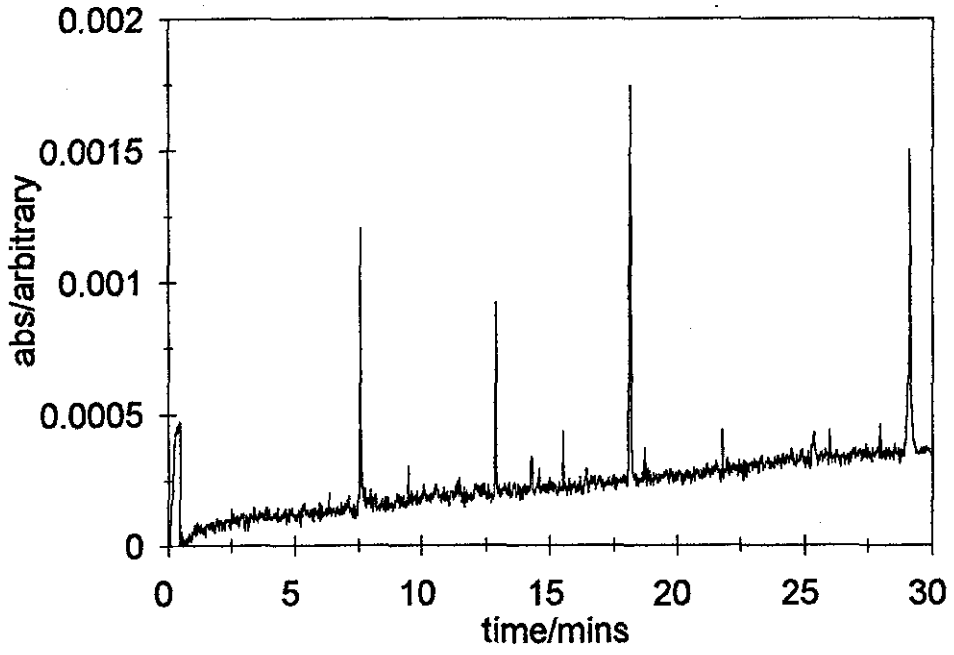


Fig. 8.21 Electropherogram of degraded RA4 developer solution, using MEKC buffer (pH 7.48 phosphate buffer with SDS (0.0057 M) and acetonitrile (10 % v/v), +15 kV, detection at 254 nm)

In the electropherogram of degraded RA4 (Fig. 8.21) the developing agent CD-3 has fully degraded and Phorwite is still present as a series of peaks with migration times of 12.6 mins, 18.0 and 29.2 mins. It is difficult to see any specific degradation products from CD-3.

8.7 CE of Fotospeed CKRA4 developer solution

As Fotospeed CKRA4 contains the same components as Kodak RA4 an identical carbonate buffer was used for analysis. But it would also be possible to use the MEKC buffer system used for RA4. Degradation had been monitored using HPLC and it was shown that the developing agent in CKRA4/D degraded to polar and non-polar products whereas the stain-reducing agent Phorwite did not appear to degrade. This degradation pattern was similar to that seen for Kodak RA4. An electropherogram of a degraded Fotospeed CKRA4 sample is shown in Fig. 8.22. The concentration levels of Phorwite are lower and the developing agent CD-3 has degraded. The main signals for Phorwite migrate at 10.7 mins and 15 mins. It is difficult to identify the other signal which is normally seen for Phorwite. Several other smaller signals are seen from 6 mins to 10 mins and are possibly due to degradation products from the developing agent CD-3.

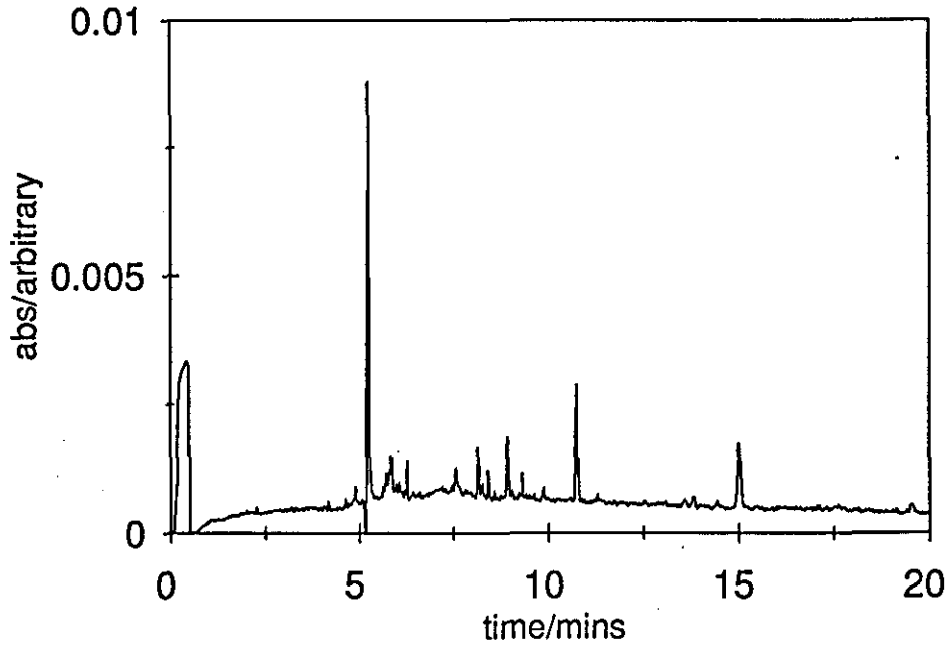


Fig. 8.22 Electropherogram of degraded Fotospeed CKRA4 developer solution (pH 10 carbonate buffer, + 15 kV, detection at 254 nm)

A degraded Fotospeed CKRA4 sample which had been stored in the dark is shown in Fig. 8.23. The developing agent CD-3 has degraded but the main Phorwite peaks are still identifiable at 10 mins and 13.9 mins. It is difficult to identify the other signal which is usually seen for Phorwite. Again numerous small peaks are present with migration times ranging from 5 mins to 10 mins. No individual peak is prominent.

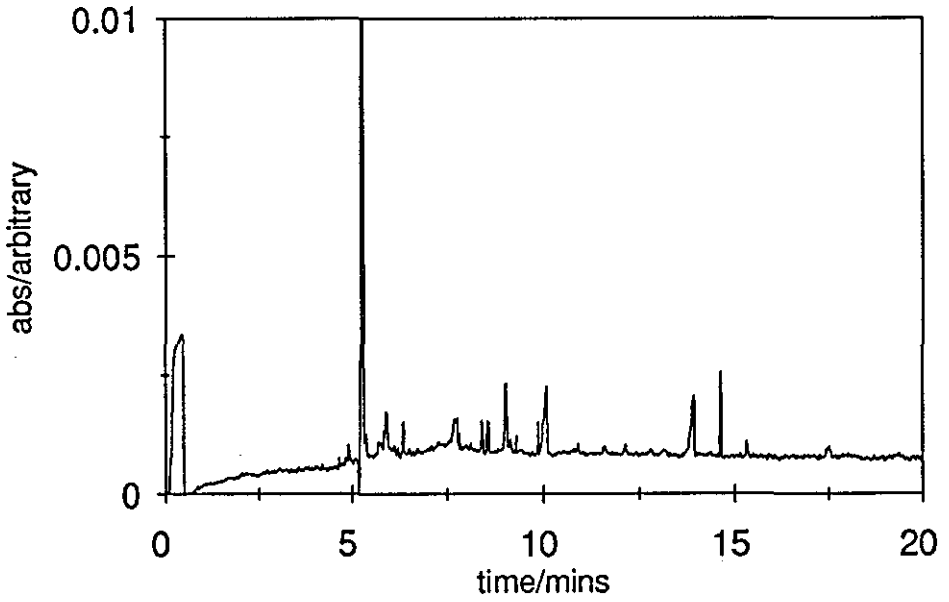


Fig. 8.23 Electropherogram of degraded Fotospeed CKRA4 developer solution which had been stored in the dark (pH 10 carbonate buffer, + 15 kV, detection at 254 nm)

8.8 Summary

Capillary electrophoresis was found to be a good technique for the analysis of CD-4 developing agent and C-41 developer solution as the developing agent and the developing agent sulphonates (the main degradation products in C-41 developer solutions) were charged polar species and separated before and after the electroosmotic flow. Peak shapes were good and separation times were relatively short. However the situation was different with CD-3 developing agent and RA4 developer solutions. It was necessary to deprotonate and separate CD-3, the developing agent and Phorwite, the other main component of RA4 developer solutions, at high pH. However, this did not give an ideal separation. Some investigations, using MEKC, were undertaken but time did not permit a more detailed examination. CE has a lot of potential for use in the analysis of photographic solutions and further investigations will have to be carried out.

Conclusion

The major components from the degradation of the colour developing agents CD-3 and CD-4 have been isolated and identified. Chromatographic and electrophoretic methods have been developed for the determination of individual components of RA4, RA100 and C-41 colour developer solutions. Some of the analytical techniques used in the project included HPLC, capillary electrophoresis, solid phase extraction, preparative TLC. Identification of degradation products was supported by MS, NMR, Raman spectroscopy, FT-IR spectroscopy, UV spectroscopy and fluorescence spectroscopy.

Liquid chromatography methods, using ion-pair reagent and diode array detection have been developed for the analysis of the major components of colour developer solutions. In the case of RA4 and RA100 colour developer solutions, the major components were CD-3 developing agent, Phorwite stain reducing agent and the polar and non-polar degradation products of the developing agent. In the case of C-41 colour developer solution, the major components were CD-4 developing agent, the sulphonates of the developing agent and, to a lesser degree, the non-polar degradation products of the developing agent. The methods developed for Kodak developer solutions were successfully applied to the analysis of used photographic developer solutions and similar developer solutions from competitors.

Capillary electrophoresis was successfully applied to the analysis of developer solutions and proved especially useful for the analysis of CD-4 developing agent and CD-4 sulphonates in fresh and degraded C-41 developer solutions. Electrophoresis was also applied to the analysis of RA4 developer solutions.

Degradation studies were carried out to examine the degradation of the developing agents and other components in developer solutions. The studies clearly show that the degradation of the solutions is zero order with respect to the developing agents and that other components such as Phorwite do not appear to degrade. Degradation studies involving additives such as sulphite successfully mimicked their effect in authentic developer solutions.

Isolation and identification of the most prominent non-polar degradation products from the precipitates of solutions containing CD-3 developing agent gave a valuable insight into its degradation. The yellow azo dimer was previously postulated [52] but the blue quinoneenamine and purple phenazine compounds had not been seen before. There was a

multitude of other peaks but they proved too unstable or too insignificant to isolate and identify.

Similar degradation studies were carried out on the precipitates from solutions containing CD-4 developing agent. Similar yellow azo dimer and blue quinoneimine compounds to those seen in CD-3 developing agent, were isolated from degraded solutions containing CD-4 developing agent. Again further compounds were isolated but were too unstable for identification.

Many of the components in used colour developer solutions have been identified. The principal degradation routes of the degradation products are the formation of sulphonates, which remain in solution, and of condensation products which precipitate as an insoluble tar. An understanding has been gained of the mechanisms for the degradations and thus of additional compounds that are probably present but were not isolated.

Future work could include more investigations of the methods developed and application of these methods to different types of developer solutions. More studies will have to be undertaken on the application of LC-MS to developer solutions. This area may prove invaluable for the investigation of other degradation products, particularly the polar degradation products. Capillary electrophoresis is an area with a lot of potential for the routine analysis of developer solutions. Further investigations into the analysis of CD-3 developing agent and RA4/RA100 developer solutions, using MEKC, will have to be undertaken. Reproducibility studies need to be performed before CE can be applied to the routine analysis of developer solutions.

It may be possible to isolate and identify further non-polar degradation products from the precipitates of developer solutions and to investigate the possiblity of identifying some of the polar degradation products. Some environmental work has been carried out but more will have to be done on the fate of developer solutions in the environment.

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Papers and presentations

1. T. P. Desmond and R M. Smith, "Chromatographic Investigation of Photographic Developer solutions", talk presented at the "Research Topics in Chromatography" meeting organised by the Chromatographic and Electrophoresis Group and the Western Region of the Analytical Division Royal Society of Chemistry, at Chepstow, 11th March 1993 (presented by P. Desmond).

2. T. P. Desmond, R. M. Smith and T. Bumfrey, "Investigation of photographic developer solutions", poster presented at 17th International Symposium on Column Liquid Chromatography, organised by the Fachgruppe Analytische Chemie, Gesellschaft Deutscher Chemie, in Hamburg 9th - 14th May 1993.

3. T. P. Desmond, R. M. Smith and T. Bumfrey, "Investigation of photographic processing solutions", poster presented at the Research and Development Topics in Analytical Chemistry meeting organised by the Analytical Division, Royal Society of Chemistry, Bradford, 13th-14th July.

4. T. P. Desmond, R.M. Smith and T. Bumfrey, "Investigation of the degradation of photographic processing solutions", poster presented at 20th International Symposium on Chromatography, organised by the Chromatography, Bournemouth, 20th - 24th June 1994.

5. T. P. Desmond, R. M. Smith and T. Bumfrey, "Investigation of the degradation of photographic processing solutions", poster presented at Research and Development Topics Meeting, organised by the Analytical Division, Royal Society of Chemistry at the University of Hertfordshire, 18th and 19th July, 1994.

6. T. P. Desmond, R. M. Smith and T. Bumfrey, "Investigations into the chemistry of colour photographic solutions", *Analytical Proceedings, 31 (1994) 69.*

Investigations into the Chemistry of Colour Photographic Developer Solutions

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Two non-polar degradation products of a colour developing agent have been isolated and identified by the use of thin-layer chromatography, nuclear magnetic resonance spectroscopy and mass spectrometry.

Photographic developer solutions contain very specific reducing agents, termed developing agents, which reduce silver ions to metallic silver. One of the most commonly used photographic colour developing agents is Kodak developing agent CD-3, a phenylenediamine based reducing agent (Fig. 1). In the developer solution, the developing agent is used in conjunction with antifoggants, halide salts, optical brightening agents and a number of other minor components, in a buffered solution of pH 10.5.

Although they have been used for many years, relatively little is known about the reaction products formed in developer solutions. Some earlier studies[1–8] examined potential routes for the reactions of developing agents on oxidation, but usually the products from the reactions were not isolated nor structures confirmed. The present project employed thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) and mass spectrometry for the isolation and identification of the non-polar water insoluble products formed by the spontaneous degradation of the Kodak developing agent CD-3 and developer solutions on exposure to air, which could lead to a better understanding of the reaction pathways of these developing agents.

Experimental

HPLC separations were carried out on a Hewlett-Packard HP1084B system with a Hewlett-Packard 1040A diode array detector, using a Supelco (150 × 4.6 mm) LC-18-DBS octadecylsilyl column, with a Supelco octadecylsilyl (10 × 4.6 mm) guard column. The components were separated using an ammonium acetate buffer (pH 4.8) and acetonitrile eluent. The concentration of acetonitrile was increased from 17% (v/v) to 60% (v/v) over the first 10 min, followed by an isocratic period of 15 min. The separation shown was detected at 243 nm.

TLC separations were carried out on 100 × 20 cm preparative TLC plates coated with a 2 mm thick layer of TLC grade silica, which were prepared in the laboratory. The degradation products were eluted using 90 + 10 (v/v) dichloromethane–acetonitrile solvent mixture. The bands were extracted from the silica by use of acetonitrile and evaporated to dryness under nitrogen. The extracts were repurified by using TLC.

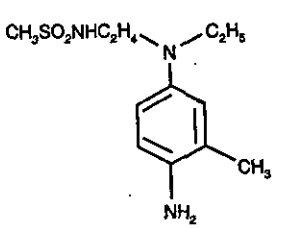


Fig. 1 Kodak colour developing agent CD-3

Results

A complex mixture of products was formed by the oxidation of an aqueous solution of Kodak developing agent CD-3 on exposure to air (Fig. 2). A very similar chromatogram was obtained for the oxidation products of a photographic developer solution, containing CD-3, suggesting that it had followed a similar reaction pathway. The mixture from the developing agent was fractionated by preparative TLC and two major non-polar products (yellow and blue) were isolated. These were analysed by using mass spectrometry and NMR spectroscopy. The electron impact mass spectrum of the

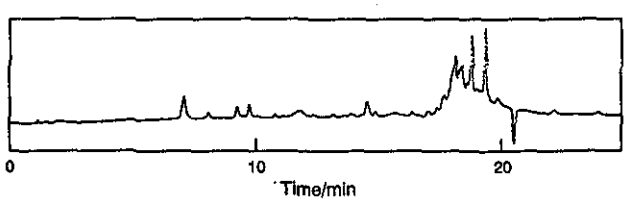


Fig. 2 HPLC chromatogram of a solution of degraded Kodak colour developing agent CD-3. Conditions as Experimental

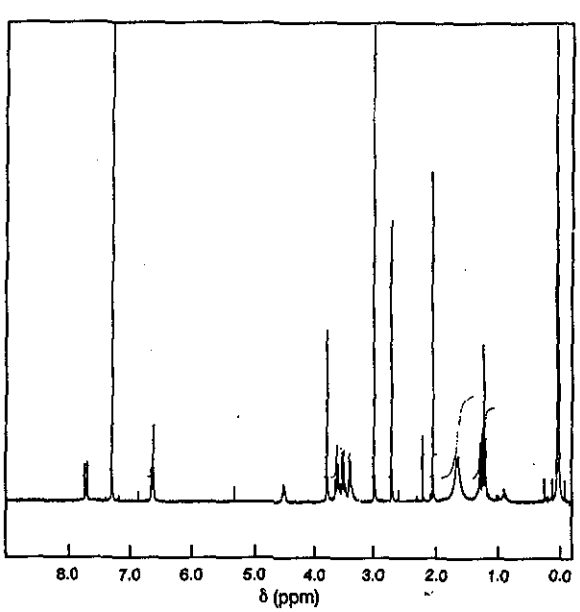


Fig. 3 ^{1}H-NMR spectrum of yellow degradation product run on a Brüker AC250 250 MHz NMR spectrometer

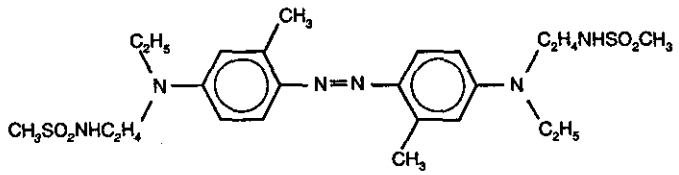


Fig. 4 Yellow degradation product

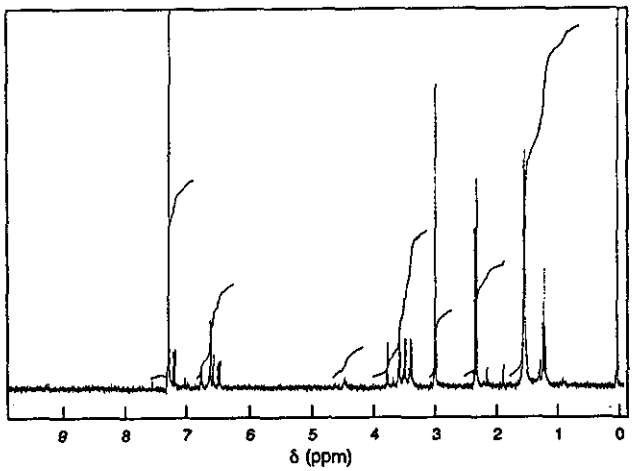


Fig. 5 ^{1}H-NMR spectrum of blue degradation product run on a Jeol GX-400 400 MHz NMR spectrometer

yellow product gave strong ions at *m/z* values of 430 and 163 and a molecular ion at *m/z* 538, which was assigned the formula $C_{24}H_{38}O_4N_6S_2$. The mass spectrum of a blue compound gave ions at *m/z* values of 269 and 240 and a molecular ion at *m/z* 377, which was assigned to $C_{18}H_{24}O_3N_3S$.

The ^{1}H-NMR spectrum of the yellow product (Fig. 3) was similar to the spectrum of the original developing agent. Both contained signals for the CH_3 of an ethyl group attached to a tertiary amine, the CH_3 of an SO_2CH_3 group and an aryl CH_3 group. However, the signals found for the primary amino-group protons of the developing agent were absent from the spectrum for the yellow product. A characteristic pattern of an ABX coupled system of aromatic protons appeared as three discrete signals in the spectrum of the developing agent but was present in the yellow product as two complex bands at 6.60 and 7.67 ppm with relative integrations of 2H and 1H. This compound was identified as the azo-derivative (Fig. 4), the formation of which by oxidative dimerization was predicted in the earlier studies.[1] It had a similar ultraviolet spectrum to that reported previously for compounds of this type.[1]

The ^{1}H-NMR spectrum of the blue compound was very different in appearance (Fig. 5). A triplet at 1.10 ppm integrated as a methyl group, which was assigned to the CH_3 of an ethyl group. The singlets at 2.30 and 2.35 ppm were identified as methyl groups attached to an aromatic ring and to a conjugated system, which had been de-shielded by an electron withdrawing group, such as a carbonyl. The sharp singlet at 2.95 ppm integrated as a CH_3 group and was assigned to the SO_2CH_3 system because of its chemical shift. The signals in the 3.00–3.60 ppm region appeared to be a triplet of quartets and integrated to 6H. These signals

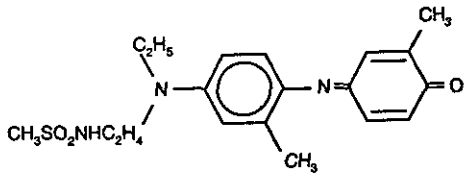


Fig. 6 Blue degradation product

probably originated from the three N-CH_2 groups and similar signals were present in the spectrum of the developing agent. There was a broad signal at 4.45 ppm, which integrated to an amido NH group. The signals from 6.60 to 7.15 ppm, which integrated to 6H, were more complex than those of the yellow compound. Based on these results this compound has been assigned the structure shown in Fig. 6. This compound can be formed by condensation between a quinone imine, formed by hydrolysis of the substituted side chain of the developing agent, and a second developing agent (CD-3) molecule. This route had been proposed previously as a degradation mechanism for related phenylenediamines.[2,3]

Conclusion

Two of the major non-polar degradation products of CD-3 photographic developing solution have been isolated and identified. Work is continuing on identification of the other degradation products.

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