DECLARATION OF CANDIDATE

I, Ayesha Hansa, declare that unless indicated, this dissertation is my own work and that it has not been submitted for a degree at another Technikon or Institution.

A Hansa

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ABSTRACT

An environmental problem facing the textile industry is the coloured effluent from the dyeing of cellulosic fibres with reactive dyes. Reactive dye loss during dyeing operations is about 10 to 40 %, indicating the need to learn more about the fate of these dyes. Increasing environmental regulations are driving technical innovation to manage this problem. Good analytical techniques for the separation and detection of reactive dyes and their derivatives are necessary for monitoring dye-house effluent, as well as in the optimisation of dye synthesis, purification, formulation and application.

This project concerns the development of analytical techniques for the analysis of reactive dyes and their hydrolysed derivatives in dye-bath effluent. The dyes selected for the study were: C I Reactive Green 19, C I Reactive Blue 171, C I Reactive Yellow 84, C I Reactive Red 120 and C I Reactive Red 141. Two analytical systems, high performance liquid chromatography (HPLC) and capillary electrophoresis (CE), were chosen for the study. The HPLC technique used was reversed phase chromatography with an ion pairing reagent. The CE techniques used were capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC).

The HPLC separation of the 5 selected dyes showed limited separation efficiency with C I Reactive Blue 171 and C I Reactive Green 19 co-eluting. The most commonly used technique in CE is capillary zone electrophoresis (CZE). CZE provided superior separation efficiency than HPLC with all 5 selected dyes being well separated.

Analysing the residual dye-bath liquors by HPLC resulted in the dye-bath samples being non-retained on the HPLC column, implying that dye hydrolysis or dye degradation was occurring much earlier than expected. Upon further investigations, the non-retention of the dye-bath samples was found to be related to the high salt content of the dye-bath. An investigation into the effect of salt on dye retention showed an inverse relation between the dye peak area versus the salt content, i.e. a decrease in the dye peak area with an increase in the salt content. Since ionic dyes are known

THE DEVELOPMENT OF TECHNIQUES FOR THE ANALYSIS OF REACTIVE DYES IN TEXTILE DYEING WASTEWATER

by

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to aggregate in the presence of salts, it was proposed that the non-retention of the dye-bath samples was due to dye aggregation in the presence of salts.

Using dialysis to remove the salt, separation of the hydrolysis products from the starting dye was investigated using the HPLC and CZE methods developed previously. However, for C I Reactive Red 120 and C I Reactive Red 141, CZE was unable to resolve the hydrolysis products from the parent dye. Hence an alternate CE technique, micellar electrokinetic chromatography (MEKC) was investigated, which afforded the necessary separation.

In analysing the residual dye-bath and residual rinse liquors by HPLC, significant levels of the parent dye were found in the residual dye-bath liquors. This indicated that incomplete dye hydrolysis occurred during the application of the reactive dyes. This is of concern in terms of its toxicity, and points strongly to more effort required in monitoring these dyes in aquatic systems. The HPLC and MEKC methods presented herein show potential for studying these pollutants.

One of the disadvantages of the CE techniques used was the long analysis time of 25 to 35 min compared to 12 min by HPLC. Methods to reduce analysis time by adding an ion-pairing reagent to the MEKC buffer were investigated. The increase in the ion-pairing reagent concentration reduced analysis time significantly and also improved resolution.

The main problem in the analysis of polar reactive dyes with high water solubility is the extraction step from aqueous solution. Thus, the final stage was to develop techniques for the extraction and concentration of the dyes from water systems. A liquid-liquid extraction method with an ion pairing reagent was investigated for the extraction of C I Reactive Red 141 in tap and river water.

Recoveries from tap water were approximately 100 %. However, poor and inconsistent recoveries were achieved from river water. These inconsistencies were attributed to interferences from other organic compounds co-extracted from the river water that interfered with the analytical procedure. Solid phase extraction was tested for the elimination of the interference. Preliminary investigations using this technique with an ion pairing reagent resulted in successful removal of the interferent and in achieving recoveries close to 100 %.

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GLOSSARY

Acid dye	An anionic dye characterised dye by substantivity for protein fibres and often applied from an acid bath
Aerobic	The condition of living or acting only in the presence of molecular oxygen.
Anionic dye	A dye that dissociates in aqueous solution to give a negatively charged coloured ion.
Anthraquinone dyes	Dyes based on the structure of 9,10-anthraquinone, with powerful electron donor groups in one or more of the four alpha positions.
Auxiliary	A chemical or formulated product which enables a processing operation in preparation, dyeing, printing or finishing to be carried out more effectively or which is essential if a given effect is to be obtained.
Auxochrome	A substituent group in a chromogen that influences its colour.
Azo dyes	Dyes which contain a least one azo group (-N=N-), and can contain up to four azo bonds.
Azoic dyeing	The production of insoluble azo compound on a substrate by interaction of a diazotised amine (azoic diazo component) and a coupling component (azoic coupling component).
Basic dye	A cationic dye characterised by its substantivity for the acidic types of acrylic fibre and for tannin mordanted cotton.
Batchwise processing	Processing of materials as lots or batches in which the whole of each batch is subjected to one stage of the process at a time.
Bleaching	The procedure, other than by scouring only, of improving the whiteness of textile material by decolourising it from the grey state, with or without the removal of natural colouring and or

extraneous substances.

Bioelimination — The mechanism for removal of soluble dyes by an aerobic biological treatment may be straightforward biodegradation, but is mainly by adsorption on to the biomass and destruction in subsequent sludge processing. The term *bioeleimination* may be used to describe collectively these different mechanisms.

Carcinogenic — Cancer-causing.

Cationic dye — A dye that dissociates in aqueous solution to give a positively charged coloured ion (see also basic dye).

Chrome dye — A mordant dye capable of forming a chelate complex with a chromium ion.

Chromogen — A chemical compound that is either coloured or can be made coloured by the attachment of suitable substituents. The *chromophore* and the *auxochromes(s)* are part of the chromogen.

Chromophore — A chemical group which when present in a compound (the *chromogen*) is responsible for the appearance of colour. *Colourants* are sometimes classified on the basis of their chief chromophore, e.g. azo dyes contain the chromophore -N=N-.

Colour Fast — Said of colours which are not affected by the normal conditions of use (i.e. light, heat and chemical action) to which they are subjected.

Colour Index — An authoritative, descriptive catalogue of natural and synthetic *colourants* and intermediates in terms of generic name, and constitution where disclosed.

Colour yield — The depth of colour obtained when a standard mass of *colourant* is applied to a substrate under specified conditions.

Colourant — A colouring matter, a dye or pigment.

Detergent — A substance normally having surface-active properties specifically intended to cleanse a substrate.

Direct dye — An *anionic dye* having *substantivity* for cellulosic fibres, normally applied from an aqueous dye-bath containing an electrolyte.

Disperse dye — A water-insoluble dye having *substantivity* for one or more hydrophobic fibres, e.g. cellulose acetate, and usually applied from fine aqueous dispersion.

Dyeing auxiliaries — Chemicals used in the dyeing process to aid the dyeing of the cloth / yarn.

Exhaust dyeing — A batchwise dyeing process in which the dye-bath is discarded on completion as opposed to the use of a standing bath.

Exhaustion — The proportion of dye or other substance taken up by a substrate at any stage of a process to the amount of originally available.

Fixation — The dyestuff that is chemically bound to the fibre.

Levelling — *Migration* of dye leading to a more uniform colouration of a substrate.

Liquor : fibre ratio — The ratio of mass of liquor employed in any treatment to the mass of material treated. *Note* : 'Short' and 'long' are often used to describe low and high liquor : goods ratios, respectively.

Metal-complex dye — A dye having a coordinated metal ion in its molecule.

Migration — The movement of a dye or pigment from one part of material to another.

Mordant — A substance, usually a metallic compound, applied to a substrate to form a complex with a dye which is retained by the substrate more firmly than the dye itself.

Mutagens — Certain chemical or physical agents that cause mutations to

occur.

Pigment — A substance in particular form which is substantially insoluble in a medium, but which can be mechanically dispersed in this medium to modify its *colour* and / or light-scattering properties.

Range — A group of dyes that have a common structural characteristic.

Rate of dyeing — The rate at which a dye is absorbed by a substrate under specified conditions. *Note* : It may be expressed quantitatively in several ways, such as the mass of dye absorbed in unit time, or the time taken for the substrate to absorb a given fraction of the amount of dye which it will absorb at equilibrium.

Reactive dye — A dye that, under suitable conditions, is capable of reacting chemically with a substrate to form a covalent dye-substrate bond.

Scouring — Freeing textile materials or wool sheepskins from natural or other non-fibrous constituents by treatment with aqueous solutions or organic solvents.

Sizing agents (size) — Gelatinous film-forming substances that are applied to the individual yarns during weaving in order to coat and protect the yarns from the abrasive effects of the filling yarns, as these are positioned by shuttle action of the weaving loom.

Substantivity — The attraction between a substrate and a dye or other substance under the precise conditions of test whereby the latter is selectively extracted from the application medium by the substrate.

Surfactant — An agent, soluble or dispersible in a liquid, which reduces the surface tension of the liquid.

Textile finishing — A collection of processes in which raw cloth / yarn is cleaned and prepared for dyeing and printing.

Vat dye	A water-insoluble dye, usually containing keto groups, which is normally applied to the fibre from an alkaline aqueous solution of the reduced enol (leuco) form, and which is subsequently oxidised in the fibre to the insoluble form.
Xenobiotic	A compound not found in nature.

LIST OF ABBREVIATIONS

ADMI	American Dye Manufacturers Institute
CE	Capillary electrophoresis
CI	Colour Index
CID	Collision induced decomposition
CMC	Critical micelle concentration
COD	Chemical oxygen demand
CTAB	Cetyltrimethylammonium bromide
CZE	Capillary zone electrophoresis
DOC	Dissolved organic carbon
EOF	Electroosmotic flow
ESI	Electrospray ionisation
ETAD	Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry
GC	Gas chromatography
HPLC	High performance liquid chromatography
HV	High voltage
i.d.	Internal diameter
LC	Liquid chromatography
LLE	Liquid-liquid extraction
LSIMS	Liquid secondary ion mass spectrometry
MEKC	Micellar elctrokinetic chromatography
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
ODS	Octadecyl silane
RSD	Relative standard deviation
SDS	Sodium dodecyl sulphate
SPE	Solid phase extraction
TBAB	Tetrabutylammonium bromide
TOC	Total organic carbon
TSP	Thermospray
UV	Ultraviolet
UV-VIS	Ultraviolet-visible
USEPA	United States Environmental Protection Agency

Chapter 1

INTRODUCTION

Large quantities of dyes are produced and used in diverse applications, including textiles, paint pigments, printing inks and food colouring. The largest consumer of these dyes is the textile industry accounting for two thirds of the dyestuff market. It is estimated that approximately 15 % of dyes produced are lost in the synthesis and processing of dyes. The main source of this loss, 10 to 20 %, is to be found in residual liquors, because of incomplete exhaustion of dyes (Reife, 1993).

Dye containing waste became a source of concern when some azo dyes were implicated as being carcinogenic. In addition, azo dyes can be reduced in the environment and *in vivo* to produce aromatic amines that may be carcinogenic (Weber, 1988). Therefore, the presence of azo dyes in wastewater is of considerable interest because of the potential for contamination of ground water and drinking water supplies by compounds that may produce a health risk.

Reactive dyes are typically azo-based chromophores combined with different types of reactive groups. They differ from all other classes of dyes in that they bind to the textile fibres such as cotton to form covalent bonds. But not all the dye reacts with the cellulose, the reaction with water to form the hydrolysed dye is a competing reaction (Richardson et al., 1993). Hence, this fact is important when conducting environmental analysis. However, it is possible that a portion of the original dye may escape the dye-bath and enter the textile waste stream.

Presently, fixation rates vary from 60 to 90 %, leaving significant levels of unfixed dyes in the textile wastewater (Camp and Sturrock, 1990). Unfortunately, most commonly used wastewater treatment procedures do not remove these dyestuffs sufficiently. Thus, the dyes pass directly through the treatment plant into the receiving river.

This results in aesthetic problems which initiates public concern. Moreover, it restricts the downstream application of this water. The latter is extremely serious in water restricted countries such as South Africa, which rely on the extensive recycling of water to meet the ever increasing demand for water by agricultural, industrial and domestic sectors.

It is, therefore, necessary to identify which components are present before a successful removal procedure can be developed. In addition, an analysis system for dyes is important for the evaluation of the efficiency of dye removal by conventional and proposed treatment systems.

Furthermore, the possible long term health effects of a few dyes and dye degradation products are becoming of increasing concern. Analysis of specific dyes may be important in identifying and quantifying possible health risks.

Published analytical methods for dyes are frequently based on ultraviolet-visible spectra of these compounds (Venkataraman, 1977). Liquid chromatography with either ultraviolet-visible [(Tincher and Robertson, 1982); (Bailey, 1985); (Shaul et al., 1991)] or mass spectrometric [(Voyksner, 1985); (Henion at al., 1989), (Borgerding and Hites, 1994); (Camp and Sturrock, 1990);(Bruins, 1994); (Slater et al., 1995)] detection has been used for the analysis of these dyes. Only few of these studies have measured dyes in environmental samples [(Tincher and Robertson, 1982); (Borgerding and Hites, 1994)], with only one study on reactive dyes and their hydrolysed derivatives (Camp and Sturrock, 1990).

The aim of this study is to develop an analysis system that will aid in determining whether appreciable amounts of reactive dyes and their derivatives enter the textile waste stream and subsequent quantitative determination in river waters in order to determine efficiency of dye removal by treatment processes.

1.1 PROJECT APPROACH

The project was divided into a number of phases:

i) Select a range of dyes, which are known to be problematic with regard to treatment, to target for the study.

ii) Review the literature to establish the most appropriate analytical techniques to use for the study.

iii) Develop these techniques for the analysis of the selected dyes and their hydrolysed derivatives in dye-bath effluent.

The latter phase was further divided as follows:

a) Develop the analytical techniques for the separation of the selected dyes.

b) Apply these techniques for the separation of the parent dye and their hydrolysed derivatives. Modify or develop new techniques where appropriate and apply these techniques to analysing residual dye-bath liquors.

c) Evaluate an extraction method for these dyes in water systems.

1.2 THESIS ORGANISATION

The thesis begins with a review of the history of reactive dyes, their structure and chemistry, which is presented in **Chapter 2**. Due to the lack of literature on reactive dyes in residual dye-baths and in the environment, **Chapter 3** focusses on the analysis of dyes in general with emphasis on sulphonated azo dyes. The rationale behind this was that reactive dyes rely heavily on the azo chromophore and are generally sulphonated in order to confer the necessary water solubility, factors which are discussed in **Chapter 2**. **Chapter 4** begins with the criteria for selecting the dyes, followed by the structures of these dyes where disclosed, and then the choice of analytical techniques chosen for the investigation. **Chapter 5** presents the development of the analytical techniques chosen for the analysis of the 5 selected reactive dyes. The chapter is concluded with a comparison of the two techniques and their applicability to dye analysis. **Chapter 6** deals with the development of a suitable methodology for the analysis of reactive dyes and their hydrolysed derivatives in residual dye-bath liquors. **Chapter 7** presents the results of the extraction of one reactive dye from tap water and river water by liquid-liquid extraction and its extraction from river water by solid phase extraction. The thesis is concluded with a summary of the experimental work presented and includes recommendations for future research in **Chapter 8**.

Chapter 2

REACTIVE DYES

This chapter discusses the development of reactive dyes, their structure and chemistry.

The objective of dyestuff chemistry is to produce dyes which will impart a permanent colouration to a textile material, and an important criterion is that the colour should not fade or discolour on laundering. While by the 1950's satisfactory solutions to these problems had been provided for synthetic fibres, e.g. polyamide and polyacrylonitrile, and for fibres of animal origin, e.g. wool and silk, the dyeing of cellulosic fibres still posed significant problems (Zollinger,1987).

The choice lay between forming an insoluble dye on the fibre and by relying purely on the insolubility of the dye produced, aided by aggregation of the dye particles, or by building a large water soluble dye, linear in shape and carrying a multiplicity of sites capable of hydrogen bonding with the cellulosic fibre. The first of these two choices, used in the cases of azoic and vat dyes, solved the fastness problem but entailed the use of dyeing methods which were complicated. The second, on which the direct dyes rely, gave only a poor level of fastness but presented a very simple application method (Rys and Zollinger, 1989). Thus, neither method was entirely satisfactory. It had long been perceived that an attractive approach to this problem would lie in constructing a dye which would react chemically with the fibre, thus firmly attaching the dye to the fibre by a covalent bond (Stead, 1990).

The requirement was for a dye that could easily be applied to the cellulosic fibre from aqueous solution under mild conditions. The solution to this problem arose from observations made during investigations into the properties of dyes carrying a dichlorotriazinylamino group, and it was upon this group that the first range of reactive dyes for cellulose were developed (Rattee and Breuer, 1974).

2.1 HISTORY OF REACTIVE DYES

Reactive dyes are dyes which have groups capable of forming covalent bonds between a carbon or phosphorus atom of the dye ion or molecule and an oxygen, nitrogen or sulphur atom of a hydroxy, an amino or a mecapto group, respectively, of the textile substrate (Rys and Zollinger, 1989).

The first three dichlorotriazinyl dyes were marketed in April 1956, by ICI under the trade name Procion. An example of the first group of reactive dyes is Procion Orange MX-G (C I Reactive Orange 1), shown in Fig 2.1and the inset shows the general structure of this range of dyes.

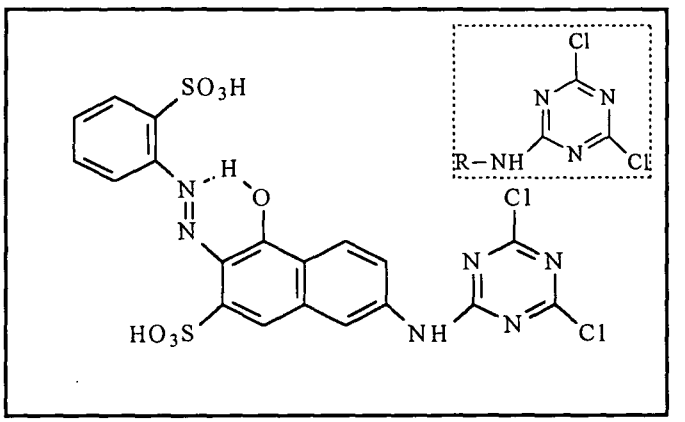

Figure 2.1 : Structure of C I Reactive Orange 1, see text for inset.

Following the introduction of these dyes, at least one new reactive dye range (i.e. an introduction of a new reactive group on the dye) was introduced commercially every year (except 1969) until 1971, however, between 1972 and 1988, only five new reactive ranges were introduced (Rys and Zollinger,1989).

During 1957, mainly due to a recognition of the short storage life of textile pastes made from dichlorotriazinyl dyes, further research led to the introduction of the more stable, hence, less reactive monochlorotriazinyl derivatives. This requires employing a dyeing temperature of 85 °C compared to 40°C and in general a slightly higher pH value in order to react with cellulose. Ciba and ICI introduced these dyes jointly as the Cibacron and Procion H (H for *Hot*) dyes, respectively (Zollinger, 1987).

Since that time the chlorotriazinyl dyes have accounted for more than half of the world consumption of reactive dyes for cellulosic fibres and have generally been regarded as the most important group of cellulosic reactive dyes (Stead, 1990).

An entirely different type of reactive system was introduced in 1958 by Hoechst under the trade name Remazol. These dyes contain a β-sulphatoethylsulphonyl group shown in Fig 2.2 (Venkataraman, 1977).

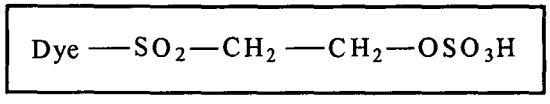

Figure 2.2 : Structure of β-Sulphatoethylsulphonyl group.

These dyes display a reactivity between the mono- and dichlorotriazines, requiring an application temperature of 70°C. The β-sulphatoethylsulphonyl group does not provide the same facility of hydrogen bonding with cellulose as does the halogenoheterocyclic group, thus physical adsorption is considerably lower. In the printing processes, however, they present an acceptable level of reactivity, and the weak physical attraction for the fibre enables the loose dye to be washed out easily.

Dyes containing two reactive groups, to achieve higher fixation values, were introduced soon after with Remazol Black B (C I Reactive Black 5) shown in Fig 2.3, being one of the first bi-functional commercial dyes to be introduced (Stead, 1990).

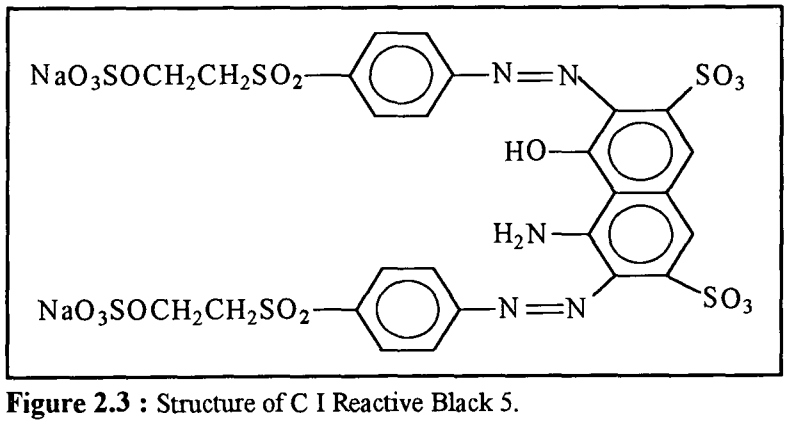

Figure 2.3 : Structure of C I Reactive Black 5.

Later, ICI developed the Procion Supra dye range with two monochlortriazine groups per molecule. The replacement of the chlorine atom by the more reactive fluorine atom into the reactive system lead to the introduction of the Cibacron F (CGY) range shown in Fig 2.4 (a), which embodies the monofluorotriazine reactive system. In the Levafix E-A (BAY) and Drimarene K (S) dye ranges the reactive group is a mixture of the isomers of 5-chlorodifluoropyrimidinyl shown in Fig 2.4 (b) and (c).

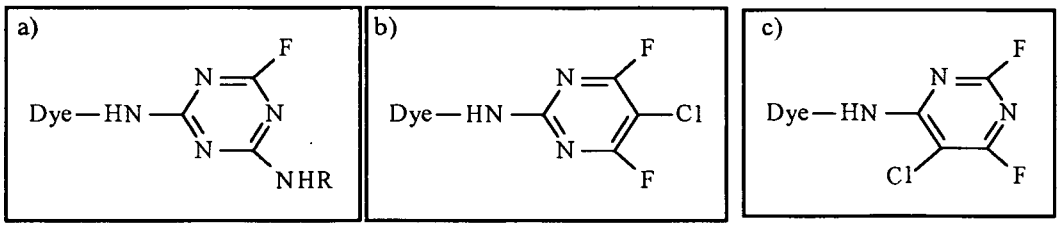

Figure 2.4 : Structures of (a) the monofluorotriazine reactive system used in the Cibacron F (CGY) range, and (b &c) the isomers of 5-chlorodifluoropyrimidinyl reactive group used in the Levafix E-A and Drimarene K ranges.

The first fibre reactive range to consist entirely of heterobifunctional, i.e. two different reactive groups, dyes was introduced by Sumitomo in 1980 under the trade name Sumifix Supra (NKS). Since then the number of heterobifunctional dyes used in reactive dyeing has increased.

All the reactive dyes discussed above have been designed to react with cellulose under alkaline conditions. The ready ionisation of cellulose together with its stability to alkaline treatments makes this choice of pH an ideal situation. Application processes call for very different levels of reactivity. At one extreme, printers, for economic reasons, look for print paste stability of up to 4 weeks and this is provided by, e.g, monochlorotriazine dyes. On the other hand, pad-batch dyeing processes call for reaction with the fibre at ambient temperature and much greater reactivity is needed to ensure adequate dye-fibre reaction within acceptable dyeing times. This is provided by Remazol and Procion MX dyes (Rys and Zollinger,1989).

However, in dyeing polyester-cotton blends it is necessary to colour the polyester with a water insoluble disperse dye. An alkali medium is not suitable for disperse dye uptake, thus, making concurrent dyeing of cotton with the above dyes difficult. To meet this need, reactive dyes which can fix to cellulose under acidic conditions have been developed. These dyes, marketed in 1978 under the trade name Procion T dyes shown in Fig 2.5 (a), rely upon a phosphoric acid group for their

effect (Duff and Sinclair, 1990). The most recent approach to the problem stems from the finding that quaternary derivatives of the monochlorotriazines and nicotinic acid will react with cellulose at high temperatures under neutral conditions. Dyes of the type shown in Fig 2.5 (b), contain two such groups, and form the basis of the Kayacelon React range marketed by Nippon Kayaku for application to the fibre blend in conjunction with a disperse dye without any addition of alkali (Stead, 1990).

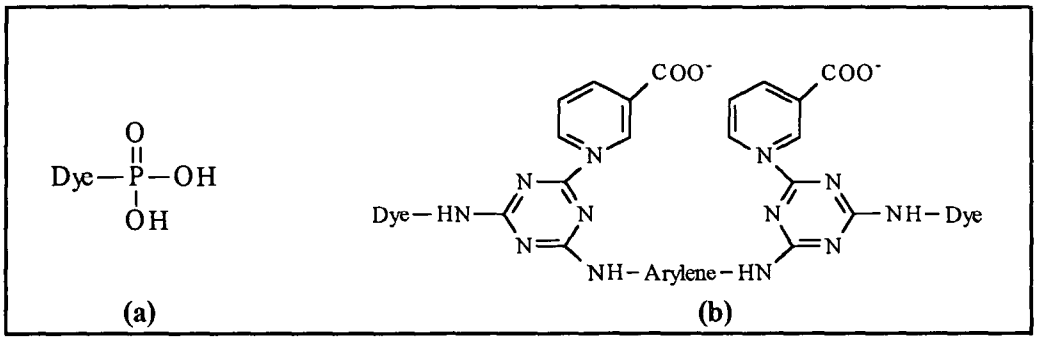

Figure 2.5 : Structures of (a) Procion T dye range and (b) Kayacelon React dye range.

2.2 THE STRUCTURE OF REACTIVE DYES

The characteristic structural features of a reactive dye are shown below in Fig 2.6. In some cases the reactive group is attached directly, i.e. without a bridge, to the dye molecule.

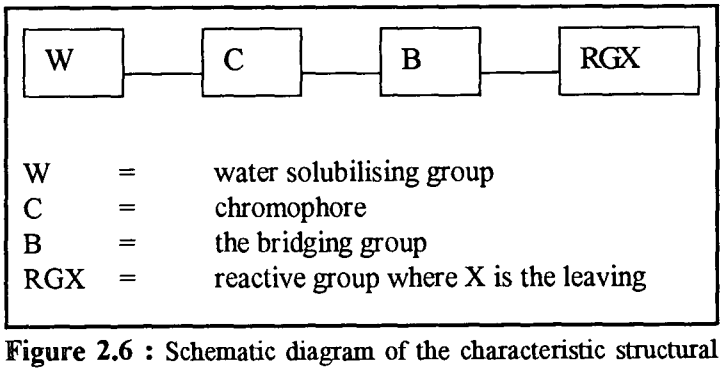

Figure 2.6 : Schematic diagram of the characteristic structural features of reactive dyes.

2.2.1 The Reactive Group

Although, there are a number of reactive groups that are marketed, two groups dominate the market viz. (a) reactive systems based on cyanuric chloride and (b) vinyl sulphone and vinyl sulphonamide dyes.

2.2.1.1 Reactive Systems Based on Cyanuric Chloride

This group is quite stable at near neutral pH values but will slowly hydrolyse under acid conditions. Such hydrolysis liberates hydrogen chloride and is, therefore autocatalytic. To guard against this, a suitable buffer, usually an equimolar mixture of mono- and dihydrogen phosphates, is added to the dye solution before isolation. In manufacture an inorganic diluent, usually sodium chloride, is then added to adjust the dye content to a standard value, followed by an agent such as dodecylbenzene to give cleaner handling properties by preventing dustiness (Stead, 1990). Thus, the commercial dye powder contains in addition to the dye, sodium chloride, phosphate buffer and the dedusting agent. Since for economic reasons, no purification stage is included, the commercial dye will also contain minor amounts of organic debris from the final reaction stages. The result is a complex mixture characterised by the dye itself and other compounds.

Dichlorotriazine Dyes

The dichlorotriazine dyes are highly reactive and can be applied to cellulosic textiles at temperatures of 30 to 40 °C. In the presence of alkali, the following reaction occurs as illustrated in Fig 2.7.

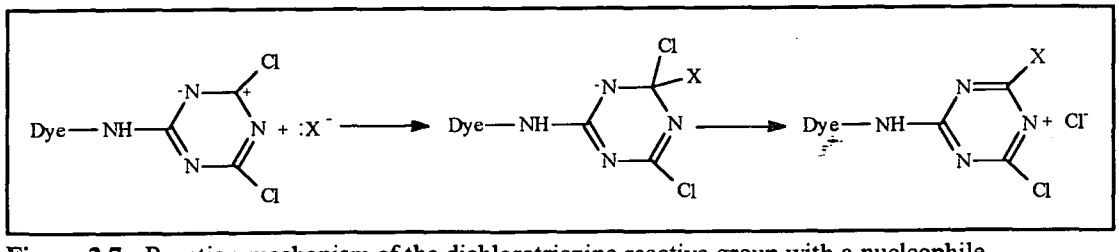

Figure 2.7 : Reaction mechanism of the dichlorotriazine reactive group with a nucleophile.

The attacking nucleophile can be either a cellulosate anion or a hydroxide ion, the former resulting in fixation of the dye to the fabric as shown in Fig 2.8 (a), whilst the latter merely leads to the hydrolysis of the dye shown in Fig 2.8 (b), and its loss from the dyeing process. The chlorine in the *fixed* species shown in Fig 2.8 (a), may remain unchanged, or may be hydrolysed or may react further with the fibre to produce the crosslinked form shown in Fig 2.8 (c). The hydrolysed species shown in Fig 2.8 (b), does not react further since under alkaline conditions the ionisation of the acidic hydroxy group leads to feedback of negative charge into the ring which stops the build-up of a positive charge on the ring carbon atoms and thereby deactivates the remaining chlorine (Rys and Zollinger,1989).

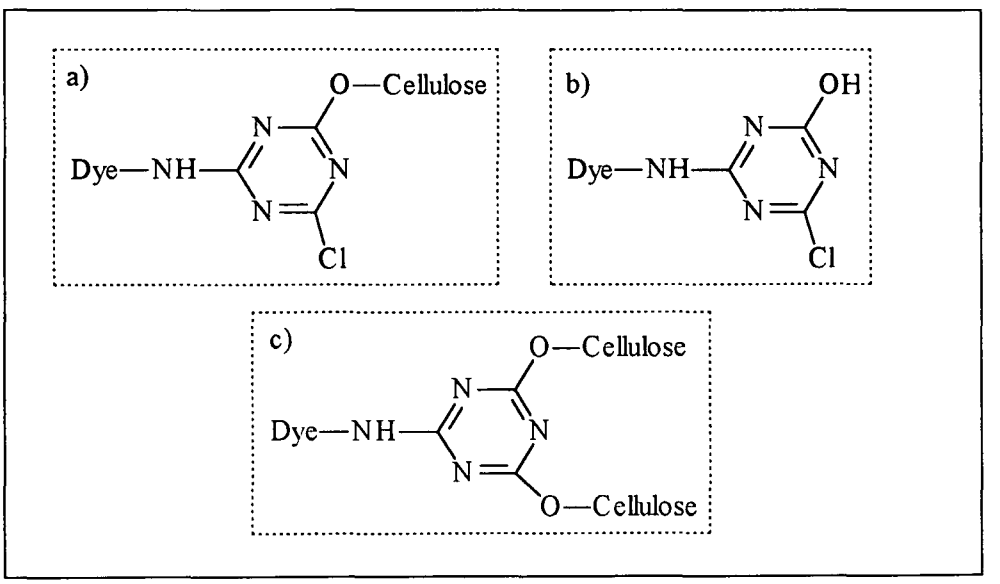

Figure 2.8 : Possible structures of the dichlorotriazine reactive group after reaction with (a) a cellulosate anion, (b) a hydroxide ion and (c) with two cellulosate anions resulting in the crosslinked form.

Since the dyeing process with the dichlorotriazine dyes is achieved at low temperature, fairly small chromogens are required to ensure adequate mobility of the dye on the fibre during the exhaustion stage. This requirement makes these dyes suitable for producing bright shades but leads to difficulties in obtaining satisfactory deep tertiary shades.

Monochlorotriazine Dyes

The monochlorotriazine dyes shown in Fig 2.9 (a), reacts with cellulose in exactly the same manner as the dichlorotriazine dyes, except that a higher temperature and slightly higher pH conditions are required, as mentioned previously. Closely related to these dyes are the more reactive monoalkoxy-monochlorotriazine dyes shown in Fig 2.9 (b). These dyes are applied at a slightly lower temperature of about 70°C.

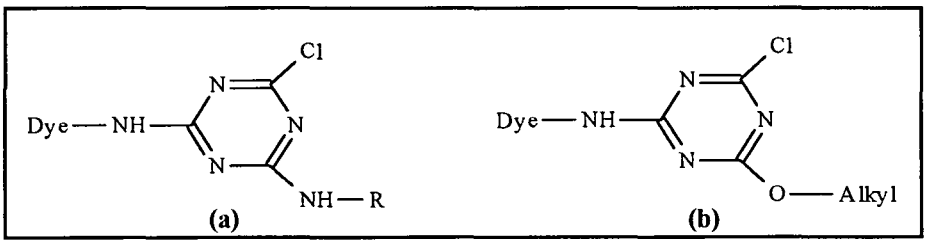

Figure 2.9 : Structures of dyes containing the reactive groups, (a) monochlorotriazine and (b) monoalkoxy-monochlorotriazine.

In the case of small dye molecules there is no advantage to offset the heating costs incurred in operating with the less reactive dye. However, the higher temperature can be advantageous in allowing the use of larger dye molecules, which at lower temperature would have insufficient mobility for dye adsorption. This feature is particularly exploited in the case of the Procion HE dyes discussed in the next section.

Bis-monochlorotriazine Dyes

If two monochlorotriazine groups are incorporated into the dye molecule, then since reaction of either group will result in fixation of the dye, a higher fixation efficiency would be expected. There are three possible arrangements available which are shown in Fig 2.10.

Dyes which have the arrangement shown in Fig 2.10 (a) belong to the Procion Supra range (now withdrawn) whereas the arrangements shown in Fig 2.10 (b) and (c) make up the Procion HE range of high exhaust efficiency dyes. These dyes have about twice the molecular size of chlorotriazinyl dyes, are much more substantive and this allows them to achieve excellent exhaustion on to the

substrate at the preferred dyeing temperature of about 80°C, leading to fixation values of 70 to 80 %.

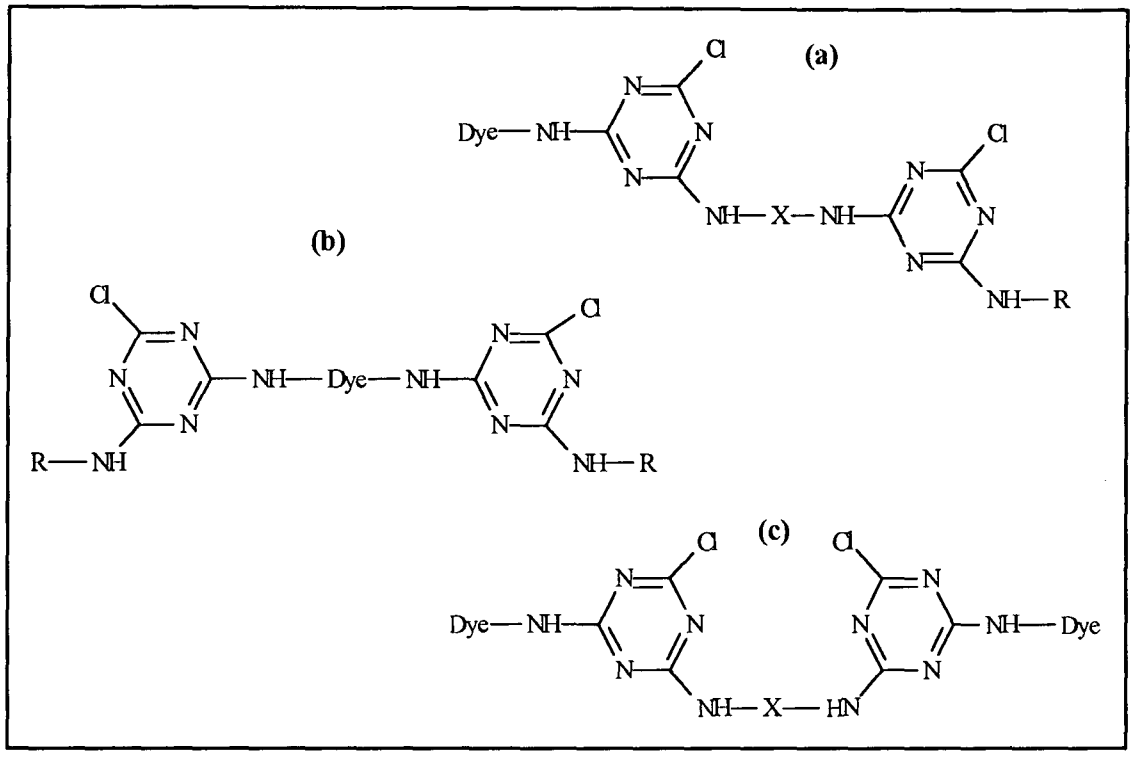

Figure 2.10 : The three possible arrangements for the bis-monochlorotriazine dyes.

2.2.1.2 Vinyl Sulphone and Vinyl Sulphonamide Dyes

These dyes differ from the halogenoheterocyclic reactive dyes in that they react by a nucleophilic addition mechanism rather than a nucleophilic substitution mechanism. They contain a β-sulphatoethylsulphone group, which in the presence of alkali, generates the reactive form of the dye, the vinyl sulphone. The dye-fibre adduct is formed in a subsequent reaction in which the nucleophilic fibre (Cell-O^-) adds to the vinyl sulphone by a Michael-type 1,4-addition shown in Fig 2.11. Here again, competing with this reaction is the reaction with water to give the hydrolysed derivative. The substantivity of these dyes is markedly lower than that of the halogenoheterocyclic dyes, since the vinyl sulphone group has little affinity for the fibre. Unlike the chlorotriazines, the bond formed with the fibre is at its weakest at alkaline pH values (Stead, 1990).

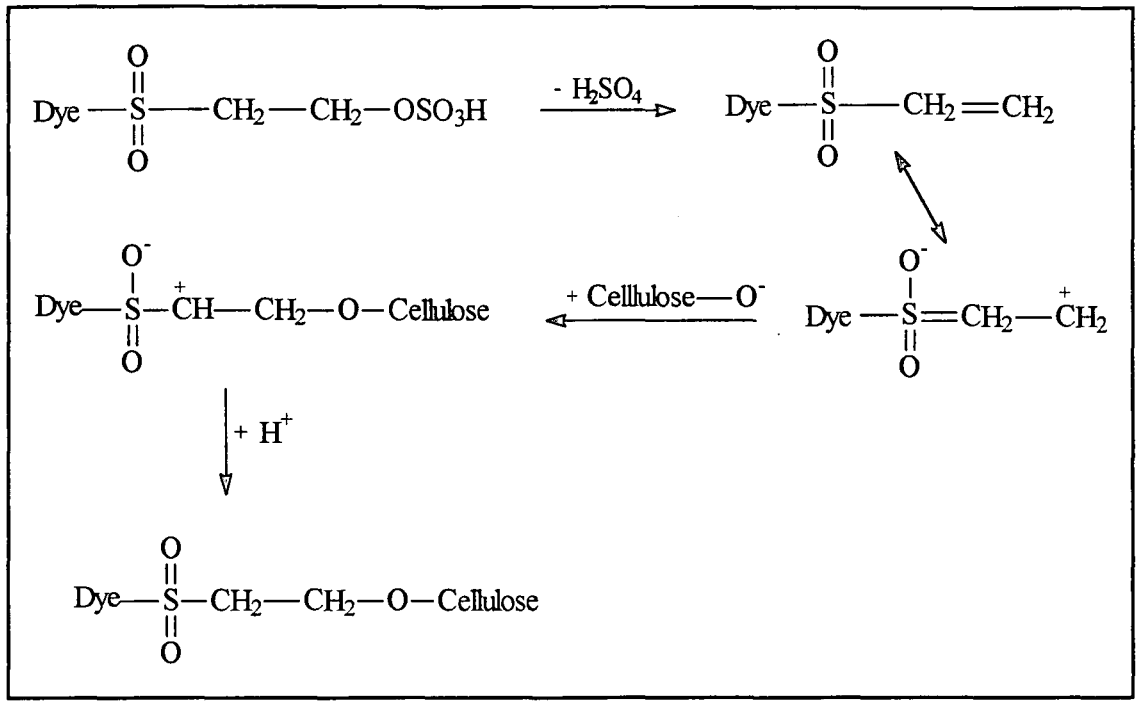

Figure 2.11 : Reaction mechanism for the β-sulphatoethylsulphone group with a cellulosate anion.

2.2.2 The Leaving Group

The leaving group should form a stable ion or molecule after dissociation. This criterion is fulfilled by all leaving groups in practical use. If the chlorine leaving group in a monochlorotriazine dye is replaced by fluorine there is a 100-fold increase in reactivity. Thus, monofluorotriazine dyes can be dyed batchwise at 40°C instead of 80°C. Apart from the increase in reactivity there is a gain in efficiency of reaction which probably contributes an additional 10 % to fixation, other factors being unchanged (Rys and Zollinger,1989).

2.2.3 The Bridging Group

The bridging group can influence the reactivity of the reactive system as well as selectivity and substantivity, e.g. the reactivity of the reactive system may be reduced by several orders of magnitude by the dissociation of the imino bridge as shown in Fig 2.12 , (Zollinger 1987). This could lead to a decrease in substantivity and hence fixation.

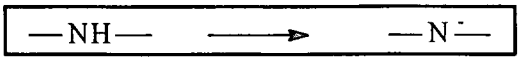

Figure 2.12 : Dissociation of the imino linkage results in lower fixation of the dye molecule.

Reactive groups that are linked via -NH- bridges to the dye are present in tautomeric form, shown in Fig 2.13, of varying selectivity (Rys and Zollinger, 1989). The reactive group has the greatest selectivity when the imino bridge is alkylated (Zollinger, 1987). On the other hand, alkylation generally reduces substantivity and hence degree of fixation.

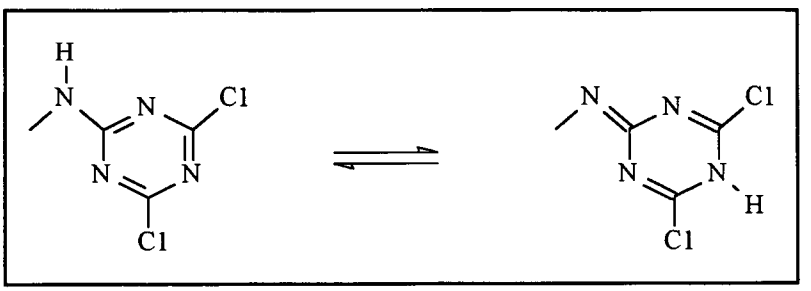

Figure 2.13 : Tautomeric equilibrium of reactive group isomers containing the proton on the imino bridge.

2.2.4 The Chromophore

The introduction of ensuring fixation of the dye by means of forming a covalent bond with the textile substrate, freed the colour chemist from many of the structural limitations implicit in the design of most other ranges of dyes, so that virtually any chemical class could be employed as a chromogen. The demands placed on the chromophore are primarily that it must contain a number of sulphonic acid groups to give the dye the technically necessary water-solubility and also that it must contain an amino group to provide the site to which to attach the reactive system.

In practice, however, the reactive dye chemist has relied heavily on the azo chromophore, accounting for 70 % of the reactive dye class, usually unmetallised but sometimes as metal-complexes in the duller hues, such as navy, brown and black. Anthraquinone, dioxazine, formazan and phthalocyanine chromophores in the bright blue-green sector are the exceptions, but in every other hue sector azo dyes account for over 95 % of reactive dye structures (Stead, 1990).

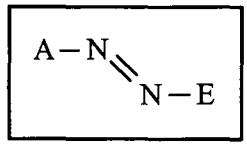

Figure 2.14

The presence of one or more (-N=N-) groups is the characteristic feature of this class. The azo group is attached to two components in the trans form as shown in Fig 2.14, where A is an electron withdrawing group and E is an electron donating group.

2.2.5 The Water Solubilising Group

With dyes for cellulose and protein fibres, 1 to 4 sulphonic acid groups are necessary to confer sufficient solubility for the dyeing process. The high solubility of the dyes makes them very well suited for pad applications and exhaust dyeing at very low liquor ratios.

2.3 CONCLUSION

A brief explanation of dyestuff commercial names which are used would perhaps be worthwhile, since these sometimes seem a source of mystery. The name starts with a range name (e.g. Procion), usually a registered trade mark of the manufacturer, followed by an indication of the colour (e.g. Red), and finishes with a collection of numbers and letters. These suffixes are used to specify the exact shade and possibly highlight a feature of use of the dyestuff and are important in naming a dye. Thus, the letters B and G after a colour indicate the degree of blueness or yellowness (German, *gelb*) of a particular shade, and in the Procion range the designation HE indicates suitability for hot exhaust dyeing processes (Shore,1990).

The major manufacturers report all new dyes to the Society of Dyers and Colourists (SDC), who classify the dyes in the *Colour Index*, awarding to each structurally distinct dye a specific *Colour Index* classification number. Thus, the SDC classify Procion Red HE-3B as C I Reactive Red 120. The principal system by which colourants are classified, is the internationally accepted *Colour Index* classification.

The use of *Colour Index* generic names for commercial dyes and pigments is now widespread among trade organisations, producers and user firms throughout the world. These designations avoid the duplication implicit in quoting the numerous commercial names from individual suppliers and facilitate the comparisons between chemically similar products. Warning against assuming that dyes or pigments with the same C I generic name will necessarily behave identically when subjected to application or fastness tests have been sounded in recent years (Shenai,1997).

Chapter 3

LITERATURE REVIEW

Reactive dyes constituted one of the fastest growing groups of commercial dyes in use in 1993 (Richardson, 1993). Dye classification is based on the major functionality of the dye, however, reactive dyes can encompass any dye class but has relied heavily on the azo, anthraquinone and phthalocyanine chromophores as mentioned in **Chapter 2**, with azo dyes accounting for 70 % of the reactive dye class. These dyes are generally sulfonated in order to confer the necessary water solubility. Due to the lack of literature on analysis of reactive dyes in the environment this chapter will focus on the analysis of dyes in general with emphasis on sulfonated azo dyes.

The review begins with some case studies of the fate of dyes in the environment where analysis of dyes has predominately been by high performance liquid chromatography (HPLC), followed by the use of mass spectrometry (MS) for the analysis of sulfonated azo dyes. This is followed by use of capillary electrophoresis (CE) as an alternative to conventional HPLC methods for the analysis of sulfonated azo dyes and its combination to MS is commented upon. Finally, current trends in extraction methods for sulfonated azo dyes from water using liquid-liquid and solid phase extraction (SPE) are presented. Where possible, the literature is arranged chronologically so as to show trends/changes over the years.

3.1 INTRODUCTION

Interest in the environmental behaviour of dyes arose largely from concerns about carcinogenicity. Some dyes are carcinogens and others after transformation or degradation yield compounds such as aromatic amines which may be carcinogenic or otherwise toxic [(Weisburger, 1976) and (Weber, 1988)].

Both aromatic sulphonic acid and azo groups are rare among natural products and thus confer a xenobiotic character to sulfonated azo dyes. In conventional sewage plants, certain aromatic compounds carrying sulphonic groups as substituents resist biodegradation or are incompletely

degraded. As a consequence 5 to 15 % of the organic carbon content is caused by sulfonated compounds and its secondary products (Knackmuss et al., 1991).

Until 1990, few studies were performed on the fate of dyes in the environment. Work is being done primarily by the US Environmental Protection Agency (USEPA) and the Ecological and Toxicological Association of the Dyestuff Manufacturing Industry (ETAD) (Reife, 1993). One of the reasons for the lack of literature was probably because environmental analysis depended heavily on GC-MS, which is not suitable for most dyes because of their lack of volatility. However, significant progress is being made in analysing nonvolatile dyes by newer mass spectral methods discussed later.

However, most of the studies reviewed paid little or no attention to detection limits, the reasons may be twofold (a) because these compounds are not regulated and (b) due to difficulties associated with their analysis, emphasis has been mainly on developing the techniques. Since these compounds are not regulated and therefore have no maximum concentration levels, according to Barcelo et al. (1997) *it would be acceptable to develop analytical methods that can quantify these analytes at 10 to 50 ppb in water samples, which is in the order of other ionic pollutants already regulated.*

3.2 HPLC AND THE ANALYSIS OF DYES IN THE ENVIRONMENT

Analytical systems using ion pair reversed phase and normal phase liquid chromatography were established for the analysis of 8 acid and 7 disperse dyes, respectively, to study dye removal from carpet dyeing process wastewater (Tincher and Robertson, 1982). All samples were concentrated 100 fold prior to analysis. The results indicated higher concentration levels of acid dyes than disperse dyes. Two possible factors were cited for the observed differences. First, acid dyes are used with increasing frequency in the carpet industry and second, that disperse dyes are more readily removed by biological waste treatment than acid dyes. Generally, they found wide fluctuations in the removal of the acid dyes and speculated that when removal did occur the removal mechanism was probably adsorption onto sludge rather than biodegradation. They concluded that carbon adsorption in conjunction with biological treatment appeared to be particularly effective for removal of dyes

from wastewaters. It should be noted that the acid dyes studied were similar to azo reactive dyes, except the omission of the reactive group, thus similar results would be expected.

In a later study by Tincher (1988) using the previously established analytical systems the removal of 7 acid, 4 direct, 3 reactive and 10 disperse dyes from a simulated landfill facility filled with sludges from textile dyeing wastewater treatment plants was studied over a 3 year period. The detection limits, after a 100 fold concentration step, were reported to be in the range of 1 to 10 ppb for most of the dyes. Effluent from the simulated landfill facility as well as the water from the dye column interior showed no evidence of dyes at the detectable concentrations. Attempts at removing the dyes from the sludge by solvent extraction were relatively unsuccessful with direct and reactive dyes exhibiting the lowest recoveries. From the results obtained, he concluded that most of the classes of dyes studied appeared to be strongly adsorbed and tenaciously held by the sludge.

In a USEPA report regarding the fate of reactive dyes (anthraquinone dye) in the aquatic environment, Camp and Sturrock (1990) positively identified derivatives of Reactive Blue 19 (the hydroxy and the vinyl sulphone) in a textile wastewater using gradient HPLC with a novel type of electrochemical detector (swept-potential electrochemical detector). The detection limits for the vinyl sulphone and hydroxy derivatives were 0.45 ppb and 0.85 ppb, respectively. The concentration of the vinyl sulphone derivative in the textile mill effluent was estimated to be 760 ppb and that of its hydroxy derivative to be 80 ppb.

In a later paper on this study, Weber et al. (1990) reported that the hydroxy derivative could not be detected in the effluent of the wastewater treatment facility receiving the textile wastewater, however, the vinyl sulphone derivative was present at 89 ppb. They estimated that 25 % of the amount of vinyl sulphone entering the treatment plant was passing through the municipal wastewater treatment plant without degrading and into the natural surface water. This was based on the knowledge that the textile mill effluent accounts for 50 % of the flow entering the wastewater treatment plant. Because the vinyl sulphone is electrophilic there is concern over the possible ecotoxicity and muta-genecity of the molecule. Thus, reactive dyes have become a source of concern of the USEPA and methods are sought to characterize and identify these reactive dyes and their transformation products, in order to predict their potential fate and effects in receiving water. Some progress at identifying these dyes by MS has been achieved, this is discussed in the next section.

In order to assess the environmental fate of the vinyl sulphone derivative, the hydrolysis kinetics was studied. The estimated half-life for base-mediated hydrolysis of the vinyl sulphone derivative at a pH value of 7, was approximately 46 y at 25°C. Consistent with this estimate was the observation that no loss of the vinyl sulphone derivative from a filtered natural water sample could be detected over a 3 week period. The half-life of the vinyl sulphone derivative in an anaerobic sediment-water system was 2.5 d. However, they were unable to detect any transformation products, including the hydrolysis product, the hydroxy derivative. They speculated that the vinyl sulphone derivative, reacted with nucleophilic sites on the sediment surface through the same mechanism that the dye binds to cellulose.

Shaul et al. (1991) studied the fate of water soluble azo dyes, 15 acid and 3 direct dyes, in an activated sludge process (ASP). The partitioning of the dyes was achieved by ion pair reversed phase high pressure liquid chromatography with UV-visible detectors. Mass balance calculations were made to determine the amount of dye in the waste activated sludge (WAS) and in the activated sludge effluent (ASE). Of the 18 dyes studied, 11 compounds were found to pass through the ASP substantially untreated, 4 were significantly adsorbed onto the WAS and 3 were apparently biodegraded. He speculated that the non-removal of dyes in the ASP was related to the relatively high sulphonic acid substitution (a characteristic feature of reactive dyes). However, the removal of some dyes by WAS seemed to be related to the positioning of the sulphonic acid functional groups and the molecular mass of the compound. They concluded that further investigations into the effect of sulfonation versus molecular mass were necessary before a relationship, if any exists, could be developed.

In a study of the occurrence and persistence of dyes in a Canadian River by Maguire (1992), three dyes were positively identified: Disperse Blue 79, Disperse Blue 26 and Disperse Red 60. This was achieved by establishing a retention time - spectral library of 17 acid, basic, disperse, mordant, and reactive dyes using paired-ion liquid chromatography with diode array spectrophotometer. Although detection limits were not mentioned Disperse Blue 79and Disperse Blue 26 were found in water at concentrations of 3 to 17 µg/ℓ. A further twelve dyes were found but could not be identified. In addition a mutagenic degradation product of Disperse Blue 70, 2-bromo-4,6-dinitroanaline, was identified in sediment samples. The reactive dyes included were, Reactive Blue 19, Reactive Black 5 and Reactive Orange 13.The author recognised in the case of reactive dyes that short of accidental discharges of large volumes of chemicals, it is the hydrolysis products rather than the parent

compound which is likely to be present in effluents. However, no attempt was made at identifying the hydrolysed products.

Food and cosmetic dyes, Acid Blue 9, Acid Violet 17, Quinoline Yellow, Acid Red 51, Acid Red 87, and Acid Red 92 along with *N*-benzyl-*N*-ethylaniline sulfonic acid (BEASA), a synthetic precursor, were identified and measured in coloured wastewater samples from a municipal treatment plant (Borgerding and Hites, 1994). Continuous-flow fast-atom bombardment (FAB) mass spectrometry was used only to analyse BEASA, since it was the only analyte identified that responded well to FAB. Liquid chromatography with ultraviolet detection was used to analyse the other dyes, but its lack of selectivity required prior isolation of the analytes from interfering compounds by solid phase extraction onto C_{18} extraction disks and onto cartridges packed with strong anion-exchange resins.

The results showed that sorption is much more important than degradation for removing some dyes from wastewater in the municipal treatment plant. Compounds which have a very high ionic character, are not removed from the wastewater at all. On the other hand, weaker anions, such as the xanthenes dyes, are removed entirely and remain attached to the sludge in the plant at least until the sludge is treated in the anaerobic digesters. It should be emphasized that none of the dyes studied had azo-based chromophores, however, the reported non-removal of very highly ionic compounds from wastewater would be applicable to reactive dyes in general due to their highly polar ionic character.

In summary, the analytical system most commonly employed for monitoring sulphonated azo dyes or other polar ionic compounds in the environment was reversed phase liquid chromatography with an ion pairing reagent combined with a UV-VIS detector. The addition of an ion pairing reagent was necessary to reduce peak tailing, the most common ion pairing compounds used were the tetraalkylammonium salts. In general, polar ionic compounds were not efficiently removed from wastewater by conventional treatment methods.

3.3 MASS SPECTROMETRY AS A DETECTOR

With all non-substance specific chromatographic detector systems, standards are indispensable to enable the comparison of the retention times after separation for peak identification. The situation is quite different if a mass spectrometer (MS) is to be applied as a detector after chromatography. Spectra with fragment- (particle beam), molecular- or cluster ions (thermospray; electrospray and atmospheric pressure chemical ionisation) are generated according to the ionisation method applied and the interface used for coupling (Betowski et al., 1996). Spectra from fragment ions provide details of the structure whereas molecular and cluster ions only give information on the molecular mass of the ionised compound.

The generation of structure specific fragments after soft ionisation is essential for identification, and can be realised by collisionally induced dissociation (CID). However, for this purpose an instrument suitable for MS/MS is required. After generation of structure specific fragments ions (daughter ions), by MS/MS after HPLC separation or by mixture analysis from ions of precursors (parent ions), identification of many compounds can still give rise to considerable problems because of the lack of commercially available and universally compatible daughter-ion libraries. This hinders identification. However, if there is no identical standard available, or a known compound forms similar fragments, an experienced spectroscopist may make an identification by a time-consuming spectral interpretation (Schröder, 1996).

Since structural analysis of all components in dye analyses is so time consuming, most analysis are by default, target searches (Betowski et al., 1996). A target search is an analysis in which several specific analytes are targeted for identification. An authentic sample of each target analyte is usually available, and the mass spectrum, solubility, and other characteristics are also known.

3.3.1 Analysis of Dyes by Mass Spectrometry

In early work reported by Voyksner (1985), he found that certain azo dyes were amenable to analysis by HPLC/MS using the thermospray interface. This method of analysis produced primarily $[M+H]^+$ ions with few fragment ions. The HPLC/MS method was shown to detect dyes down to 10 ppt in

wastewater, 100 ppb in soil and 1 ppm in gasoline. On the other hand, azo dyes containing sulphonic acid or sulphonate groups did not produce any positive or negative ions under the conditions used. This makes sulphonated azo dyes challenging test compounds that are not easily analysed by thermospray or other mass spectrometric methods.

Henion et al. (1987) used a mobile phase of acetonitrile and 0.1 M ammonium acetate in water to separate a mixture of 6 monosulphonated (Acid Red 397, Acid Red 151, Acid Black 52, Acid Red 88, Acid Yellow 151 and Acid Orange 7) and 3 (Acid Red 1, Acid Black 1 and Acid Blue 113) disulphonated azo dyes, with UV detection at 254 nm on a reversed phase column. Under these HPLC conditions, thermospray HPLC/MS mass spectra could be obtained for monosulphonated azo dyes but not for the disulphonated azo dyes. After limited success by thermospray for the analysis of sulfonated dyes, atmospheric pressure ionisation using three different HPLC/MS interfaces: (1) heated pneumatic nebulizer, (2) electrospray or ion evaporation and (3) the home-made ion spray interface were examined. All three interfaces led to the detection of sulfonated azo dyes to some degree. The heated pneumatic nebulizer was used to detect the $[M-H]^-$ ion of the free acid of all the monosulphonated dyes examined and fragments ions of low relative abundances were also observed. The disulphonated dyes were more difficult to analyse, and Acid Blue 113 did not show an $[M-H]^-$ ion peak. This dye did give fragment ions that may be due to thermal degradation in the heated nebulizer. Using electrospray, monosulphonated azo dyes gave $[M-H]^-$ ion peaks from the free acid, while the disulphonated azo dye gave $[M-2H]^{2-}$ as the base peak, together with a very weak $[M-H]^-$ ion peak. Very little or no fragmentation was observed in the electrospray.

Sensitivity for sulfonated azo dyes by ion spray was found to be appreciably higher than by ion evaporation or by atmospheric pressure chemical ionisation using the heated pneumatic nebulizer. The ion spray mass spectra of sulfonated azo dyes were identical with those obtained by electrospray. Additional structural information could be obtained using collision induced decomposition in the transport region of these atmospheric pressure interfaces. An important observation was that the sensitivity for sulfonated azo dyes by liquid phase ionisation is strongly reduced when ammonium acetate at a concentration of 0.01 M or more is added to the mobile phase

to improve peak shapes[1]. A 0.001 M ammonium acetate ion concentration gave adequate chromatographic separation without sacrificing sensitivity. Liquid phase ionisation by ion spray or ion evaporation shows less than a factor of 10 difference in sensitivity between mono- and disulphonated azo dyes. Whereas, gas phase ionisation was 20 to 50 times less sensitive for disulphonated azo dyes compared with monosulphonated azo dyes. Here again, no detection limits were given, however, in the total ion current profiles the sample size was reported as 1 to 2 μg per component.

Researchers have tried various techniques to overcome the problems associated with the analysis of sulphonated azo dyes using thermospray (TSP). Mclean and Freas (1989) modified a TSP-HPLC-MS system in order to improve sensitivity by restricting the vaporiser exit orifice and adding a needle tip repeller to the ion source. The 5 disulphonated dyes studied were: Direct Yellow 4, Direct Red 39, Direct Red 81, Acid Red 114 and Direct Violet 32. Detection limits were not reported. An increase in signal response was achieved for disulphonated azo dyes in the negative ion mode. An efficient chromatographic separation procedure for five disulphonated azo dyes, which is compatible with thermospray mass spectrometric detection, was also presented. This entailed employing a mobile phase containing a low concentration of ammonium acetate as an ion-pairing reagent. Concentrations higher than 0.01 M were detrimental to sulphonated azo dye detection due to neutralisation reactions occurring in the ion source. Results indicated that both restriction of the vaporiser exit aperture and the needle tip repeller are needed to obtain mass spectra of sulphonated azo dyes.

Some progress has been achieved, by the USEPA, with respect to the identification of Reactive Blue 19 and its derivatives using negative ion liquid secondary ion mass spectrometry/tandem mass spectrometry (LSIMS/MS/MS). Using this technique, Reactive Blue 19 (an anthraquinone dye) and its derivatives were examined, characteristic ions observed were SO_3^- ions, ions due to central amino cleavage and reactive group cleavage, and ions due to loss of SO_3 and SO_2 (Richardson et al., 1993). They concluded that because of the central amino cleavage, ions thus produced could be used to distinguish between anthraquinone reactive dyes and other classes of

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1 As mentioned previously, ion pairing reagents were added to the liquid chromatography mobile phases to reduce peak tailing. However, when combining HPLC to MS, mobile phase additives are limited to volatile buffers only.

reactive dyes. Further, the reactive group cleavage provided information about which form of reactive group was present in the structure. Similarly, the reactive form could be distinguished from its unreactive parent form and its hydrolysed form.. They reported LSIMS/MS/MS was found to be less sensitive than traditional GC/MS methods, which implied that environmental samples will have to be concentrated prior to analysis, however, no detection limits were given.

It should be noted that although the latter study was not on sulfonated azo dyes, the authors referred to a previous study by them using this technique to determine structural information on some sulfonated azo dyes (direct and acid).

A paper by Voyksner and Lin (1993) described the determination of environmental contaminants using a commercial electrospray which was interfaced to an ion trap mass spectrometer (ITMS). Optimisation of the ITMS and its capabilities to detect non-volatile environmental contaminants, including pesticides, herbicides and azo dyes (non-sulphonated) were emphasized. In particular, the collision induced decomposition(CID) capabilities within the electrospray and within the ITMS were compared for the detection of these environmental contaminants. CID spectra from both processes were similar. Collisional activation in the electrospray transport region required high sample purity. The MS/MS spectra generated in the ITMS were less susceptible to interference from matrix components. However, the acquisition of the MS/MS spectra required extensive instrument set-up. On the other hand, CID spectra generated within the electrospray transport region were easily obtained. The repeller voltage was set and a CID spectra was acquired. But this method was useful on relatively pure samples or samples showing minimal background, placing more of an emphasis on HPLC separation prior to MS analysis. Electrospray also proved very useful for obtaining both molecular mass and structural information on solvent dyes, disperse dyes and organic pigments. The detection limit of the electrospray ITMS system enabled the full-scan detection of 20 to 40 pg of material introduced into the system.

Significant progress toward the analysis of polysulphonated azo dyes was reported by Slater and co-workers (1995). The dyes included in the study were: Acid Yellow 23, Acid Orange 52, Direct Blue 98, Potassium Indigo Tetrasulphonate, Direct Green 26, Direct Red 80, Direct Red 81, Tetrasodium-Meso-Tetra-(4-sulphonatophenyl) Porphine and Reactive Red 120. They found that the addition of an amine base sensitizes the polysulphonated azo salts towards electrospray ionisation mass spectrometry. They proposed that the base enhanced the removal of the cations, this results in

combination of an ion peak series into a single acid peak for each charge state which in turn increases the intensity of these ions. They do acknowledge that the cation removal mechanism was not fully understood. They concluded the paper with, *We consider that this increase in electrospray sensitivity should be of some assistance to the management of industrial effluent containing azo dye residues.* However, concentrated solutions of the dyes were used for the study so that most of the peaks in any ion series could be observed and detection limits were not reported.

In summary, in most cases thermospray gave poor structural information apart from molecular mass and some losses, e.g. SO_3^-, for sulfonated dyes. With regard to sensitivity, although most classes of dyes can be determined, good detection limits for monosulphonated and specially for polysulphonated azo dyes are difficult to achieve without modifications to the thermospray interface. Electrospray in the negative ion mode offers higher sensitivity for sulfonated azo dyes over other techniques. Here again, mono- and di- sulphonated azo dyes are more readily analysed than polysulphonated azo dyes. The addition of an amine base is reported to enhance sensitivity for polysulphonated azo dyes in electrospray. The major problem in combining liquid chromatography to mass spectrometry for the analysis of highly sulphonated azo dyes is achieving tailing free separation while employing a mobile phase that is compatible with the mass spectrometer.

3.4 Capillary Electrophoresis and Dye Analysis

Capillary electrophoresis (CE) has been compared very favourably with high performance liquid chromatography for the analysis of ionic compounds [(Moodley, 1995) and (John, 1997)]. Electrophoretic mobility, which is expressed as a function of the ratio of net charge to molecular size, plays an important role in separations by capillary electrophoresis. CE is a versatile analytical technique that has gained much attention particularly from those working with biologically active molecules. More recently, CE has been successfully applied to synthetic colourants by dye chemists and environmental chemists.

Croft and Hinks (1992) reported high-resolution separation of some cationic dyes using capillary electrophoresis. The analysis of 4 acid and 5 reactive dyes were compared by capillary zone electrophoresis (CZE) and HPLC, with CZE offering better separation efficiency with all dyes well

separated. All analyses were carried out using uncoated silica capillaries and a borate buffer (10mM $Na_2B_4O_7$, 50 mM H_3BO_3 pH = 10.0). The reactive dyes used in this study were all derived from the twin-chromophore monochlorotriazinyl (C I Reactive Red 120) type. In some cases the reactive chlorines were replaced with various quaternary amino, primary amino and hydroxy groups. Using HPLC, 2 of the derivatives co-eluted whereas with CZE all 5 derivatives were separated. The results obtained indicate that buffer control can considerably increase the resolution achieved.

A method for determining some synthetic food dyes using CE was investigated by Suzuki et al. (1994). In this study 7 synthetic red dyes, all permitted as food additives in Japan, were examined. They were separated using a buffer mixture of 25 mM sodium phosphate buffer and 25 mM sodium borate buffer (1:1) containing 10 mM sodium dodecyl sulphate (SDS) at a pH value of 8. Three of the dyes (Amaranth, New Coccine and Allura Red AC) studied belonged to the sulfonated azo dye class. At these conditions the isomeric dyes Amaranth and New Coccine co-migrated and no separation was achieved by either raising the capillary temperature or by the addition of tetraalkylammonium salts to the buffer. Substitution of β-cyclodextrin for SDS in the buffer was effective for the separation of Amaranth and New Coccine. The peak due to Allura Red showed peculiar fronting and variations of buffer pH values, buffer composition or sample concentration showed no effect at improving peak shape.

Application of capillary electrophoresis and capillary liquid chromatography/mass spectrometry to environmental analysis was reported by Brumley et al. (1994). Separations obtained by capillary HPLC were compared with those obtained by CE, using micellar electrokinetic chromatography (MEKC), for 7 selected dyes. Of the 7 dyes studied 4 dyes were sulfonated azo dyes: Acid Red 151, Acid Orange 8, Orange II and Tropaeolin O. Both techniques were reported to separate the selected compounds with high efficiency. Although capillary HPLC was coupled with continuous-flow liquid secondary ion mass spectrometry, interfacing CE to the mass spectrometer was reported to be more difficult. Although detection limits were not reported, standard concentrations ranged from 0.1 to 0.25 ppm.

The separation of 6 dyes, Remazol Black B, Remazol Red RB, Remazol Navy Blue GG, Remazol Golden Yellow RNL, Cibacron Red C-2G and Cibacron Orange CG, by reverse-polarity capillary electrophoresis was attempted by Oxspring and co-workers (1995). Only four of the starting dyes could be separated by this technique and variations in pH, temperature, acetonitrile concentration,

temperature, ionic concentration and micelle concentration failed to separate Remazol Black B and Remazol Red RG. However, the separation of the hydrolysed derivatives of the 5 dyes was achieved using a buffer containing 50 mM citric acid with 10 % acetonitrile at a pH value of 3.25. Remazol Black B and Remazol Navy Blue GG belong to the sulfonated azo class of dyes. The structures of the dyes were not given except Remazol Black B.

Tapley (1995) monitored the activation and subsequent hydrolysis of Remazol Black B (a vinyl sulphonyl dye), under different pH and temperature conditions, using MEKC. The 5 major components (the parent dye and the 4 hydrolysis products) were separated using acetonitrile at a ratio of 1:9 with the aqueous micelle buffer, 10 mM sodium dodecyl sulphate, 10 mM sodium tetraborate and 6 mM potassium dihydrogenphosphate.

Blatny et al. (1995) reported the separation of 9 synthetic dyes, including 6 sulphonated azo dyes (Acid Red 27, Acid Red 26, Acid Orange 52, Acid Orange 7, Acid Orange 12, Acid Red 88 and Acid Red 2) using CE. It was reported that using a phosphate buffer insufficient separation occurred, but the addition of a counter ion with complexing ability (in this case, 1,3-bis[tris (hydroxy-methyl) amthylamino] propane) showed a significant increase in the separation for the 9 selected dyes. However, not all the dyes were separated. A buffer system containing bis-tris-propane as a complexing ion with different linear polymers (polyethylene glycol and polyvinylpyrrolidone acting as pseudo phases) permitted the separation of all analytes. However, the migration time for Acid Red 88 was too long using this buffer system. They also demonstrated that the relative increase in the migration times of the dyes correlates with the number of benzoaromatic rings in the molecule.

Barcelo et al. (1998) reported the separation of 1 monosulphonated (Mordant Yellow 8) and 7 disulphonated (Acid Redl, Mordant Red 9, Acid Red 13, Acid Red 14, Acid Red 73, Acid Yellow 23 and Acid Blue 113) azo dyes using micellar electrokinetic chromatography. The buffer consisted of 10 mM ammonium acetate and 0.05 % Brij 35 at a pH value of 8.6. The detection limits ranged from 1 to 6 mg/ℓ. The method was reported to be feasible for the determination of these dyes in water after solid phase extraction (details of the extraction are discussed in **Section 3.5**).

The latter paper also reported success at interfacing the CE to the mass spectrometer. The previously determined conditions were used except no Brij 35 was added to the buffer solution as it was found

to suppress the ionisation, hence, separation was in the CZE mode. The make-up solvent consisted of isopropyl alcohol/water (80 : 20) containing 0.1 % ammonia delivered at a flowrate of 10 μℓ/min. It was found that in order to increase sensitivity and improve detection limits it was necessary to employ a low cone voltage. The main ions formed were by the losses of 1 to 2 Na^+ cations and their replacement by H^+ ions. The mass ion which gave the best signal to noise ratio for each compound was chosen for CE/MS analysis. It should be noted that this ion did not always correspond to the ion with maximum abundance because at higher masses the noise levels were lower. In the case of Mordant Yellow 8, which gave a base peak at m/z 178, but the ions selected for the analysis were at m/z 200 and 401. This study suggested that acquisition at 2 mass ions is preferable when problems of stability in the spectrum occur and consequently the relative abundances of the ions may change. This technique was also found to help in the confirmation of the selected dyes in real samples.

In summary, since sulfonated azo dyes are ionised at most pH values, their electrophoretic mobility cannot be influenced by buffer pH adjustments. Thus, a method commonly used for this class of dye is micellar electrokinetic chromatography (MEKC) with SDS as the micelle additive. Although most of the authors reported that separation of dyes by CZE is not possible and MEKC had to be employed, in some studies, these compounds have been successfully separated by CZE (Croft and Lewis, 1992). The use of MEKC enables the analysis of neutral compounds as well as strong acids and polar compounds in a single anàlytical technique. It should be noted that in the studies reviewed in this section, little or no emphasis was placed on detection limits except the most recent paper by Barcelo et al. (1998).

3.5 Sample Handling

The main problem in the analysis of polar reactive dyes with high water solubility is the concentration step from aqueous solution. In a study by Tincher and Robertson (1982) on the analysis of some acid and disperse dyes, the extraction of dyes from water was by adsorption onto macro reticular resins. These resins were copolymers of styrene with divinylbenzene. Of the available resins, Amberlite XAD-2 was found to give the best removal and recovery of dyes from wastewater. The recovery efficiencies for 1 ppm solutions of the 15 acid and disperse dyes ranged

from 70 to 100 %. However, this technique was not satisfactory for direct and reactive dyes (Reactive Orange 4, Reactive Red 2 and Reactive Blue 19). This approach was abandoned in favour of concentration by solvent evaporation. Using this approach recoveries were as follows: Reactive Orange 4 - 15 %; Reactive Red 2 - 86 % and Reactive Blue 19 could not be recovered.

Henion et al. (1987) reported on the extraction of 4 monosulphonated (Acid Red 337, Acid Red 151, Acid Red 88 and Acid Orange 7) and 3 disulphonated (Acid Red 1, Acid Black 1 and Acid Blue 113) azo dyes using solid phase extraction with C_{18} cartridges. The addition of triethylamine to all solutions prior to extraction was found necessary to prevent irreversible adsorption of the dye onto the column. Recoveries were determined by spiking the dyes into filtered Cayuga Lake water. The recoveries of 0.1 ppm solutions of the monosulphonated dyes ranged from 87 to 103 %.The procedure was modified for the disulphonated dyes since they were only slightly retained by the bonded silica; a larger C_{18} column was used and washing of the column with 25 % methanol in 0.01 % triethylamine/water was omitted. Recoveries ranged between 65 to 100 %.

Schramm et al. (1988) described a method for the extraction of Reactive Red 4 and its hydroxy and methoxy derivatives from aqueous solution by addition of an ion pairing reagent followed by extraction with cyclohexanol then back extracted into a phosphate buffer at a pH value of 6. Analysis were carried out using reversed phase ion pair HPLC. Different amounts of a nutrient mixture (consisting of malt extract 80.0 g, pepton from meat 16.0 g, pepton from casein 14.0 g, glucose 8 g, extract of yeast powder 1.0 g, sodium chloride 6.0 g, glycine 5.0 g, ammonium phosphate dibasic 2.0 g, potassium hydrogen phosphate 2.0 g, magnesium sulphate 0.2 g, calcium carbonate 0.1 g, lignine without sulphur 1.4 g and lignine with 13 % sulphur 1.4 g) was dissolved in distilled water. These solutions were spiked with the dye and extracted. The recovery experiments showed a decrease in recovery with an increase in nutrient content, thus the method was recommended only for water of low suspension content. Recoveries of 90 - 95 % were reported for C I Reactive Red 4 from aqueous solutions (no added nutrients) by the above method.

Nielen (1993) reviewed trace enrichment of environmental samples by capillary zone electrophoresis. Emphasis was placed on the injection and preconcentration of very large sample volumes using the field amplified injection method. According to Nielen sample pretreatment can be minimised by using C_{18} membrane disks for simultaneous filtering and solid phase extraction. Desorption from the disks can be achieved by using acetonitrile-buffer mixtures, thus providing a sample matrix having a

sufficiently low and constant conductivity. The potential of this technique was demonstrated by the determination of phenoxy acid herbicides in drinking and different river water samples with recoveries of 98 to 103 % at the 0.5 ppb level in drinking water and 55 to 81 % in river water samples. The lower recoveries from some river water samples were attributed to the higher content of particulate matter in that particular river. He concluded that the system can be adapted for trace analysis of other ionic or ionisable pollutants, this class includes reactive dyes, in aqueous samples.

Brumley et al.(1994) selected 4 dyes for studies of the extraction/clean-up of dyes from water and soil matrices: Cresol Red, Acid Blue 40, Acid Orange 8 and Tropaeolin. The latter two dyes are mono-sulfonated azo dyes. Empore extraction disks from Varian were employed for recoveries using two techniques: pH adjustments and ion-pairing methods. The ion pairing reagent used was cetyldiethylmethylammonium bromide at a concentration of 5 mM. Extraction of the 4 dyes from water at a concentration of 1 ppm of the dyes ranged from 73 to 111 % using pH adjustments and 43 to 85 % for the ion pairing method with Tropaeolin being the least quantitatively recovered by ion pairing. They concluded that use of pH adjustment (to pH 1) was the most direct approach for isolating the dyes, but ion pairing offered an alternative approach for compounds that are sensitive to strongly acidic conditions.

A comparative study by Puig and Barcelo (1996) reported on the performance of 4 sorbents (PLRP-S, LiChrolut EN, Isolut ENV and porous graphite carbon) for on-line solid phase extraction of phenolic compounds. Recoveries in ground water were in the range of 55 to 105 % and detection limits down to 0.1 µg/ℓ were achieved. A few differences were found when comparing sorbents from different suppliers, which they attributed to the different physio-chemical properties of the sorbents. Binding phenomena among phenols and humic substances were detected, leading to interferences in the determination.

Recovery studies were carried out for 1 monosulphonated (Mordant Yellow 8) and 7 disulphonated (Acid Red1, Mordant Red 9, Acid Red 13, Acid Red 14, Acid Red 73, Acid Yellow 23 and Acid Blue 113) azo dyes, spiked in groundwater at the 3 mg/ℓ level by Barcelo et al. (1998). Disposable 6 mℓ cartridges packed with 200 mg Isolute ENV and 3 mℓ cartridges packed with 200 mg Lichrolut EN were employed for the recovery experiments. The addition of an amine base such as triethylamine to the extraction solvent resulted in better recoveries. According to the authors the triethylamine assists in the total removal of cations, resulting in better elution of the compounds. For

Acid Yellow 23 and Acid Black 113 recoveries were always below 50 % whereas recoveries of the remaining 6 dyes ranged from 64 to 83 %. Although the 2 sorbent materials are based on the same organic structure, recoveries were higher with Isolute ENV. The differences between the sorbents, according to the authors, could only be traced back to the different physio-chemical characteristics of the sorbent material.

In summary, solid phase extraction was found to be more suitable than liquid-liquid extraction for the preconcentration and clean-up of environmental samples. Two techniques have been employed, pH adjustments and ion pairing methods. The use of pH adjustment is the most commonly used approach for isolating dyes, but ion pairing offers an alternative approach. The latter approach has not been fully explored. In general, mono-sulfonated dyes gave better recoveries than their di-sulfonated counterparts.

3.6 CONCLUSION

Until the 1990's, most researchers have used reversed phase HPLC with an ion pairing reagent combined with UV-VIS detection for monitoring these dyes in the environment. However, with the development of HPLC/MS, this trend is changing. Due to the importance of sulphonated azo dyes and their environmental impact, a number of researchers have dedicated effort in developing mass spectral methods as seen by the studies cited.

The use of thermospray HPLC/MS has been successfully utilised for the determination of azo dyes, but was less successful for sulphonated azo dyes. Some improvement in sensitivity was achieved by modifications of the thermospray interface. With the introduction of atmospheric pressure interfaces, for coupling HPLC to MS, good sensitivity and structural information can be achieved.

In the studies cited, it was reported that mono and disulphonated azo dyes could be detected with high sensitivity using HPLC/MS with an electrospray ionisation interface. However, difficulties arose in their separation by HPLC. This is because of the incompatibility of the commonly used mobile phases with the HPLC/MS interface. Furthermore, the sensitivity was shown to decrease with increasing degree of sulphonation. Research by Slater et al.(1995), showed improved sensitivity for

electrospray by the addition of an amine base. Thus, the improved sensitivity of electrospray should lead to more extensive use of atmospheric pressure interfaces in environmental analysis.

In general, capillary electrophoresis (CE) has shown improved separation efficiency compared to HPLC, with most researchers reporting the need to add a micelle to the CE buffer to effect separation. The potential of CE for dye analysis is suggested by the number of research articles addressing this application. Although initial attempts at coupling CE to MS interfaces were unsuccessful, the recently reported success of combining CE to MS should see the difficulties encountered with HPLC separation a problem of the past. However, the addition of the micelle agent to the CE buffer system, was found to suppress the ionisation. A further problem cited in coupling CE to MS, is poor sensitivity associated with the small injection volumes employed. Thus, future focus will most likely be on improving the extraction of these polar ionic compounds from wastewater using solid phase extraction and combining this with application of CE/MS.

In the analysis of dyes, solid phase extraction was found to be more suitable than liquid-liquid extraction for the preconcentration and clean-up of environmental samples. In general, mono-sulfonated dyes gave better recoveries than their di-sulfonated counterparts.

In summary, various applications have been published describing the analysis of dyes using capillary electrophoresis and high performance liquid chromatography, but there have been relatively fewer publications of sulphonated azo dyes and even fewer on reactive dyes. The analytical problems encountered with, especially, the di-sulphonated and poly-sulphonated azo dyes have always been greater than those of other dye classes.

Chapter 4

CHOICE OF REACTIVE DYES AND ANALYTICAL TECHNIQUES

The aim in this chapter is to identify which dyes to target for the study and review the literature to identify the most appropriate analytical techniques to use for the investigation

4.1 CHOICE OF DYES

The objectives in this section are:

(a) To determine the criteria for selecting the dyes to be investigated.

(b) To select a range of dyes with these criteria.

(c) To establish their *Colour Index* generic names and, where disclosed,also their structures.

The following criteria were used for choosing the target dyes:

i) Do the dyes give rise to aesthetic problems at low concentrations?

ii) Are the dyes known to be problematic with respect to both loading and treatability?

iii) Are the dyes used in high volumes throughout the year?

iv) Are the dyes based on the azo chromophore?

Dyes, because they are intensely coloured, present special problems in effluent discharge, since even a small amount is noticeable. However, the effect is aesthetically displeasing rather than hazardous, e.g. red dyes discharged into rivers and oceans (Gregory, 1993). Hence, red dyes generally meet the first criterion.

In a report investigating the issue of colour removal (McCurdy et al., 1991) it was stated that *the treatment plant had no trouble treating the textile wastewater until mills started using reactive dyes.* Furthermore, C I Reactive HE dyes are known to be more slowly biodegraded in water in

comparison to conventional reactive dyes due to the orientation of the two reactive groups at the different positions of the dye molecule (Chardraborty, 1990). Hence, these dyes are problematic with regard to wastewater treatment. A survey of textile mills in the Hammersdale and Pinetown regions showed a high consumption of the C I Reactive HE dye range, thus, this dye range was chosen for the investigation.

The azo chromophore was targeted because certain azo dyes have been implicated as being the causative agents for bladder tumors among workers in the dye industry (Betowski et al., 1996). In addition, azo dyes can be reduced in the environment and *in vivo* to produce aromatic amines that may be carcinogenic (Weber,1988).

Taking the above factors into consideration and the fact that red dyes are used in combination with other colours, and upon consultation with water authorities and textile manufacturers in the Hammersdale and Pinetown regions 5 reactive dyes were chosen for the investigation belonging to the bis-monochlorotriazine dye range. These dyes were introduced to the market by ICI (now Zeneca) under the trade name Procion HE. However, cheaper dye equivalents (i.e. colourants having essentially the same chemical composition), Evercion HE dyes, are used by the textile mills, in both the Pinetown and Hammersdale regions. The Evercion HE range is marketed by Everlight Chemical Industrial Corporation, Taipei. Hence, 5 dyes from Evercion range were chosen:

Trade name	:	**C I Generic names**
Evercion Green HE4BD	:	C I Reactive Green 19
Evercion Red HE7B	:	C I Reactive Red 141
Evercion Red HE3B	:	C I Reactive Red 120
Evercion Navy HER	:	C I Reactive Blue 171
Evercion Yellow HE4R	:	C I Reactive Yellow 84

Using the *Colour Index* Handbook, the *Colour Index* Generic Names given above, were established. Structures for the above dyes were not given in the handbook, hence the literature was searched to determine the structures, see Fig 4.1 to 4.4.

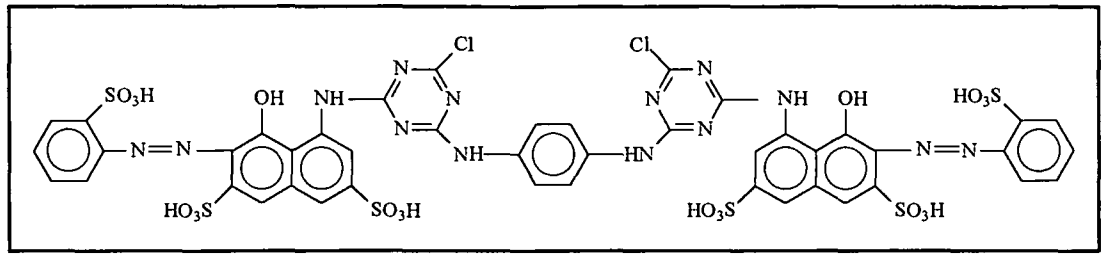

Figure 4.1 : Structure of C I Reactive Red 120, (Bent et al., 1982).

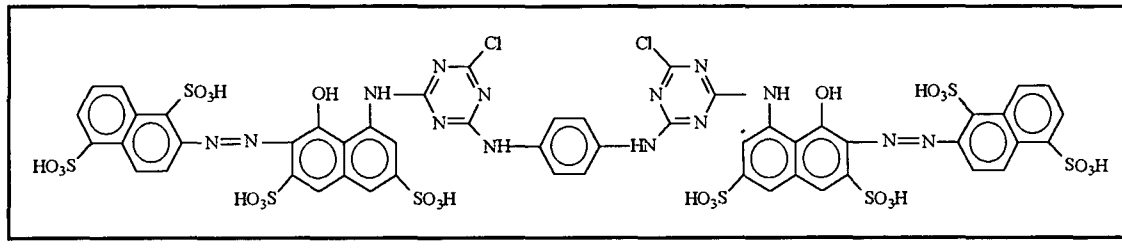

Figure 4.2 : Structure of C I Reactive Red 141, (Carliell, 1993).

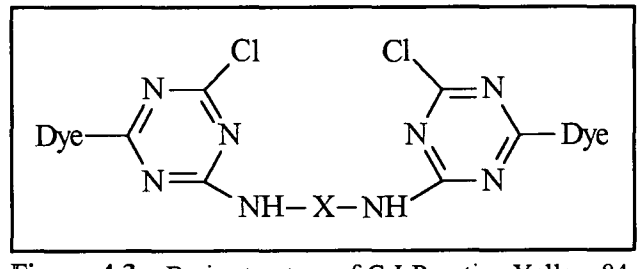

Figure 4.3 : Basic structure of C I Reactive Yellow 84, (Glover, 1991).

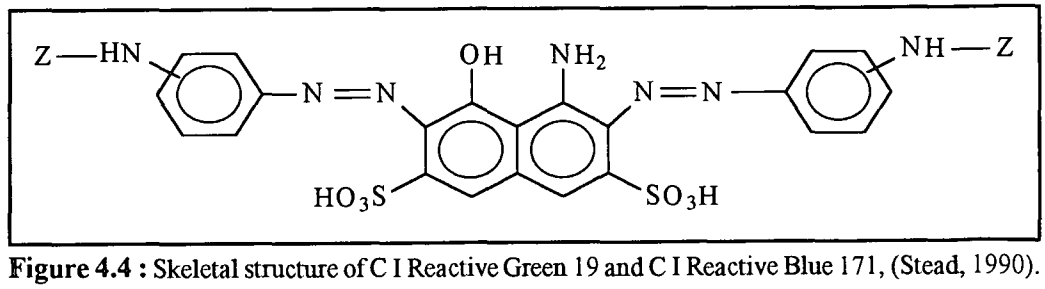

Figure 4.4 : Skeletal structure of C I Reactive Green 19 and C I Reactive Blue 171, (Stead, 1990).

4.1.1 Purification of Dyes

The objective in this section is to purify the commercial dyes to prepare samples of pure dyes to use as analytical standards, since commercial dyes are not pure compounds as cautioned in the *Colour Index* (see below). In general, most of the studies reviewed regarding analysis of dyes have used the commercial dyes as supplied with the exception of Tincher and Robertson (1982), Tincher, (1988) and Shaul et al. (1991).

The *Colour Index* cautions : *Dyes and pigments are marketed as preparations, which include, as well as the essential colourant, diluents, dispersants or other chemicals. It cannot be too strongly stressed that inclusion of different commercial preparations under the same C I generic name implies nothing more than that their essential colourants have the same chemical constitution. Moreover, not all preparations are manufactured to the same degree of purity or consistency of strength. Thus, the application, fastness and ecotoxicological properties may vary from one preparation to another. It should, therefore, not be assumed that one commercial brand can replace another in every respect* (Shenai, 1997).

However, attempts at purifying the dyes using the precipitation purification (Venketaraman, 1977), resin adsorption (Tincher, 1988) and column chromatography techniques were unsuccessful. Details of the methods used are given in **Appendix 7**. It was thus, decided to use the dyes without further purification for the current investigations.

4.2 Choice of Analytical Techniques

The objective in this section is to select appropriate analytical techniques to target for the investigation.

Due to the lack of literature on the analysis of reactive dyes in residual dye-bath liquors and in the environment, studies on the analysis of sulphonated azo dyes in the environment were reviewed since reactive dyes are generally azo based chromophores with sulphonic acid or sulphonate groups. This

class of dye could be separated by ion exchange liquid chromatography. However, separations are usually difficult and reproducibility is poor with ion exchange techniques (Poole and Poole, 1991).

Reversed phase high performance liquid chromatography is a versatile mode of chromatography and is used to separate both non-polar and polar compounds. To effect separation of ionic species, they must be converted to an electrically neutral form. Two common methods for this conversion are ion suppression and paired-ion chromatography.

Ion suppression techniques require converting the sulphonate groups to the non-ionized free acid by adjusting the pH of the buffer. Since these strongly acidic dyes remain ionized even at pH values of 2.5, they do not lend themselves to ion suppression methods.

In paired-ion chromatography, an organic ion is added to the mobile phase to produce an *ion pair complex* (McKone and Ivie, 1980) with the analyte. The resulting complex behaves as an electrically neutral species and is separated by standard reversed phase methods. Paired-ion chromatography with mobile phases mixtures of methanol/water or acetonitrile/water containing tetraalkylammonium salts has been used extensively for the analysis of sulphonated azo dyes and some reactive dyes. Hence, paired-ion chromatography was chosen for the investigation.

In recent years, capillary electrophoresis (CE) has been compared very favourably with HPLC. Two CE techniques, capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) have been employed for the separation of aromatic sulphonic acids and related dyes with most researchers reporting insufficient separation with CZE. However, Croft and Hinks (1992) compared CZE very favourably for the analysis of reactive dyes and their derivatives. Since CZE is the simplest and most commonly used CE technique, this technique was chosen for the initial investigations and depending on separation results the use of MEKC would be considered.

Thus, high performance liquid chromatography (HPLC), using paired ion chromatography, and capillary electrophoresis (CE), using capillary zone electrophoresis, were the techniques chosen for the investigation. The first experimental stage was to develop the chosen analytical techniques for the analysis of the target dyes. The rationale behind this approach was that once methods were developed, these techniques could be modified for the next stage which was to analyse for the parent dye and their hydrolysis products in residual dye-bath liquors.

Thin layer chromatography and HPLC have been used to determine impurities during dye synthesis while ultraviolet-visible absorbance measurements has primarily been used to obtain dye-bath exhaustion data (Luttringer, 1993). Although analysis of reactive dye hydrolysis by thin layer chromatography (Gasparic, 1977), by HPLC (Morita et al., 1996) and by CE (Tapley, 1995) have been described, the application of these methods to the analysis of residual dye-bath liquors and residual rinse liquors has not been reported in the literature reviewed.

Thus, the second experimental stage was to prepare the hydrolysed derivatives then apply the previously developed techniques for the separation of the hydrolysis products from the parent dye. Modify or develop new techniques where appropriate. Then, apply these techniques for the analysis of the residual dye-bath liquors and rinse liquors.

Finally, in determining health risks and studying the fate of dyes in the environment, methods for analysing these dyes in environmental matrices at the parts per billion level are important. Analytical techniques for dyes using UV-visible absorbance detectors can readily detect dyes at the parts per million level (Tincher and Robertson, 1982). In addition environmental matrices are known to interfere with the analytical system. Therefore, an extraction procedure is required for preconcentration and in eliminating interferents.

Current analytical methods of polar ionic compounds from water consist of acidification of the sample, followed by liquid-liquid extraction (Soniassy et al., 1994). In the analysis of dyes in wastewater the literature reported use of solid phase extraction methods rather than liquid-liquid extraction. However, recoveries of dyes with a number of sulphonic acid groups have generally been lower than their unsulphonated or monosulphonated derivatives using this technique.

A study on the extraction of reactive dyes from water systems (Schramm et al., 1988) reported recoveries of 90 to 95 % for C I Reactive Red 4, a dye with four sulphonic acid groups, from spiked water using liquid-liquid extraction with an ion-pairing reagent. Thus, liquid-liquid extraction with an ion-pairing reagent was chosen for the investigation.

Chapter 5

DEVELOPMENT OF ANALYTICAL TECHNIQUES FOR THE ANALYSIS OF REACTIVE DYES

The main aim in this chapter is to develop and evaluate the analytical techniques chosen in **Section 4.2**, for the analysis of the selected reactive dyes.

The analysis of reactive dyes has been demonstrated by high performance liquid chromatography (HPLC) and by capillary electrophoresis (CE) with ultraviolet-visible detection [(Camp and Sturrock, 1990) and (Croft and Hinks, 1992)] and mass spectrometric detection [(Richardson et al., 1993) and (Barcelo et al., 1998)]. However, little has been published on identifying the components present within residual dye-bath liquors.

Thus, the first phase was to develop techniques for the analysis of the parent dyes, then apply these techniques to analysing residual dye-bath liquors. In order to achieve this, two analytical techniques were chosen for the investigations; high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) with UV-visible detection since a mass spectrometric detector was unavailable at the time of investigation.

During the course of this study two different HPLC systems were used. Initial investigations were performed on the Varian HPLC system with a variable wavelength detector with a Water Novapak C_{18} column. Later the Waters HPLC system with a photodiode array detector with a Lichrosorb C_{18} was used. Capillary electrophoresis was carried out on a Beckman P/ACE system. The instrumental details and conditions used on the different systems are given in **Appendix 3**.

Throughout this study commercial dyes were used without purification for the preparation of the dye standards and dye samples. Since the purity of these commercial dyes were unknown, the concentration of the dye standards and dye samples reported herein refers to the concentration of commercial dyes used.

5.1 EXPERIMENTAL

The objectives in this section are :

i) To prepare standard dye solutions to be used for developing and evaluating the selected HPLC and CE techniques.

ii) To record the absorbance spectra of the dyes to determine the wavelength of maximum absorbance, λ_{max}.

5.1.1 Dye Standards

Stock solutions of each commercial textile dye were prepared at a 1 000 mg/ℓ concentration in Milli-Q water. Individual dye standards were prepared by appropriate dilutions of the stock solutions with Milli-Q water so that the final concentration of each standard was 50 mg/ℓ. These standards were used to determine the elution order of the dyes on both the HPLC and CE systems as well as for recording the absorbance spectra of each dye to determine its wavelength of maximum absorbance (λ_{max}).

5.1.2 Dye Mixture

A mixture of the 5 dyes was prepared by taking the required volume of the stock solutions prepared above and diluting to achieve a concentration of 50 mg/ℓ of each dye. This solution was used for establishing the instrumental parameters for the separation of the dyes by HPLC and CE.

5.1.3 Reproducibility and Calibration Standards

Reproducibility of the CE and HPLC methods was tested with a single dye, C I Reactive Red 141, at a concentration of 50 mg/ℓ. Calibrations of these techniques were evaluated for the same dye for a concentration range of 10 to 100 mg/ℓ.

5.1.4 Visible Absorbance

The absorbance spectra of the individual dyes were recorded in the visible region to determine the wavelength of maximum absorbance, λ_{max} (Table 5.1). The visible absorbance spectra are given in **Appendix 8.**

Table 5.1 : The λ_{max}, of each dye determined in water.

Dye	λ_{max} visible region, nm
C I Reactive Yellow 84	406
C I Reactive Red 120	510
C I Reactive Red 141	540
C I Reactive Blue 171	605
C I Reactive Green 19	630

5.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The objective in this section is to develop and evaluate HPLC using the technique 'paired ion chromatography' for the analysis of the selected dyes.

This technique, using paired-ion chromatography with mobile phase mixtures of methanol/water or acetonitrile/water containing tetraalkylammonium salts, has been used to separate some reactive dyes and the methods used by the various researchers are given below:

Croft and Hinks (1992) separated C I Reactive Red 120 and various derivatives of it (i.e. the chlorine on the reactive group was substituted with quaternary amines) using a mobile phase of water/acetonitrile (60 : 40) containing 1 mM tetrabutylammonium bromide (TBAB) and 1 mℓ/ℓ

20 % v/v acetic acid and 1 mℓ/ℓ 5 % w/v potassium hydroxide. The analytical column used was a C_{18} Reversed Phase Apex Octadecyl 4.6 mm x 150 mm.

C I Reactive Blue 19 and its hydrolysed derivatives were separated by using a mobile phase of aqueous acetate, 20 mM, with 3 mM tetrabutylammonium perchlorate (TBAP), and methanol with 10 mM sodium perchlorate and 3 mM TBAP (Camp and Sturrock, 1990). The chromatographic column was a Phenomenex Ultrex 5 C_8 4.6 mm x 150 mm, equilibrated at 35°C.

Schramm et al. (1988) separated C I Reactive Red 4 and its hydroxy and methoxy derivatives with a mobile phase of acetonitrile/aqueous solution of 1 % TBAB (1:1.15). The chromatographic column was a Zorbax CN, 4.6 mm x 250 mm.

Naidoo (1995) reported the use of a mobile phase of methanol/water (60 : 40) with a Waters Novapak C_{18} column for the HPLC analysis of C I Reactive Red 141. He, however, reported that the addition of an ion-pairing reagent resulted in the dye precipitating onto the column, thus, the use of an ion-pairing reagent was discontinued.

However, in this study on the analysis of the 5 selected dyes, it was found that without the addition of an ion-pairing reagent the dyes showed significant tailing. The addition of an ion-pairing reagent was essential to prevent peak tailing. Preliminary studies were conducted to determine a suitable mobile phase to use for the current investigation.

During preliminary experiments 3 different mobile phases were evaluated :

1. A mobile phase of acetonitrile/aqueous solution of 1 % tetrabutylammonium bromide (1:1.15) (Schramm et al., 1988).

2. A mobile phase of aqueous phosphate, 20 mM, with 3 mM tetrabutylammonium phosphate and methanol with 10 mM sodium phosphate and 3 mM tetrabutylammonium phosphate (modification of the Camp and Sturrock buffer system).

3. A mobile phase of water/acetonitrile (60 : 40) containing 1 mM tetrabutylammonium bromide (TBAB) and 1 mℓ/ℓ 20 % v/v acetic acid and 1 mℓ/ℓ 5 % w/v potassium hydroxide (Croft and Hinks, 1992).

A mixture of the 2 red dyes, C I Reactive Red 141 and C I Reactive Red 120, were analysed at a wavelength of 520 nm using the above mobile phases at varying organic content on the Varian HPLC system with a Waters Novapak C_{18} column (conditions given in **Appendix 3**).

Using the first mobile phase with 1 % tetrabutylammonium bromide resulted in the dyes investigated in this study being retained too strongly onto the column with no peaks being eluted within 40 min. Increasing the organic content from 45 to 60 % did not resolve the problem. The column had to be flushed with warm water followed by 100 % methanol to remove the dyes. No further work was conducted with this mobile phase.

Using the second and third mobile phases with an organic composition of 40 % resulted in no peaks being eluted within 40 min. Increasing the organic content from 40 to 45 % resulted in the dyes eluting within 20 min. for the third buffer but no change for the second buffer. All further trials were conducted using the third buffer system at different organic compositions.

The C I Reactive dyes studied by Camp and Sturrock and Schramm et al. had fewer aromatic rings i.e. lower molecular masses, than either those in the present study or those investigated by Croft and Hinks. This difference in the molecular mass may explain why the dyes currently investigated were very strongly retained. In general the more non-polar the complex the greater the retention on the reversed phase packing.

From the preliminary studies, the mobile phase with water/acetonitrile containing 1 mM tetrabutylammonium bromide (TBAB) and 1 mℓ/ℓ 20 % v/v acetic acid and 1 mℓ/ℓ 5 % w/v potassium hydroxide was chosen for further investigations.

5.2.1 Analysis of Individual Dyes

The objective in this section is to establish if absorption in the visible region of the spectrum could be used as a selective parameter for the analysis of the selected dyes.

The most distinctive characteristic of dyes is their absorption in the visible region of the spectrum. Thus, by operating in the visible region of the spectrum, dyes can be detected and quantified in the presence of many other organic compounds which do not absorb strongly in the visible region. In addition, the absorption maxima of colours are very characteristic, with maxima of 400 nm for yellow colours, 500 nm for red colours and 600 to 700 nm for green, blue and black colours. Hence, selection of absorbing wavelengths makes possible the determination of, for example, yellow dyes in the presence of blue dyes. Thus, wavelength selection can greatly simplify the separation and identification process. Thus, literature on the analysis of dyes by HPLC has been primarily in the visible region.

In order to reduce analysis time the following wavelengths were chosen for the analysis; for C I Reactive Blue 171 and C I Reactive Green 19, a wavelength of 605 nm, since their absorbance spectra were very similar (see **Appendix 8**), one wavelength for the red dyes, 520 nm and 406 nm for C I Reactive Yellow 84. Using the conditions established in the preliminary studies, individual dyes were analysed on the Varian HPLC system with the detection wavelength set at the selected wavelength for each colour range to determine its retention order. The resulting chromatograms are given in Fig 5.1.

Under these conditions it is clear that C I Reactive Green 19 and C I Reactive Blue 171 would co-elute in a mixture since the retention times were very close. Each dye was then analysed at the selected absorbance wavelength of the other dyes to test for interferences. In order to reduce analysis time the acetonitrile fraction was increased from 45 to 50 %.

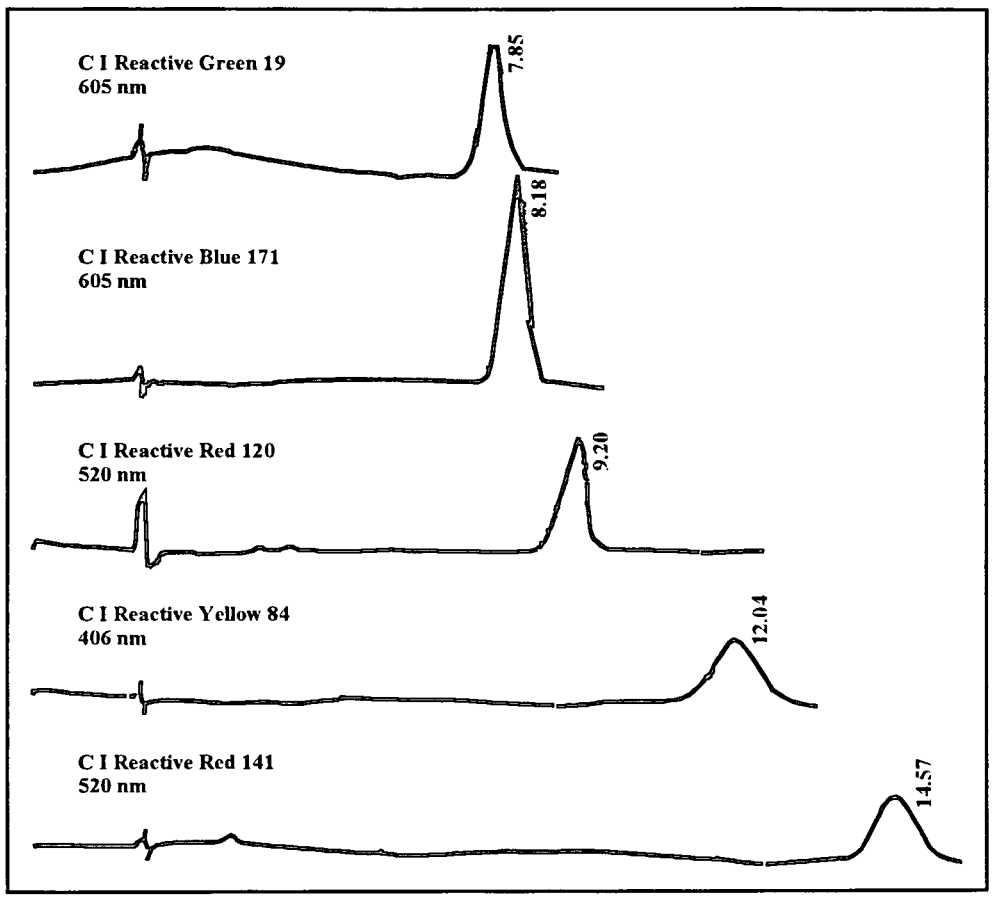

Figure 5.1 : Chromatograms of the individual dyes at the selected absorbance wavelength. Analysis conditions as described in **Appendix 3** for the Varian HPLC system except the mobile phase composition was; acetonitrile/ water (45 : 55).

From the chromatograms shown in Fig 5.2, the following observation were made:

1) At the λ_{max} of C I Reactive Green 19 and C I Reactive Blue 171; C I Reactive Yellow 84, C I Reactive Red 141 and C I Reactive Red 120 did not show any appreciable absorbance, indicating that these dyes could be measured/detected in the presence of red and yellow dyes.

2) At the λ_{max} of the C I Reactive Red 141 and C I Reactive Red 120; C I Reactive Green 19and C I Reactive Blue 171absorbed appreciably, however, these dyes were well separated when the organic fraction of the mobile phase was 45 % as observed in Fig 5.1.

3) At the λ_{max} of the C I Reactive Yellow 84 all four dyes showed some degree of absorbance but separation was not a problem.

4) C I Reactive Blue 171 showed evidence of being a mixture containing some red dye.

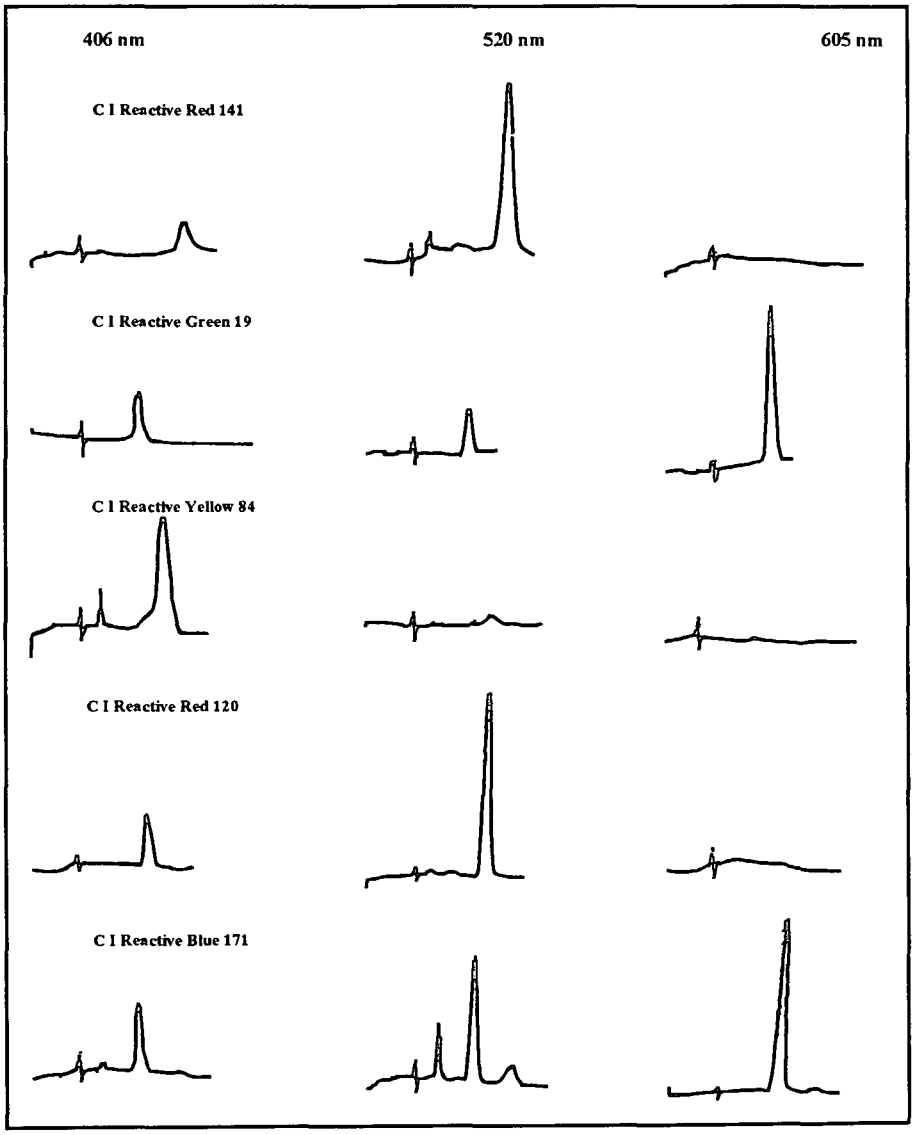

Figure 5.2 : Chromatograms of the individual dyes at wavelengths 406 nm; 520 nm and 605 nm. Analysis conditions as described in **Appendix 3** for the Varian HPLC system, except the mobile phase composition was; acetonitrile/water (50 : 50).

At this point the Waters HPLC system with a photodiode array detector (PDA) became available. The difference between a variable wavelength detector and a PDA is that the complete spectral range is recorded continuously. Using this facility, the ultraviolet-visible spectrum for each dye peak was recorded between 200 to 600 nm. From the spectra obtained (spectra given in **Appendix 8**) it was

evident that C I Reactive Blue 19 and C I Reactive Green have very similar absorbance spectra and all 5 dyes absorb more strongly in the ultraviolet (UV) region. Hence, it was decided to conduct all further investigations in the UV region. A wavelength of 290 nm was chosen since the red dyes had maxima at this wavelength and subsequently used for all further investigations. Fig 5.3 indicates the chromatograms of the individual dyes obtained at 290 nm at the conditions established on the Waters HPLC system with the Lichrosorb C_{18} column (conditions given in **Appendix 3**).

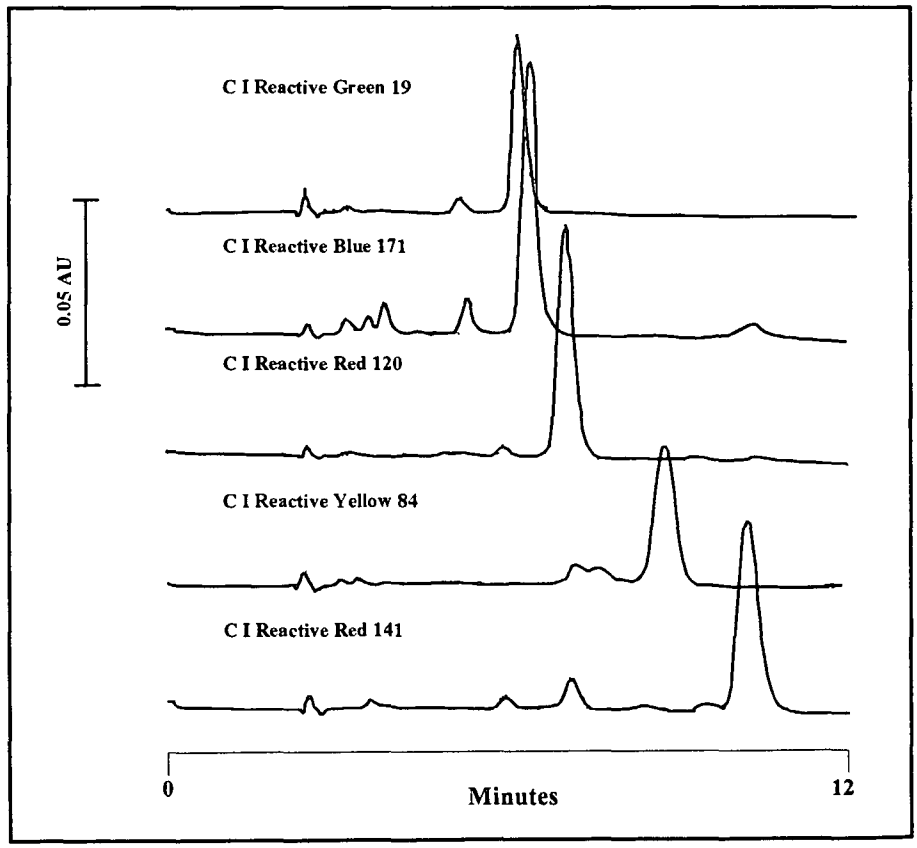

Figure 5.3 : Chromatograms of the individual dyes at 290 nm. Analysis conditions as described in **Appendix 3** for the Waters HPLC system.

From these chromatograms it is evident that all the dyes investigated have some impurities which were not apparent at the conditions used previously. It is clear that under these conditions, some impurities present in dyes could be separated using this method. Although no special attention was paid to investigating the impurities, the series of chromatograms in Fig 5.3 illustrates the possibilities of ion-pairing reversed chromatography in this area.

In summary, wavelength selection could be used to determine yellow and red dyes in the presence of the blue and green dyes and vice-versa. However, for the current investigation wavelength selection was not necessary, since these dyes were well separated. Wavelength selection could not be used for distinguishing between the blue and green dyes since their absorbance spectra were very similar. Thus, all further investigations were conducted in the UV region since the dyes absorb more strongly in this region.

5.2.2 Analysis of Dye Mixture

The objective in this section is to separate C I Reactive Green 19 and C I Reactive Blue 171 by:

i) Testing different reversed phase columns from various manufacturers.
ii) Decreasing the organic content of the mobile phase.
iii) Varying temperature.

Due to variability in selectivity between similar columns from different manufacturers, selectivity can differ. Therefore, different columns were evaluated. All analyses in this section were conducted on the Waters HPLC system, with the analysis conditions as described in **Appendix 3**.

A mixture of the 5 dyes was prepared in water for analysis on different columns in an attempt to resolve C I Reactive Blue 171 and C I Reactive Green 19. The columns tested were Phenomenex Prodigy 5 ODS 4.6 mm x 150 mm , C_{18} Reversed Phase Apex Octadecyl 4.6 mm x 150 mm and Lichrosorb C_{18} 4.6 mm x 300 mm with the previously determined mobile phase. Changing the organic content of the mobile phase and the use of different reversed phase columns did not resolve these 2 dyes. The peak tailing on the C_{18} Reversed Phase Apex Octadecyl 150 mm was high. The addition of acid reduced tailing, however the degree of interaction between the analytes and stationary phase was very poor with most dyes co-eluting. In comparing the other two columns, the Lichrosorb C_{18} gave less peak tailing than the Prodigy and hence was used for further investigations.

Some degree of separation was achieved by decreasing the acetonitrile composition from 50 to 43 %, this resulted in increasing the analysis time to 25 min. The degree of separation as shown in Fig 5.4, did not warrant the increase in analysis time. This mobile phase composition did however separate

the yellow and red dyes quite satisfactorily. Increasing the column temperature reduced analysis time and decreased peak tailing without significant loss in resolution, except for completely overlapping peaks **1** and **2**. An organic content of 43 % acetonitrile in the mobile phase and a temperature of 35°C using the Lichrosorb C_{18} column was used for all subsequent analyses.

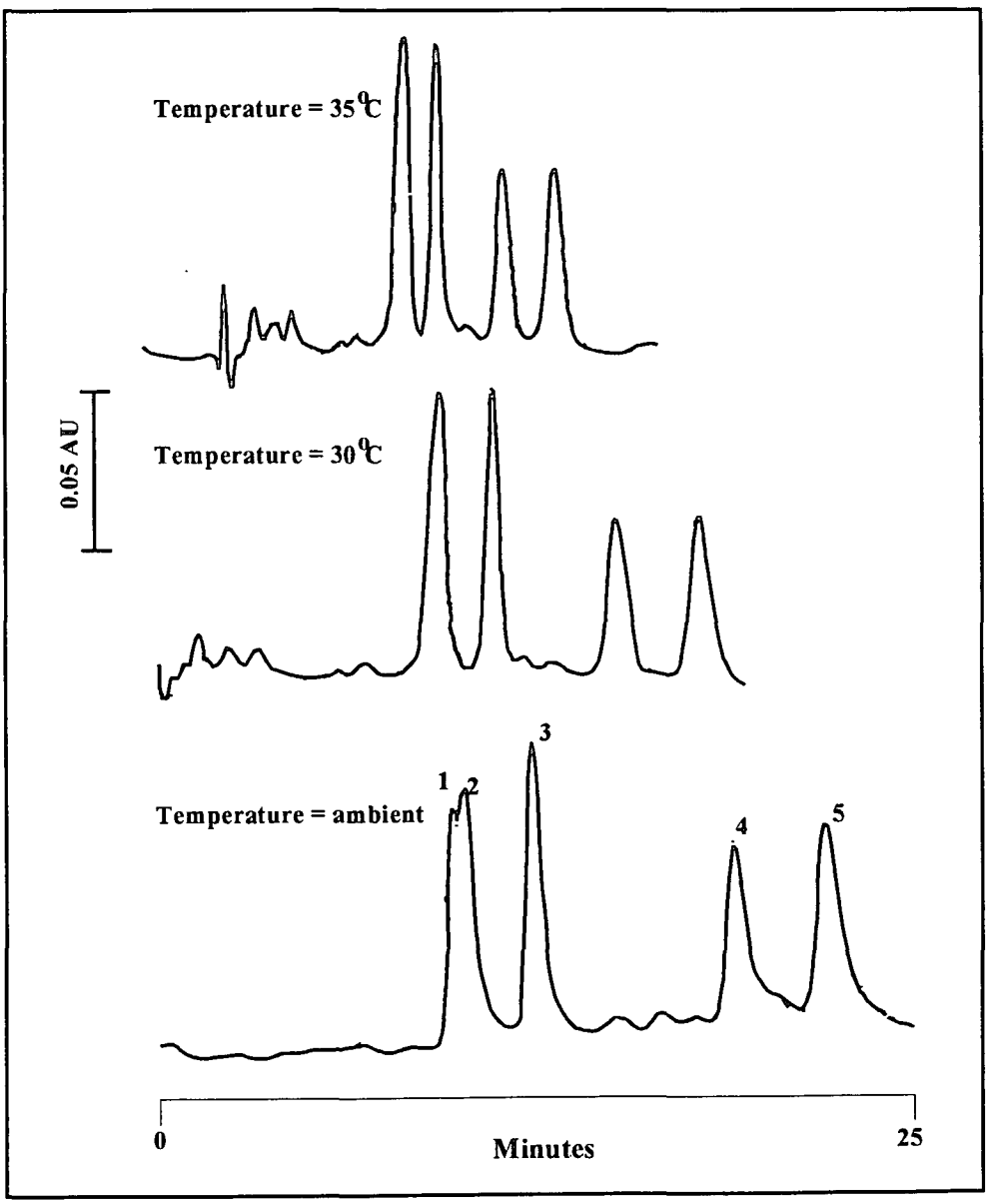

Figure 5.4 : Chromatograms of the dye mixture at 290 nm at different temperatures; where peak 1 = C I Reactive Green 19; 2 = C I Reactive Blue 171; 3 = C I Reactive Red 120; 4 = C I Reactive Yellow 84 and 5 = C I Reactive Red 141. Analysis conditions as described in **Appendix 3** for the Waters HPLC system.

The difficulty in separating C I Reactive Green 19 and C I Reactive Blue 171 highlighted the problem of separating compounds with very similar structures and UV-VIS absorbing properties. The separation achieved was under isocratic conditions, it may, however, be possible to improve the separation with gradient elution. This facility was unavailable at the time of investigation.

5.2.3 Reproducibility and Calibration Studies

Data for the reproducibility and calibration studies are given in **Appendix 4**.The relative standard deviation of the retention time and the peak areas were 0.03 % and 1.67 %, respectively (n = 19). The calibration graph was linear ($R^2 = 0.9974$) from 10 to 100 mg/ℓ. The observed column pressure was constant between 1 400 to 1 450 psi.

The retention time reproducibility achieved was excellent. However, initially retention times were not reproducible, i.e. changed with time and from day to day. The reason for this was thought to be due to insufficient time given for equilibration, normally 30 min. Ion-pairing reagents do require a much longer time to equilibrate and the problem was solved by not flushing the column at the end of the day if the system was going to be used the next day. Flushing buffers are generally necessary because salts can precipitate in the pump and column resulting in pump damage and column blocking. The buffer system used here had a very low salt content and no problems were encountered in leaving the buffer in the system overnight, however, this is not recommended for longer periods (**Appendix 3** gives more details on flushing and equilibrating the system).

5.3 CAPILLARY ELECTROPHORESIS

The objective of this section is to develop and evaluate the capillary electrophoresis (CE) technique for the analysis of the selected dyes.

Theory on the principles of capillary electrophoresis is given in the **Appendix 2**. The most commonly used technique in CE is capillary zone electrophoresis (CZE). CZE should be an efficient separation and identification method for the ionic analytes under consideration.

A study by Croft and Hinks (1992) compared CZE favourably with HPLC for the analysis of C I Reactive Red 120 and its derivatives. The buffer used was a borate buffer (10 mM $Na_2B_4O_7$, 50 mM H_3BO_3, pH = 10.0).

Preliminary work using this buffer resulted in very high currents being generated (145 μA) and currents above 75 μA are generally not recommended (Terabe, 1991) due to excessive Joule heating. Combination of phosphate-borate buffers have been used for the separation of various ionic substances (Tapley, 1995), and this buffer system at a ratio of 10 mM sodium dihydrogen phosphate/6 mM tetraborate was investigated using a mixture of the two red dyes. The two dyes were well separated and this buffer system was used for further investigations.

5.3.1 Analysis of Individual Dyes

The objective in this section is to determine if absorption in the visible region of the spectrum could be used as a selective parameter, for the same reasons as discussed earlier.

Using CZE, conditions given in **Appendix 3**, with the phosphate/borate buffer, C I Reactive Red 141 was analysed at 520 nm and 290 nm. The noise level was significantly higher in the visible region than the in the UV region of the spectrum. This could be due to the very narrow capillary through which the light has to pass, at longer wavelengths there would be greater diffractions resulting in increased noise. Hence, all further analyses were conducted at 290 nm. The analysis of individual dyes were to establish migration order, the resulting electropherograms are given in Fig 5.5. From the electropherograms of the individual dyes, it is clear that all 5 dyes are well separated at the conditions used. As noted in the HPLC chromatograms, the electropherograms of the individual dyes show evidence of impurities in the UV region.

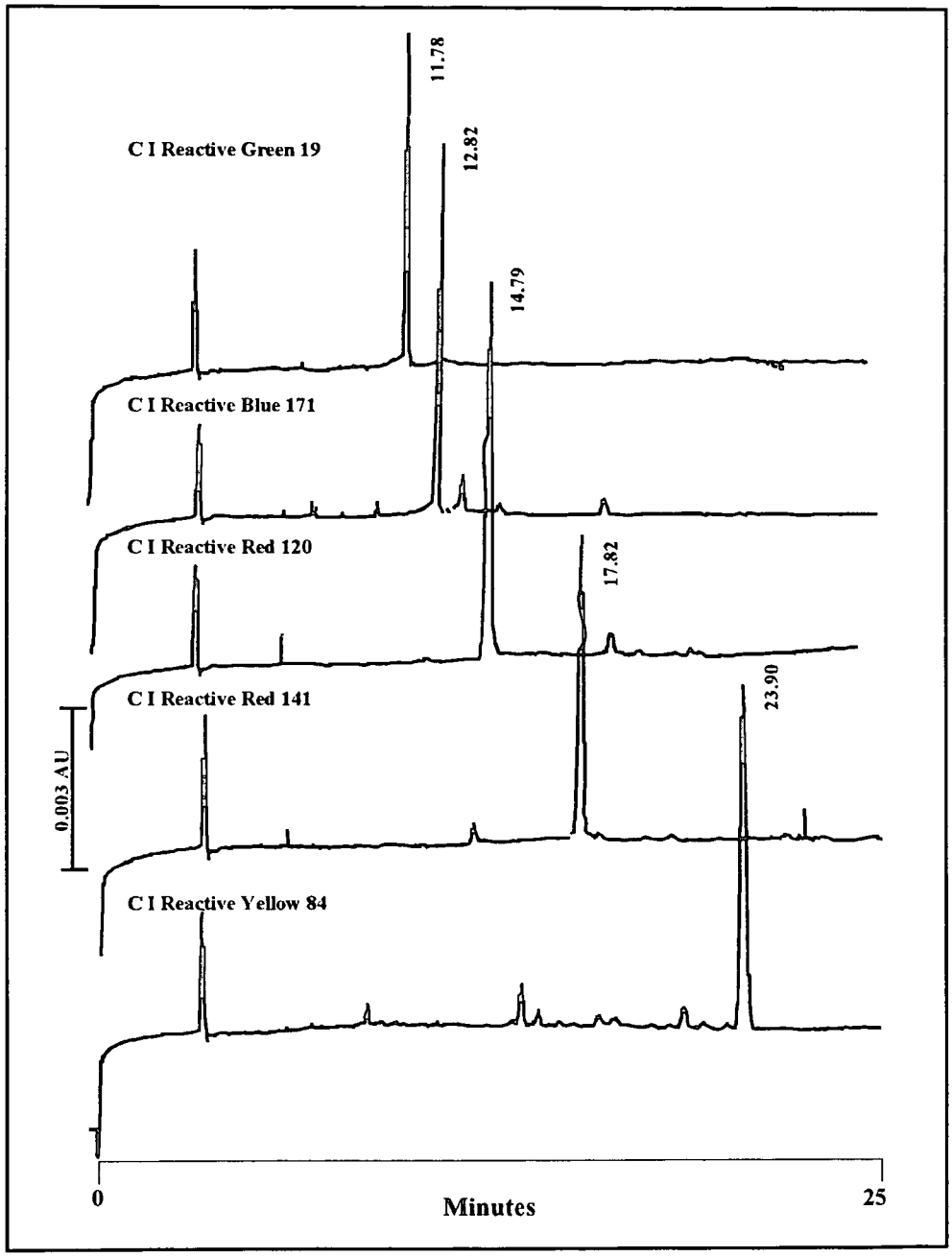

Figure 5.5 : Electropherograms of the individual dyes at 290 nm. Analysis conditions as described in **Appendix 3** for CZE.

5.3.2 Analysis of Dye Mixture

From the latter section it is apparent that all 5 dyes are well separated at the conditions used. Thus, the objective in this section is to decrease the analysis time by increasing the pH value of the buffer.

The dye mixture was analysed using the phosphate/borate buffer at the following pH values: 8, 9 and 10. As the pH increases the analysis time was expected to decrease (**Appendix 2**) and this trend was observed as shown in Fig 5.6. However, peak heights decreased significantly with an increase in the buffer pH value for some dyes with one dye peak disappearing at a pH value of 10. The phosphate/borate buffer at pH values 8 and 9 proved to be efficient for the separation of the 5 starting dyes. Because of the trade off between decreasing sensitivity and increasing analysis time with respect to buffer pH, a buffer of pH 8.5 was used for all subsequent separations.

5.3.3 Reproducibility and Calibration Studies

Reproducibility in peak area measurements and migration times are given in **Appendix 4**. The relative standard deviation of the migration time and the normalised peak areas were 5.89 % and 2.76 %, respectively (n = 11). The calibration graph was linear ($R^2 = 0.9938$) from 10 to 100 mg/ℓ. The observed electric current was constant between 40 to 42 μA.

Initially, problems were encountered with the reproducibility of the migration time, however, regeneration of the capillary with 0.1 M NaOH followed by water and then the buffer (details given in **Appendix 3**) improved the reproducibility.

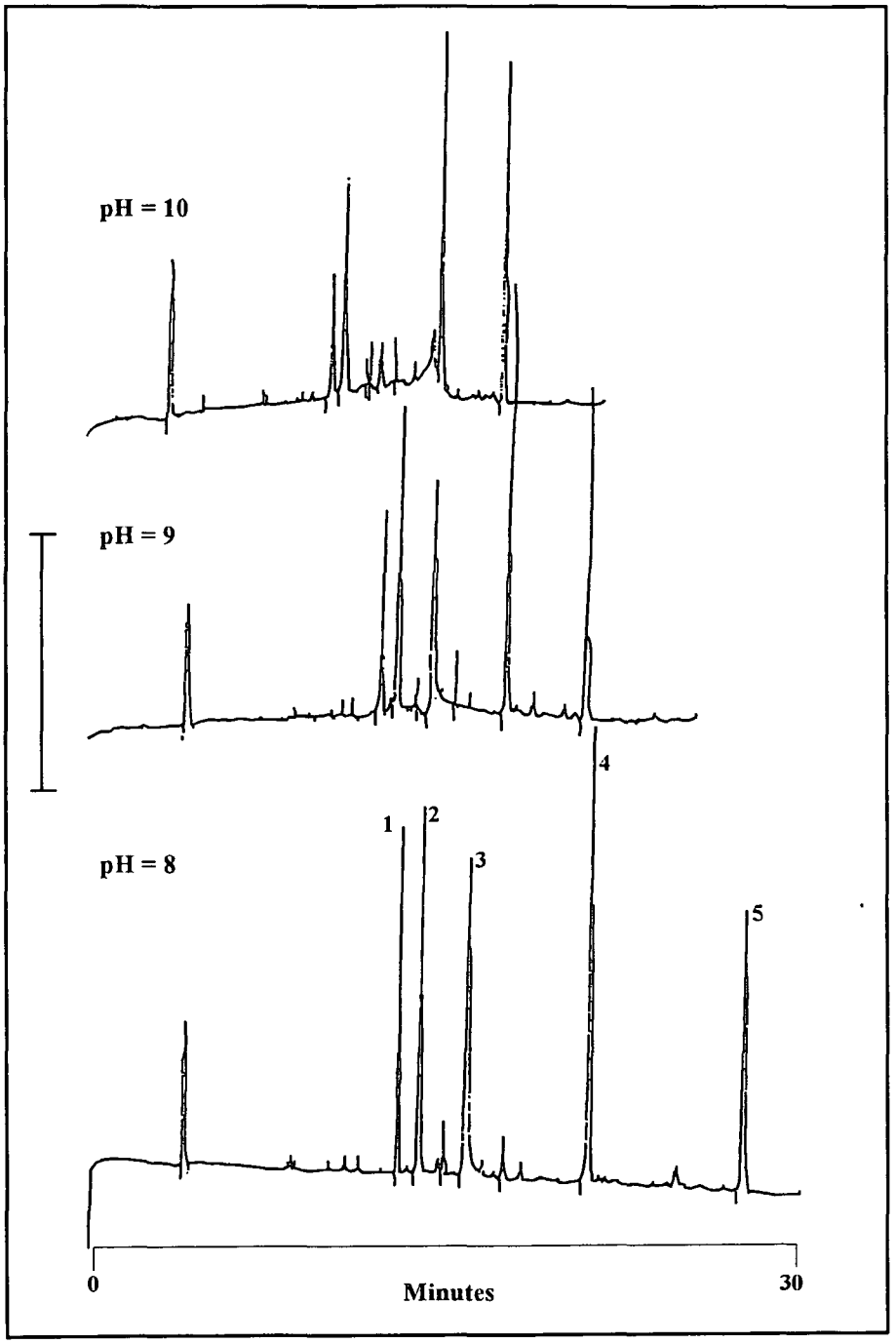

Figure 5.6 : Electropherograms of the dye mixture at different buffer pH values at 290 nm. Analysis conditions as described in **Appendix 3** for CZE. Where 1 = C I Reactive Yellow 84; 2 = C I Reactive Red 141; 3 = C I Reactive Red 141; 4 = C I Reactive Blue 171and 5 = C I Reactive Green 19.

5.4 CONCLUSIONS

Methods for the determination of 5 synthetic textile dyes were investigated using high performance liquid chromatography (HPLC) with an ion-pairing reagent and capillary zone electrophoresis (CZE). The HPLC method with an ion-pairing reagent showed limited separation efficiency with C I Reactive Green 19 and C I Reactive Blue 171 co-eluting, highlighting the problem of separating compounds with very similar structures and UV-VIS absorbance properties by HPLC. Whereas all 5 dyes were well resolved with high efficiency by CZE using a phosphate/borate buffer.

The HPLC method showed better relative standard deviation (RSD) values for retention time (0.06 %) and peak area (1.67 %) compared to the RSD values obtained for CZE for migration time (5.89 %) and normalised peak area (2.76 %). The linear dynamic ranges for both methods were comparable in the range 10 to 100 mg/ℓ. From the chromatograms shown in Fig 5.3 and electropherograms shown in Fig 5.5 of the individual dyes both techniques show potential of separating the dye compound from the impurities.

Method development time for CZE was significantly lower than HPLC, for the 5 dyes investigated. Other advantages seen with the technique were low sample consumption (~ 5nℓ) compared to 10 μℓ in HPLC, low running costs and no generation of organic waste.

Disadvantages observed were the long analysis time (25 min) for CZE method compared to 12 min for the HPLC method as well as the need to regenerate the capillary column between runs which further increased analysis time to 35 min.

The order of separation in HPLC and in CZE were similar for C I Reactive Green 19, C I Reactive Blue 171 and C I Reactive Red 120. However, the order for C I Reactive Yellow 84 and C I Reactive Red 141 were reversed, i.e. in HPLC C I Reactive Red 141 eluted last and in CZE C I Reactive Yellow 84 had the longest migration time. Thus, CZE and HPLC can be used as complimentary techniques for the analysis of reactive dyes.

Considering the separation efficiencies as currently achieved in CZE and the results of the reproducibility peak area measurements, reproducible migration times, it is apparent that CZE is a promising alternative to HPLC for the determination of sulphonated azo reactive dyes.

Chapter 6

DEVELOPMENT OF ANALYTICAL TECHNIQUES FOR THE ANALYSIS OF REACTIVE DYES AND THEIR HYDROLYSED DERIVATIVES

The aim in this chapter is to develop analytical techniques for the analysis of reactive dyes and their hydrolysis derivatives in residual dye-bath liquors. Most of the initial investigations into analysing the dye hydrolysis products involved high performance liquid chromatography (HPLC) due to instrument availability. The analysis by HPLC was performed on the Waters HPLC system except for **Section 6.1.2** where the analysis was performed on the Varian HPLC system. Two different capillary electrophoresis (CE) techniques, viz. capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC), were used in this chapter. The analysis conditions for the CE and HPLC systems used are described in **Appendix 3**.

A suitable methodology for the identification of species present in the residual dye-bath and residual rinse liquors is necessary for the following reasons: in order to reuse the residual dye-bath liquors in subsequent dyeing; in improving the dye fixation and dye-bath exhaustion; in determining what products are discharged in textile effluent. The reactive dyes under investigation have structures similar to the structure given in Fig 6.1. Under normal reactive dye-bath conditions (alkaline medium) reactive dyes undergo hydrolysis. Two products of hydrolysis would be expected, viz. where one monochlorotriazine group (mono-) is hydrolysed (Fig 6.1 b & c) or where both groups (di-) are hydrolysed (Fig 6.1c).

Reactive dye hydrolysis has been studied using thin layer chromatography (Gasparič, 1977) and later by HPLC [(Camp and Sturrock, 1990) and (Morita et al., 1996)]. These studies were successful in separating the parent dye from the hydrolysis products. However, these methods were not applied to analysing the residual dye-bath liquors. All studies associated with measuring reactive dyes in a dye-bath have been achieved spectrophotometrically [(Qu and Jamshidi-Barzi, 1992) and (Luttringer, 1993)], where dye fixation and dye-bath exhaustion were measured.

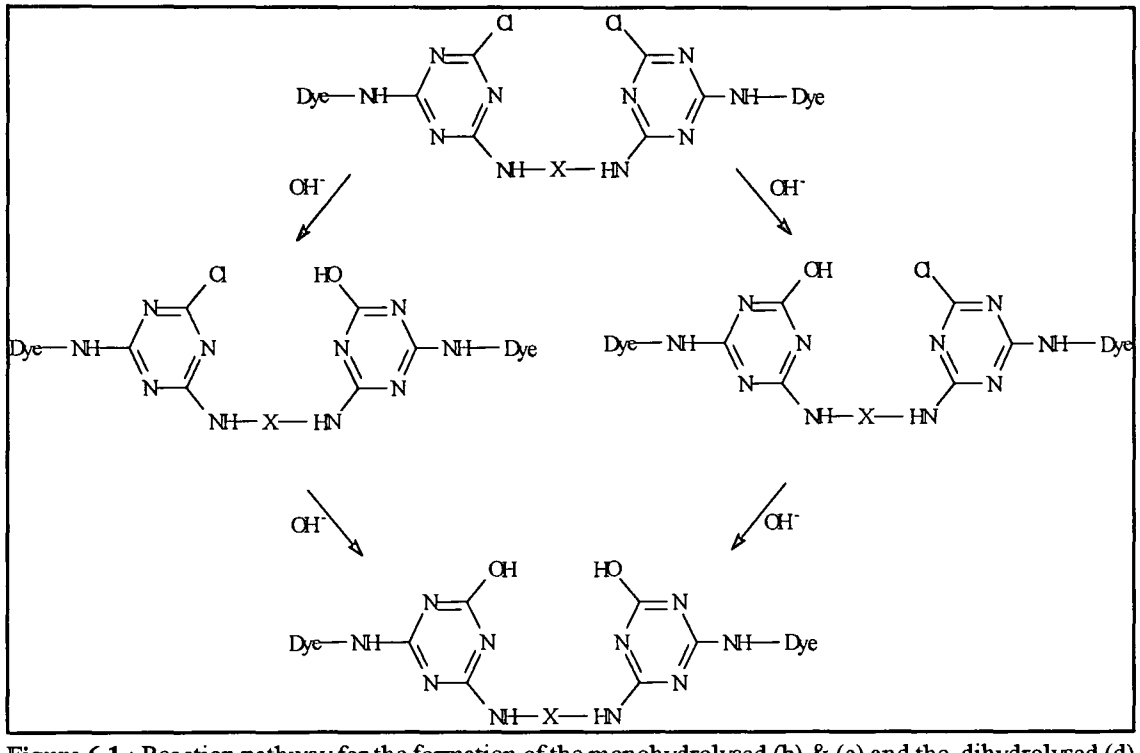

Figure 6.1 : Reaction pathway for the formation of the monohydrolysed (b) & (c) and the dihydrolysed (d) derivatives for a bis-monochlorotriazine dye (a).

The objective in this chapter is to develop suitable methodology to identify the parent dye, and the monohydroxy and dihydroxy derivatives in the residual dye-bath liquors. The approach to achieve this objective was as follows:

1) Hydrolyse the parent dyes under alkaline conditions in the laboratory. Then apply the analytical techniques developed in **Chapter 5** for the separation of the parent dye from the hydrolysis products.

2) Then apply this technique for the analysis of the residual dye-bath liquors.

The initial plan was to hydrolyse the dyes and develop appropriate analytical techniques for the separation of the hydrolysed derivatives from the parent dyes. These techniques would then be applied to analyse the residual liquors from an industrial reactive dyeing process. However, in analysing the residual dye-bath liquors, problems were encountered with the analysis, and a number of mini investigations were conducted to answer/overcome these problems. These investigations are presented independently, each subsection includes the experimental procedure and analysis of results, followed by an overall conclusion at the end of the chapter.

6.1 Preparation of the Hydrolysed Derivatives of C I Reactive Red 141

The objectives in this section are to establish the reaction conditions to prepare the hydrolysis derivatives and develop the analytical techniques for the separation of the products.

Camp and Sturrock (1990) hydrolysed C I Reactive Blue 19 by dissolving 9.0 mg of C I Reactive Blue 19 in 10 mℓ of distilled water that had been adjusted to a pH value of 10.2 with sodium hydroxide. The solution was allowed to react for 22 min, the pH was then adjusted to 6.8 with acetic acid prior to analysis by HPLC.

Using the above method for the hydrolysis of C I Reactive Red 141 no hydrolysis products were detected when analysed by HPLC. A second solution was then refluxed for 5 h. A sample was withdrawn, neutralised then analysed by HPLC, new products were detected for this sample. An excess of sodium hydroxide was added to the reaction vessel and allowed to reflux for a further 60 min. A wide range of products were shown to be formed when analysed by HPLC.

Solutions, each containing 100 mg/ℓ of C I Reactive Red 141 were prepared and the solutions adjusted to pH values 10.5; 11; 11.5 and 12, respectively, by means of aqueous 0.1 M NaOH and allowed to reflux for 3 h. The reaction solutions were neutralized with 0.1 M HCℓ. The extent of dye hydrolysis was monitored by HPLC.

6.1.1 Analysis of Hydrolysed Derivatives of C I Reactive Red 141

Under alkaline conditions the reactive dyes are converted to the mono- and dihydroxy derivatives. In the initial attempt at hydrolysing the dye, a wide range of products were formed on addition of excess sodium hydroxide. The alkalinity may have been too high, resulting in the dye breaking into smaller fragments.

The HPLC chromatograms for the hydrolysis reactions at varying pH values are given in Fig 6.2. The hydrolysed solutions showed that three components were formed by alkaline hydrolysis that had different retention times from the original dye. The peaks were assigned on the assumption that the hydrolysed derivatives (peaks **2** & **3**) will elute earlier but close to the parent dye (since the hydrolysed derivatives are more polar). The dihydrolysed derivative is more polar than the monohydrolysed derivative and, would therefore elute earlier. Besides the two expected hydrolysis components, an unknown component (peak **1**) with a very short retention time was always found in the chromatograms irrespective of the pH value of the solution. At a pH value of 12 the unknown product and the di-hydroxy derivative became the main products.

Schramm et al. (1988) reported finding two unidentified by-products, by HPLC, with very short retention times during the hydrolysis of C I Reactive Red 4, a monofunctional dye. In preliminary experiments, Gasparič (1977) reported finding an unidentified red component, by TLC, with a very short retention time during the hydrolysis of C I Reactive Red 194, a mixed bifunctional dye. This compound was formed in considerable amounts, in addition to the reaction products from the hydrolysis of the monochlorotriazine and vinyl sulfone groups. Gasparič concluded that the imino bridges between arylamines and the heterocyclic ring such as triazine and pyrimidine are hydrolysed under severe alkaline conditions. Morita et al. (1996) investigated the hydrolysis of C I Reactive Red 194, besides the ordinary hydrolysis of vinyl sulfone and monochlorotriazine groups, the hydrolysis of the imino bridge groups was confirmed. Thus, the unknown product formed during the hydrolysis of C I Reactive Red 141 is probably due to the hydrolysis of the imino bridge groups.

The results obtained imply that there are different products formed during hydrolysis, depending on the pH and duration of the reaction. It was thus decided to analyse samples from an industrial dyeing process to establish what products are formed under dye-bath conditions.

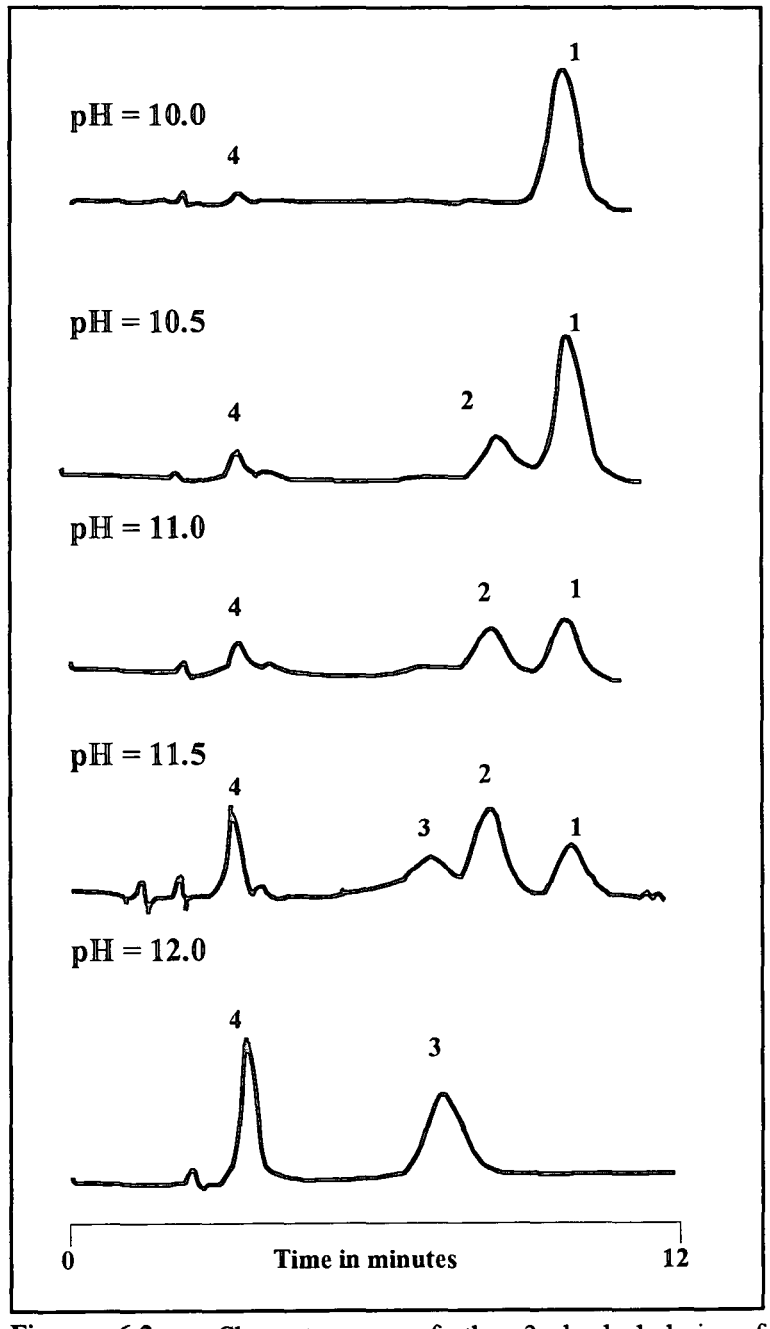

Figure 6.2 : Chromatograms of the 3 h hydrolysis of C I Reactive Red 141 at varying pH values. Where 1 = the parent dye; 2 = the monohydrolysed derivative; 3 = the dihydrolysed derivative and 4 = unidentified peak. Analysis conditions as described for the Varian system in **Appendix 3**.

6.2 Hydrolysis of Reactive Dyes in an Industrial Dyeing Process

The objective in this section is to sample a typical reactive dye-bath and determine what products are formed during the dyeing process and hence, discharged as textile effluent. A dyeing process using dyes from the selected range was targeted for analysis. Since literature was unavailable on dye-bath analysis, samples were taken at each stage of the dye application process.

Details of a typical industrial dyeing process are given in **Appendix 1**. A production dye run at Dyeco (Pty) Ltd, a textile company in Hammersdale, was monitored. Three dyes were required for the particular recipe monitored (method used by the dye-house is attached to **Appendix 1**), C I Reactive Red 141; C I Reactive Green 19 and C I Reactive Yellow 84. All components added to the dye-bath are first diluted with dye-bath solution in a side tank, mixed and then dosed into the dye-bath. Samples were taken at the different stages of the dyeing process, Table 6.1. The batchwise reactive dyeing process is illustrated in Fig 6.3.

Table 6.1 : Sampling points from an industrial dyeing process.

1)	dye solution i.e. a concentrated solution of dyes in water - before adding to side tank
2)	dye mixture in side tank i.e. the above solution added to a fraction of the dye-bath solution
3)	after dosing dye at 50°C but before increasing to 95°C.
4)	dye-bath solution just before the addition of sodium carbonate
5)	side tank sample after the addition of sodium carbonate but before dosing
6)	dye-bath solution after dosing with sodium carbonate but before the 1 h run at 80°C.
7)	dye-bath solution after the 1 h run at 80°C - this is the residual dye-bath liquor or exhausted dye-bath (effluent).

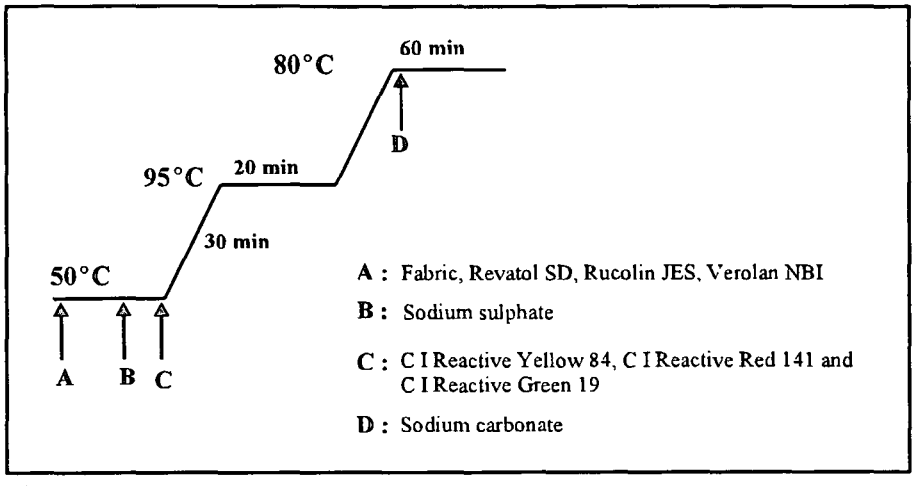

Figure 6.3 : Schematic diagram of an industrial batch-wise dyeing process for reactive dyes.

The pH values of the samples given in Table 6.2, were recorded at the time of sampling on a Knick pH-meter 766, Calimatic, at the dye-house.

Table 6.2 : pH data of samples taken from an industrial dyeing process.

Sample Number	pH
1	8.10
2	8.16
3	7.89
4	7.89
5	11.09
6	10.46
7	10.55

6.2.1 Analysis of the Dye-bath Hydrolysis

All of the above samples were analysed by the Waters HPLC system at the conditions described in **Appendix 3**. From sample 3 to 7 unexpected results were obtained, i.e. dye-bath samples were non-retained or peaks were eluting very early as shown in Fig 6.4.

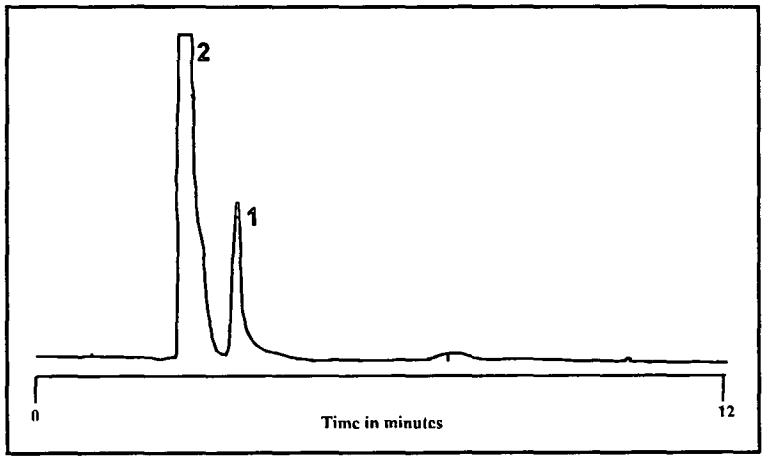

Figure 6.4 : Chromatogram of dye-bath sample 4, where 1 = the early eluting peak and 2 = non-retained peak. Analysis conditions as described in **Appendix 3** for the Waters HPLC system.

This seem to imply that hydrolysis was taking place much earlier than expected and the hydrolysis products were not the expected products. However, the implication that hydrolysis was occurring prior to the addition of the alkali did not seem feasible especially in light of the results obtained in the laboratory tests in **Section 6.1**. Furthermore, the pH values of the samples, in particular samples 3 and 4, were too low for any significant hydrolysis to occur. Further investigations were necessary to understand/explain these results. After unsuccessful efforts in resolving these samples by changing columns and mobile phases the following investigations were conducted.

6.3 INVESTIGATIONS INTO NON-RETENTION OF THE DYE-BATH SAMPLES

The objective in this section is to gain insight into dye hydrolysis by studying the effects of sodium sulphate, sodium carbonate and sodium hydroxide on dye hydrolysis. The effect of sodium carbonate and sodium sulphate were undertaken to emulate the dye-bath conditions; whereas the effect of sodium hydroxide of dye hydrolysis was undertaken to study the ease of dye hydrolysis under severe alkaline conditions.

Test 1 : Dye mixtures refluxed

The following samples, Table 6.3, were prepared in 100 mℓ of tap water and refluxed overnight:

Table 6.3 : Shows the combinations of C I Reactive Red 141 with sodium sulphate and/or sodium carbonate prepared for refluxing.

Sample	C I Reactive Red 141 (mg)	pH	Sodium sulphate anhydrous (g)	Sodium carbonate (g)
1	100	8.12	-	-
2	100	8.10	7	-
3	100	11.05	-	2
4	100	10.55	7	2

All samples were diluted 1 in 10 prior to analysis by HPLC. The sample with C I Reactive Red 141 only, gave the expected hydrolysis products (peaks **2** & **3**), shown in Fig 6.5 b, whereas the dye samples with salt and/or alkali gave chromatograms similar to the dye-bath samples (peaks **4** & **5**), shown in Fig 6.5 c. The ultrviolet-visible spectrum for each peak was recorded in the range 200 to 600 nm using the PDA detector. The spectrum of each peak was compared with the spectrum of the parent dye, however, no apparent difference was observed.

Different reversed phase columns and various mobile phase combinations were tested to achieve some interaction between the products formed and the stationary phase but to no avail.

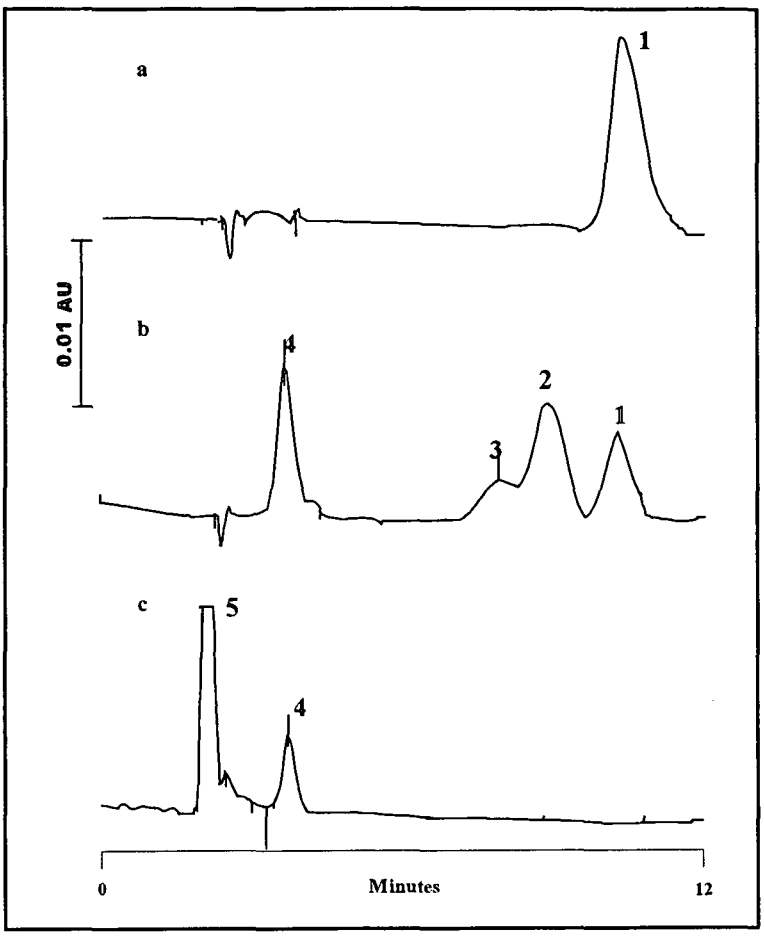

Figure 6.5 : Chromatograms of **Test 1** (a) C I Reactive Red 141, (b) C I Reactive Red 141 refluxed and (c) C I Reactive Red 141 with sodium sulphate refluxed. Analysis conditions as described in **Appendix 3** for the Waters HPLC system. Where 1 = C I Reactive Red 141; 2 = monohydroxy derivative, 3 = dihydroxy derivative, 4, 5 = unidentified peaks.

Test 2 : Analysis of dye in 0.1 M NaOH at 40°C

A 10 mg sample of the C I Reactive Red 141 was dissolved in 10 mℓ of 0.1 M NaOH (the resulting pH was 14) and heated in a water bath at 40°C. Samples were withdrawn after 2 h and 15 h for analysis. Samples were neutralized with 0.1 M HCℓ, diluted 1 in 10 and analysed by HPLC.

From the chromatograms shown in Fig 6.6, the emergence of the expected hydrolysis products (peaks **2** & **3**) is evident in the sample after 2 h with an increase in the 15 h sample. However, the original dye (peak **1**) still appeared as well as the early eluting and non-retained peaks (peaks **4** & **5**). This looked similar to the dye-bath samples except that in the dye-bath samples the original dye and expected hydrolysis products were absent. The fact that after 15 h there was an increase of the expected hydrolysis products and evidence of the original dye added weight to theory that severe/complete hydrolysis was not occurring in the dye-bath but rather some other effect/reaction was responsible for the formation of peaks **4** & **5**.

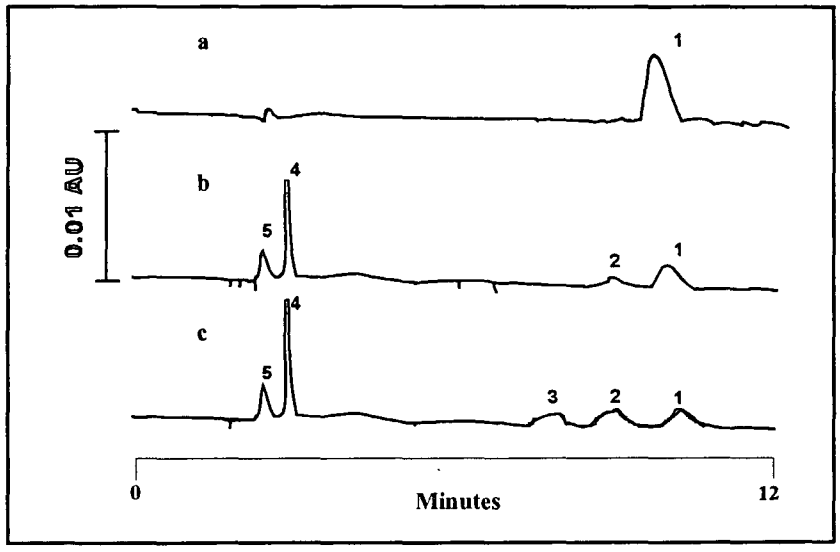

Figure 6.6 : Chromatograms of **Test 2** (a) C I Reactive Red 141, (b) C I Reactive Red 141at 40°C in 0.1 M NaOH for 2 h and (c) C I Reactive Red 141 at 40°C in 0.1 M NaOH overnight. Where 1 = C I Reactive Red 141; 2 = the monohydroxy derivative; 3 = the dihydroxy derivative; 4 = the early eluting peak and 5 = the non-retained peak. Analysis conditions as described in **Appendix 3** for the Waters HPLC system.

Test 3 : Analysis of dye in 0.1 M NaOH and 0.1 M HCℓ at room temperature

Concentrated solutions, approximately 10 000 mg/ℓ, of C I Reactive Red 141 were prepared in 0.1 M HCℓ and in 0.1 M NaOH and allowed to react at room temperature. After approximately 5 d the solutions were neutralized and diluted approximately 1 in 200 before analysis by HPLC.

The sample in acidic medium showed evidence of the expected hydrolysis products whereas the sample in a basic medium showed negligible hydrolysis as shown in Fig 6.7. There is a significant difference between **Test 2** and **Test 3** in the early part of the chromatogram. **Test 2** (Fig 6.6)

indicates both a non-retained peak (peak **5**) and an early eluting peak (peak **4**). Whereas in the more dilute samples from **Test 3** only indicate a non-retained peak.

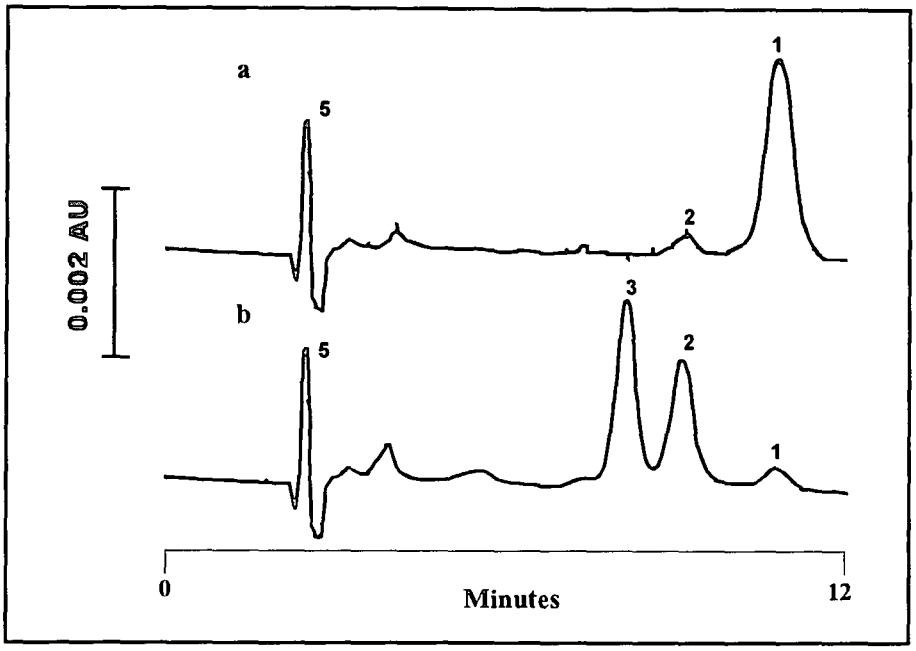

Figure 6.7 : Chromatograms of **Test 3** (a) C I Reactive Red 141 in 0.1 M NaOH and (b) C I Reactive Red 141 in 0.1 HCℓ, both after 5 d. Where 1 = C I Reactive Red 141; 2 = the monohydroxy derivative; 3 = dihydroxy derivative; 5 = the non-retained peak. Analysis conditions as described in **Appendix 3** for the Waters HPLC system.

These results once again contradict the ease of alkali hydrolysis depicted by the dye-bath samples, i.e., the evidence of the parent dye in the samples from **Test 2** above and the insignificant hydrolysis after 5 d in 0.1 M NaOH.

In studying the above results, in particular, the 2 h samples from **Test 2**, (Fig 6.6 (b)), and the alkaline solution from **Test 3** (Fig 6.7 (b)), no reasonable explanation/conclusion could be drawn with regard to the significant differences observed with respect to the non-retained and early eluting peaks. The application of heat was the only significant difference in the treatment of the dye and this did not justify the differences observed. The only logical explanation or difference was the fact that solutions in **Test 3** were diluted considerably thus reducing the electrolyte strength. From this observation it was hypothesised that the non-retained peaks were related to the high concentration of sodium sulphate (7 %) in the dye-bath samples. To test the validity of this explanation **Test 4** was undertaken.

Test 4 : Analysis of a diluted dye-bath sample

To test the above hypothesis, an aged dye-bath sample (2 months) containing a mixture of C I Reactive Red 141, C I Reactive Yellow 84 and C I Reactive Blue 171was diluted 1 in 100 and re-analysed. The chromatograms for the undiluted and diluted dye-bath samples are given in Fig 6.8 (a) and (b), respectively. The appearance of the later eluting peaks in the diluted sample confirmed that electrolyte concentration affects the chromatography. However, the early eluting and non-retained peaks were still prominent.

From the above tests it was clear that what was initially thought to be unexpected hydrolysis was incorrect but rather the dyes were behaving as predicted but some other effect/phenomenon, related to salt or electrolyte concentration, was responsible for the non-retained peaks in the dye-bath samples.

A method to remove the salts was investigated

Dimethylformamide (DMF) has been used to separate the salt from dyes using the precipitation method (Venkataram, 1977). In order to remove the salts, the dye-bath sample used in **Test 4** was evaporated to dryness on a water bath, the residue was dissolved in dimethylformamide (DMF) and filtered. The filtrate was evaporated and reconstituted in water for analysis by HPLC. The resulting chromatogram shown in Fig 6.8 (c), was quite complex with the appearance of a range of later eluting peaks (peaks 1 to 6) but the problem of non-retained peaks was partially resolved. The use of DMF was undesirable, from a health and safety perspective, and the application of heat would surely increase hydrolysis, hence, an alternate method was sought.

Since the dyes were much larger than the salts, the use of dialysis seemed a possible option. Using the samples from **Test 1** a dialysis system was set-up and samples analysed by HPLC. This method proved quite successful, with the emergence of the expected hydrolysis products as well as the original dye. A complete study of dye hydrolysis using all five dyes is presented in the **Section 6.5.**

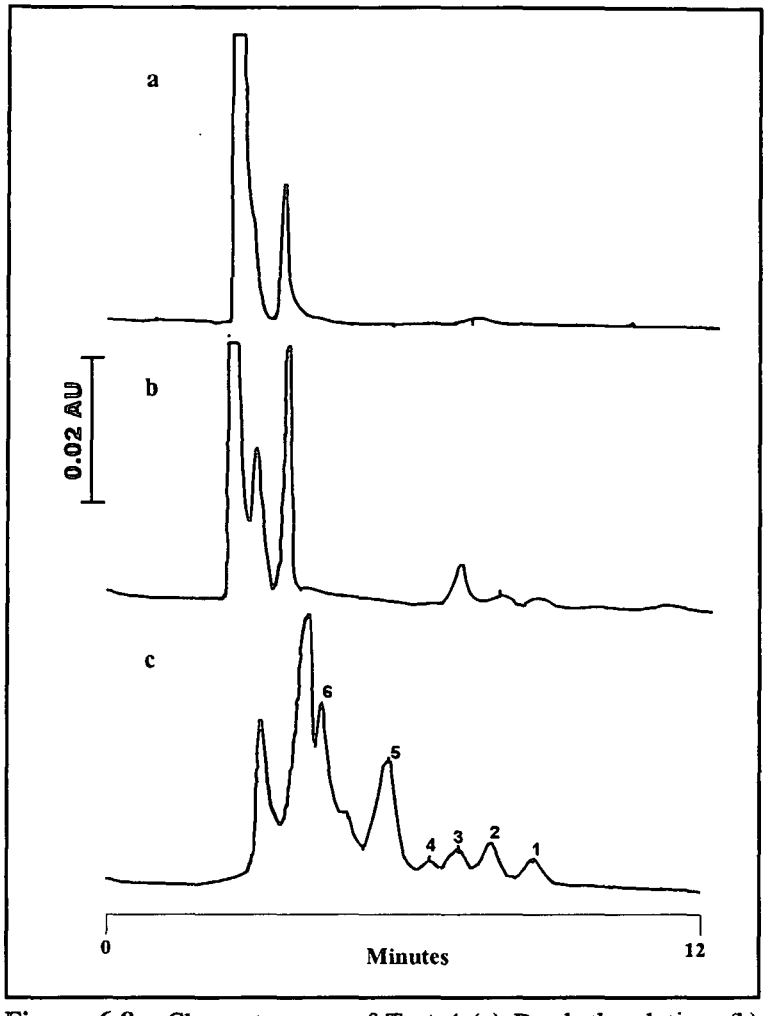

Figure 6.8 : Chromatograms of **Test 4** (a) Dye-bath solution, (b) Dye-bath solution diluted and (c) Dye-bath solution evaporated, dissolved in DMF, filtered, evaporated and reconstituted in water. Where peaks 1 to 6 are the later eluting peaks. Analysis conditions as described in **Appendix 3** for the Waters HPLC system.

From the above results, it is hypothesized that dye aggregation in the presence of salts was responsible for the non-retention of the dye-bath samples. Since the effect of dye aggregation on reverse phase chromatography has not been documented, the effect of salt on dye absorbance and retention was studied further to verify the proposed theory.

6.4 THE EFFECT OF SODIUM SULPHATE ON DYE ANALYSIS

The objective in this section is to study the effect of sodium sulphate on dye absorbance and dye retention to verify that the presence of salts was responsible for the non-retention of the dye-bath samples.

There is considerable evidence in the literature [(Coates, 1969); (Giles and Duff, 1972) and (Sumner, 1989)] that many ionic dyes form aggregates in solution. Aggregation is promoted by increasing the concentration of the dye in solution and also by the presence of added electrolyte. In many cases due to the participation of the delocalised electrons in the dye molecules in the formation of dye-dye complexes there is an observable change in the electronic absorption spectrum. It is not possible to determine from such changes the state of aggregation under equilibrium or steady state conditions (Rattee and Breuer,1974).

The sodium sulphate concentration in the reactive dye-bath is approximately 7 %. In order to test the effect of sodium sulphate on dye retention and absorbance the following were investigated.

6.4.1 The Effect of Sodium Sulphate on Dye Absorbance

Solutions of C I Reactive Red 141 at a concentration of 50 mg/ℓ were prepared at varying sodium sulphate concentrations, viz. 0; 2; 3; 4 and 5 %, and the absorbance spectra were recorded. In the UV region the absorbance values decreased with an increase in salt concentration as shown in Fig 6.9 (a), whereas in the visible region, shown in Fig 6.9 (b), the wavelength of maximum absorbance changed from 542 nm to 518 nm. The change in λ_{max} is an indication of dye aggregation; when the aggregating molecules adopt a stacked or sandwich configuration a shift of λ_{max} to shorter wavelengths is expected (Rattee and Breuer, 1974).

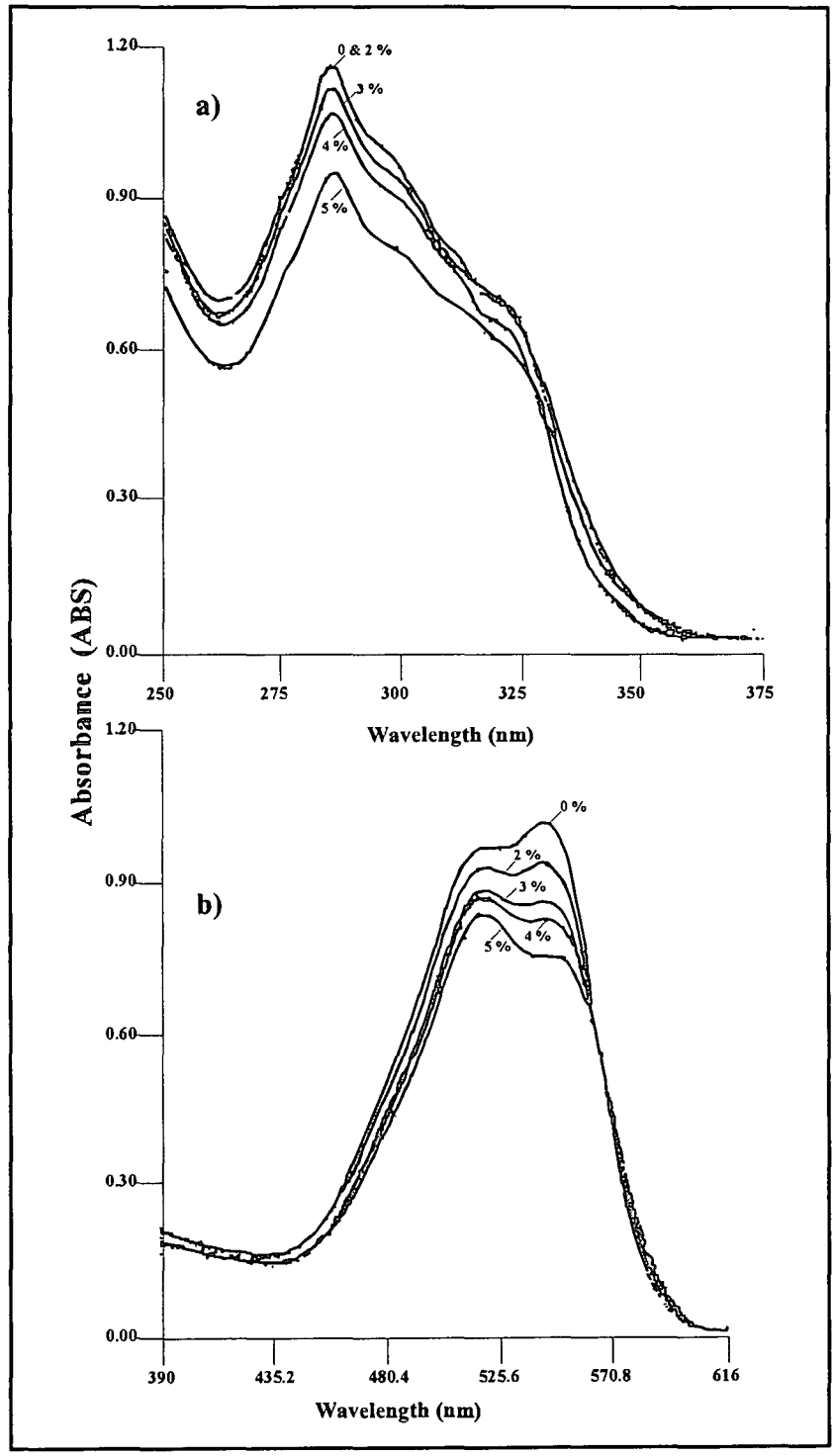

Figure 6.9 : UV-VIS absorbance spectra of a 50 mg/ℓ solution of C I Reactive Red 141 at varying salt concentrations viz. 0; 2; 3; 4 and 5 %, (a) in the UV region of the spectrum and (b) in the visible region of the spectrum. Analysis conditions as described in **Appendix 3**.

6.4.2 The Effect of Sodium Sulphate on Dye Retention

The solutions used for the absorbance investigation at varying sodium sulphate concentrations were also analysed by HPLC. However, at these salt concentrations the HPLC chromatograms gave only the non-retained peak. Therefore, solutions with much lower concentrations of sodium sulphate were prepared for the HPLC analysis. Solutions of C I Reactive Red 141 at a concentration of 50 mg/ℓ were prepared at the following sodium sulphate concentrations, 0; 0.2; 0.3; 0.4; and 0.5 %.

As seen from Fig 6.10, the area/height of non-retained peak (peak **2**) increased and the area of the dye peak (peak **1**) decreased with an increase in salt concentration.

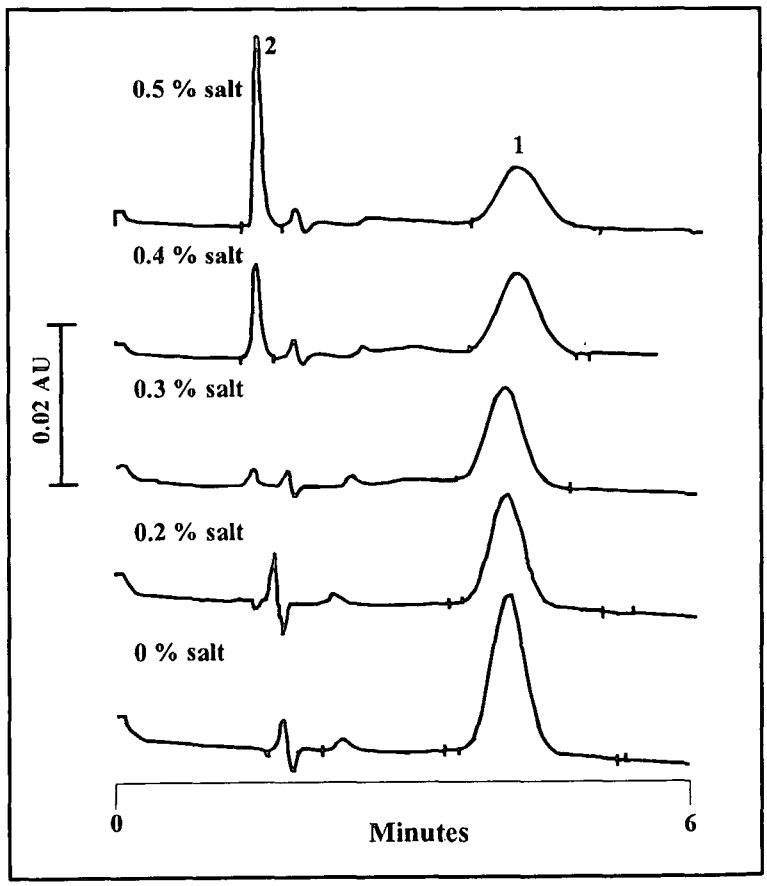

Figure 6.10 : Chromatograms of C I Reactive Red 141 at varying sodium sulphate concentrations, where 1 = the dye peak and 2 = the non-retained peak. Analysis conditions as described in **Appendix 3** for the Waters HPLC system, except the acetonitrile/water ratio used was 50 : 50.

The relationship between the dye peak area (peak 1) and sodium sulphate concentration is depicted in the graph shown in Fig 6.11. There is an inverse relationship between the peak area and salt concentration. The results indicate that the non-retained peak is due to the sodium sulphate concentration. It is proposed that the non-retained peak is due to dye aggregation or dimerisation in the presence of salts.

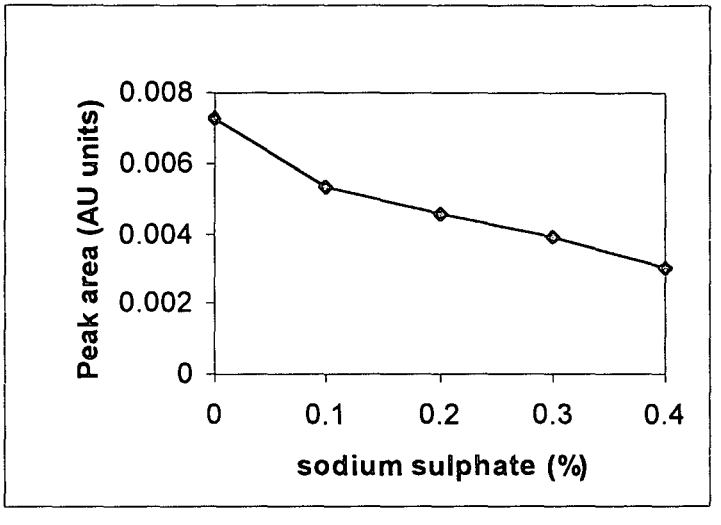

Figure 6.11 : Graph depicting the effect of sodium sulphate concentration on the dye peak area (peak 1) in HPLC.

This phenomenon of dye aggregation is comparable to self association of surfactants to micelles above the so called critical micelle concentration (Guo et al., 1994). Such a similarity does not imply that the structure of the dye aggregates resembles that of micelles, however, this could explain the non-retention of the dye-bath samples. No such effect on retention in reversed phase chromatography has been reported in the literature reviewed with regard to dyes. However, according to literature, in reversed phase chromatography, micelles generally exhibit little affinity for the stationary phase (Poole and Poole, 1991).

From the above results, there is convincing evidence that the non-retention of the dye-bath samples was due to the concentration of the electrolyte in dye-bath solution. It is proposed that this effect is due to dye aggregation in the presence of electrolytes.

6.5 Dye Hydrolysis in a Laboratory Dyeing Process

The initial plan, as reported earlier, was to prepare the hydrolysis derivatives and develop techniques for the separation of the hydrolysed derivatives from the parent dye. Due to problems associated with analysing the dye-bath effluent and the dependence of the products formed on reaction conditions reported in **Section 6.1**, the following approach was adopted.

1) To prepare the hydrolysis derivatives under dye-bath conditions and apply the techniques developed in **Chapter 5** for the separation of the hydrolysed derivatives from the parent dye.

2) Then apply these techniques to studying the dye-bath effluent by dyeing cotton with the individual dyes by the Laboratory Dyeing Process.

6.5.1 Preparation of the Hydrolysis Derivatives at Dye-bath Conditions

The objective in this section is to prepare the hydrolysis derivatives of the 5 selected dyes at dye-bath conditions and determine if the analytical techniques developed were appropriate for the separation of the hydrolysis products from the parent dye.

Each dye was hydrolysed at typical dye-bath conditions, except the textile substrate and auxiliaries were omitted:

A 100 mg sample of commercial dye and 7 g anhydrous sodium sulphate were dissolved in 100 mℓ of distilled water and heated at 95 °C in a water bath for 20 min. A 10 mℓ sample was removed for dialysis before adding 1.5 g of sodium carbonate and heated for a further 60 min at 80 °C before taking a second sample for dialysis. All samples were dialysed against distilled water for 12 h and diluted 1 in 10 prior to analysis. Samples were analysed by both HPLC and CE.

6.5.1.1 Analysis of Hydrolysed Dyes at Dye-bath Conditions

The analysis by HPLC of each dye reacted at dye-bath conditions is illustrated in Fig 6.12. The general trend shown by each dye was the insignificant level of hydrolysis before the addition of the alkali as expected. It was assumed that the molar absorptivities of the hydrolysed derivatives are similar to the parent dyes. Based on this assumption the following observations were noted. After the addition of the base, for C I Reactive Red 141, C I Reactive Blue 171 and C I Reactive Green 19, trace levels of the parent dye were observed with significant levels of the hydrolysis products. In these dyes the di-hydrolysed product was the most prominent product. For C I Reactive Yellow 84 the parent dye was still the most prominent peak and C I Reactive Red 120 also showed significant amounts of the parent dye but showed greater evidence of hydrolysis than C I Reactive Yellow 84. These variations could have been due to the conditions at different points in the water bath not being equivalent or that the dyes do behave differently. It is evident from the chromatograms that for some dyes more than two hydrolysis products are formed during hydrolysis at dye-bath conditions. As noted previously, hydrolysis of the imino bridge groups is a possibility.

The above samples were analysed by CZE to test if this technique would be able to separate the hydrolysed derivatives from the parent dye. The CZE method was first tested on the dye-bath samples prior to dialysis in order to overcome the necessity of this time consuming process. Due to the high ionic strength, zone broadening was observed and therefore, dialysed samples only were analysed. For the CZE analysis the samples had to be spiked prior to analysis to establish which peak was due to the parent dye. As illustrated in Fig 6.13, high separation efficiency was obtained for C I Reactive Green 19, C I Reactive Blue 171 and C I Reactive Yellow 83, however, for C I Reactive Red 141 and C I Reactive Red 120 separation was very poor.

In HPLC, since the hydrolysis products are more polar, they would therefore elute earlier than the parent dye and this trend was observed for all 5 dyes. In CZE, since the hydrolysis products are more negative than the parent dye they would be expected to have a longer migration time than the parent dye. This trend was observed for all the dyes except C I Reactive Yellow 84. The reason for the reversal of migration order in CZE for C I Reactive Yellow 84 was unknown. Since the structure of the dye was not disclosed, a possible explanation was not immediately apparent.

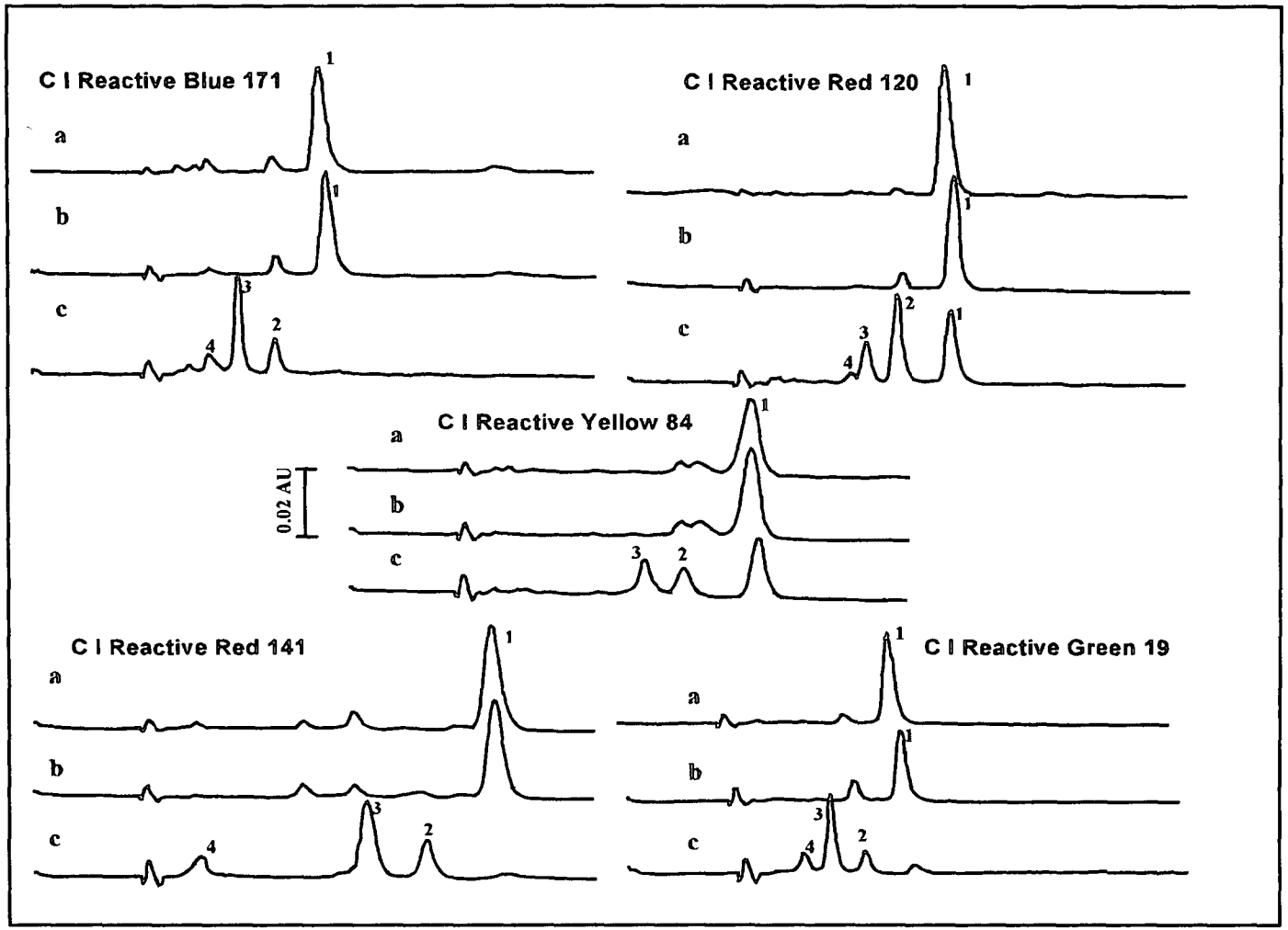

Figure 6.12 : Chromatograms of (a) the dye dissolved in water, (b) the dye and sodium sulphate dissolved in water, heated at 95°C for 20 min and (c) the latter solution after the addition of sodium carbonate and heated at 80°C for 60 min; where 1 = the parent dye, 2 = the monohydroxy derivative, 3 = the dihydroxy derivative and 4 = unidentified hydrolysis product. Analysis conditions as described in **Appendix 3** for the Waters HPLC system.

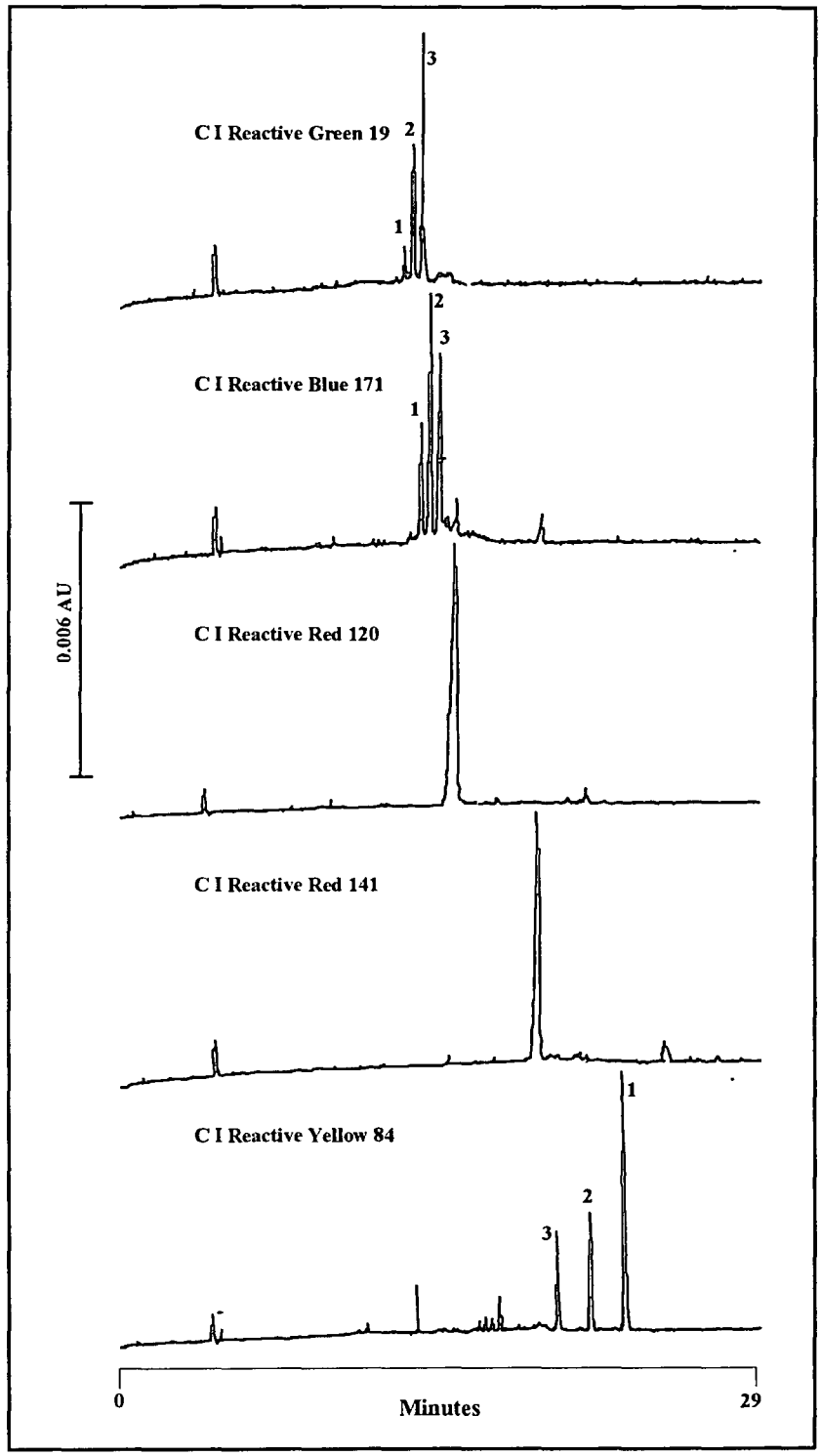

Figure 6.13 : Electropherograms of the dye solution after the addition of sodium carbonate and heated at 80°C for 1 h, where 1 = the parent dye, 2 = the monohydrolysed derivative and 3 = the dihydrolysed derivative. Analysis conditions as described in **Appendix 3.**

NB: Since the hydrolysis products of C I Reactive Red 141 and 120 were unresolved an alternate CE technique was investigated, discussed in **Section 6.6.**

6.5.2 Residual Dye-bath Liquors

Before dyeing is done in bulk at the Dye-house, the dyeing is first initiated in the laboratory. This process is known as the initial Laboratory Dyeing Process. The objective in this section was to conduct dyeings using the Laboratory Dyeing Process and establish if the analytical technique (HPLC) used in the latter section could be applied to analysing the residual dye-bath and residual rinse liquors. The experiments carried out in this section were done at Dyeco (Pty) Ltd in Hammersdale at the conditions used by the dye-house.

Laboratory Dyeing Process

The process involved here is dyeing a 10 g sample of the bulk fibre, under similar conditions to an industrial dyeing process, in a small laboratory dyeing machine. The sample dyeing is done in stainless steel dyeing tubes of 250 mℓ capacity called a dye bomb. The heating medium was polyethylene glycol. The dye bomb is constantly rotated in the medium until dyeing is complete. Laboratory dyeing and bulk dyeing are done at a fabric to liquor ratio of 1 : 10. The amount of dye to be used is dependent on the shade required. A light shade may require as little as 0.05 % of total dye. On the other hand, some dark shades require at least 5 % of the dye. All dyeings were carried out in a FONG's Laboratory Dyeing Machine with individual dyes (0.1 %) using the method shown in Fig 6.14.

At the end of the dyeing process the dyed fabric was removed from the dye-bath and excess liquor squeezed from the sample, collected and marked *residual dye-bath liquor*. The dyed sample was then rinsed with 100 mℓ of tap water at room temperature, excess liquor squeezed from the sample, collected and marked *residual rinse liquor*. The dyed sample was then treated with 100 mℓ of soap solution at the boil for 5 min, excess liquor squeezed from the sample, collected and marked *soap wash*. All samples were then dialysed against distilled water for 12 h. All samples were analysed by HPLC

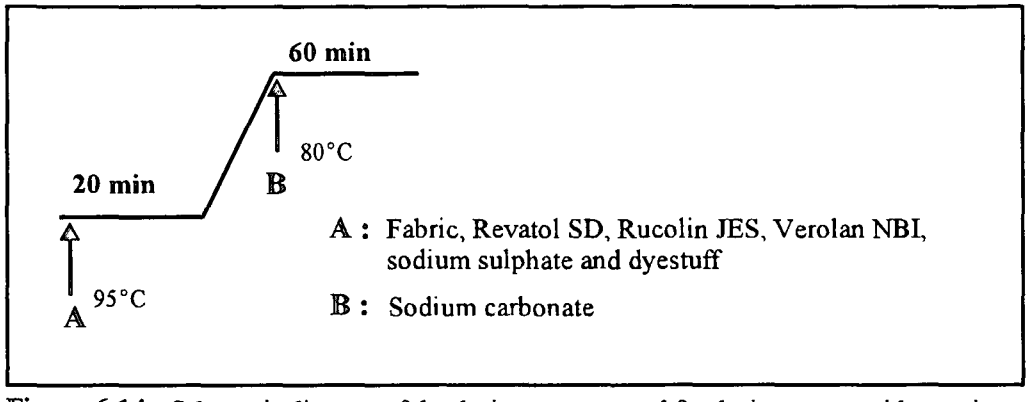

Figure 6.14 : Schematic diagram of the dyeing process used for dyeing cotton with reactive dyes by the Laboratory Dyeing Process.

The following quantities were employed:

A 100 mg/100 mℓ Revatol SD - Anti-reducing agent
100 mg/100 mℓ Rucolin SD - Lubricant (anti creasing agent)
100 mg/100 mℓ Verolin NBI - Sequestering agent
7 g/100 mℓ Sodium Sulphate
100 mg/100 mℓ dye

B 1.5 g/100 mℓ Sodium carbonate

6.5.2.1 Analysis of Residual Dye-bath Liquors

The results from the residual dye-bath liquor, shown in Fig 6.15 (b), showed significant amounts of the parent dye still present at the end of the dyeing process especially for C I Reactive Red 141and C I Reactive Green 19. Fig 6.15 (c) represents the chromatograms obtained at the end of the first cold rinse procedure that followed dye application. The rinse liquor showed similar trends observed in the residual dye-bath liquor. However, in the soap wash samples, shown in Fig 6.15 (d), the dihydrolysed derivative was the main product except C I Reactive Red 120 where both derivatives were almost equivalent. C I Reactive Blue 171 showed a much wider range of products than the other dyes but this may have been due to the initial product having a greater degree of contaminants.

The results obtained, indicated that incomplete dye hydrolysis occurred during application of the dyes to cotton. It should be noted that the total concentration of all dye species in the rinse liquor was lower than in the soap washes. This is in keeping with theory, that the dyes have a high substantivity for the substrate and very little of the unfixed dye is removed in the first cold rinse (Duff and Sinclair, 1990).

There were variations between the samples prepared for dye hydrolyses under dye-bath conditions and the dye-bath effluent from the Laboratory Dyeing Process but similarities were also evident. Reasons for the variations could be due to the omission of the various additives added during the dyeing process. This may also be due to the textile substrate not being included during preparation of the hydrolysis derivatives, hence, concentration of dye in solution was much higher resulting in more dye being available for reaction. The evidence of the parent dye at the end of dyeing process is an area of concern in terms of health effects since the reactive forms are electrophilic compounds and thus gives concern about their ecotoxicity. Furthermore, this implies that the dyeing processes are not optimised resulting in dye loss.

The chromatograms shown in Fig 6.15, indicate that the separation of the products formed, when a single dye is employed during the reactive dyeing process, can be achieved by HPLC. The results are of interest as information concerning relative dye uptake can be studied in detail. Assessment of the level of hydrolysis occurring in the bulk dye-bath and also the relative amount of hydrolysed/unhydrolyzed dyes present upon subsequent rinsing are of interest with regard to textile effluent.

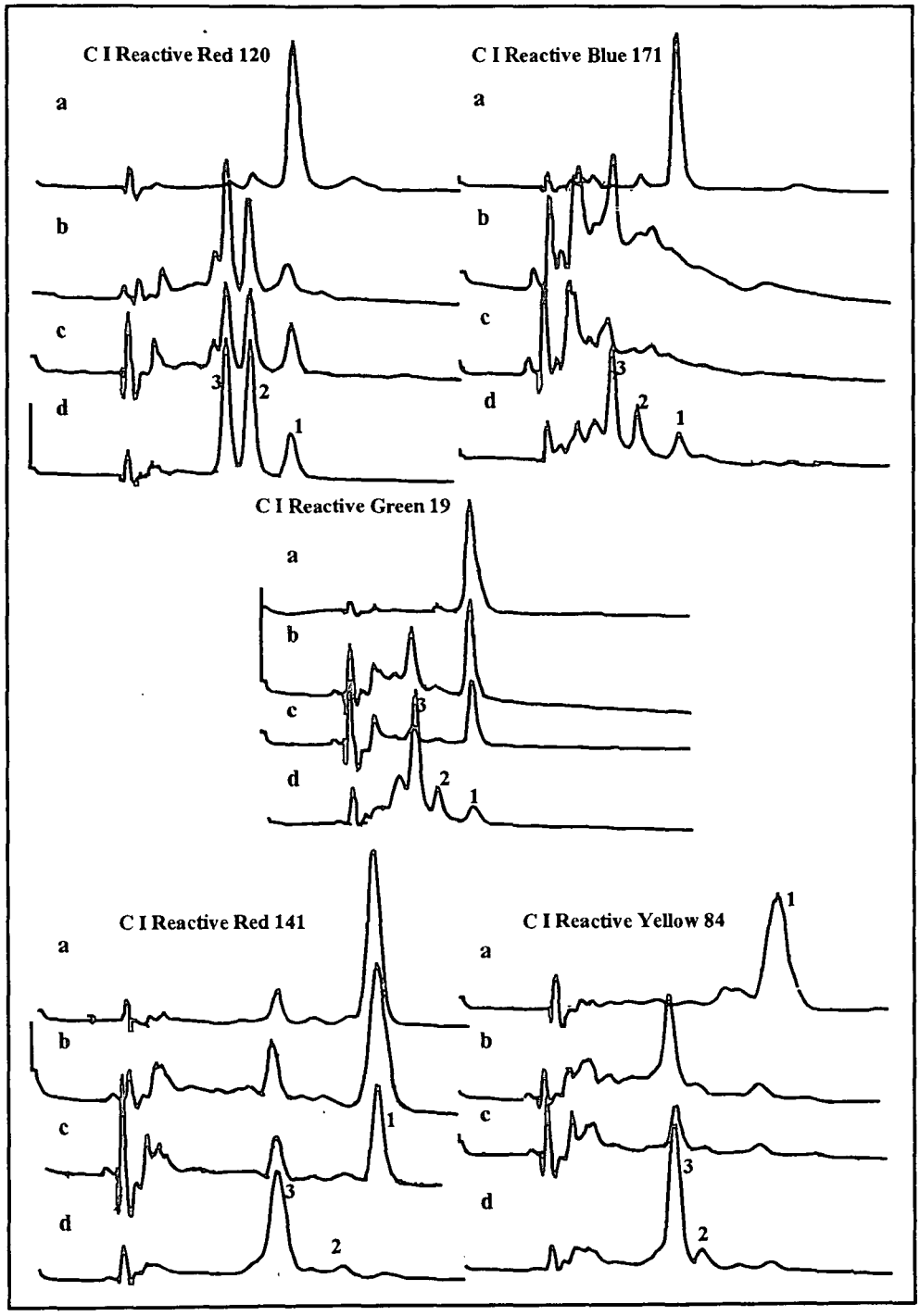

Figure 6.15 : Chromatograms of the dye-house laboratory samples, (a) dye dissolved in water, (b) residual dye-bath liquor; (c) residual rinse liquor and (d) soap wash at the boil; where 1 = the parent dye, 2 = the monohydroxy derivative and 3 = the dihydroxy derivative. Analysis conditions as described in **Appendix 3** for the Waters HPLC system.

6.5.3 Conclusions

The HPLC technique developed provides a suitable methodology for analysing both the residual dye-bath and residual rinse liquors when studying individual dyes in a dye-bath. A disadvantage of the methodology, is the need to dialyse all samples prior to analysis. Perhaps further developments in the methodology could overcome this shortfall by using CE as an analytical tool. As noted, zone broadening was observed during the analysis of the dye-bath sample by CZE prior to dialysis. Zone broadening or peak deformations are generally related to conductivity differences between the analyte zone and buffer. A possibility for future research is to consider using CZE with buffers of much higher conductivity.

From the above results it is evident that the textile effluent could include both the parent dye and the hydrolysis products, thus making it a complicated mixture, but more important is the greater health risk associated with the reactive form of the dye. The evidence of various products in the effluent indicates the need to determine the transformation pathways for these chemicals in aquatic ecosystems.

To access the environmental fate of these dyes it would be necessary to study the hydrolysis kinetics and its disappearance kinetics in natural water and in an anaerobic water system. What can, however, be said, the HPLC method used and the alternate CE method described in **Section 6.6.1** could be used to conduct these experiments for individual dyes. However, assessment of multiple dyes mixtures in a dye-bath, could prove quite difficult using the HPLC technique presented here. The CE method presented later, **Section 6.6.3** shows potential for overcoming this drawback.

6.6. MICELLAR ELECTROKINTEIC CHROMATOGRAPHY

The work in this section is aimed at separating the C I Reactive Red 141 and 120 from their hydrolysis derivatives using micellar electrokinetic chromatography (MEKC).

Micellar Electrokinetic Chromatography (MEKC or MECC) is a hybrid of electrophoresis and chromatography (see **Appendix 2** for more details). Separation is accomplished by the addition of a surfactant to the run buffer (Heiger, 1992). The separation of analytes is achieved by the difference in the partitioning between the micellar phase and the aqueous phase and also according to electrophoretic mobility. This method is reported to have advantages over CZE in the separating of both electrically neutral and ionic substances (Nielen, 1993).

6.6.1 Separation of the Hydrolysis Products of C I Reactive Red 141 and 120

The objective in this section was to evaluate the use of MEKC for the separation of C I Reactive Red 141 and C I Reactive Red 120 from their hydrolysis products by adding sodium dodecyl sulphate (SDS) at varying concentrations to the CZE buffer previously used.

The critical micelle concentration of SDS is 8.27 mM (Heiger, 1992). SDS was added at concentrations of 10 mM, 25 mM and 50 mM to the phosphate/borate buffer previously used. These buffers were tested for their separation efficiency for C I Reactive Red 141 and C I Reactive Red 120 from their hydrolysis products.

6.6.1.1 Analysis of Results using the SDS buffer

The phosphate/borate buffer at varying concentrations of SDS resolved the hydrolysis products for C I Reactive Red 141 but at 50 mM SDS the C I Reactive Red 141 peak was broad, as shown in Fig 6.16. On the other hand, C I Reactive Red 120 gave a broad peak with poor resolution at 10 mM SDS but was resolved at 25 mM SDS and a loss of resolution again at 50 mM SDS as shown in Fig 6.17.

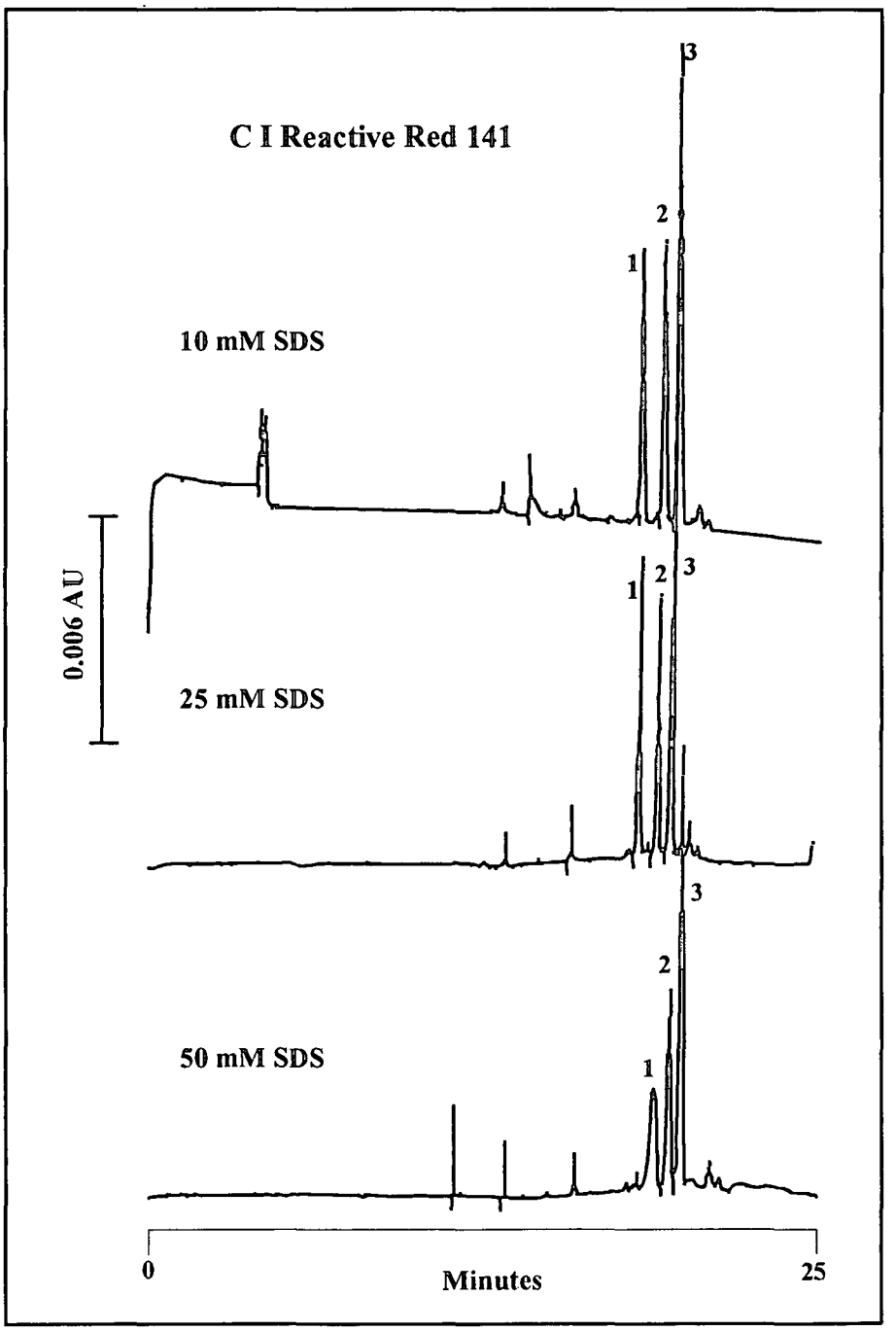

Figure 6.16 : Electropherograms of the hydrolysed dye at varying SDS concentrations in the MEKC buffer, where 1 = the parent dye, 2 = the monohydroxy derivative and 3 = the dihydroxy derivative. Analysis conditions as described in **Appendix 3**.

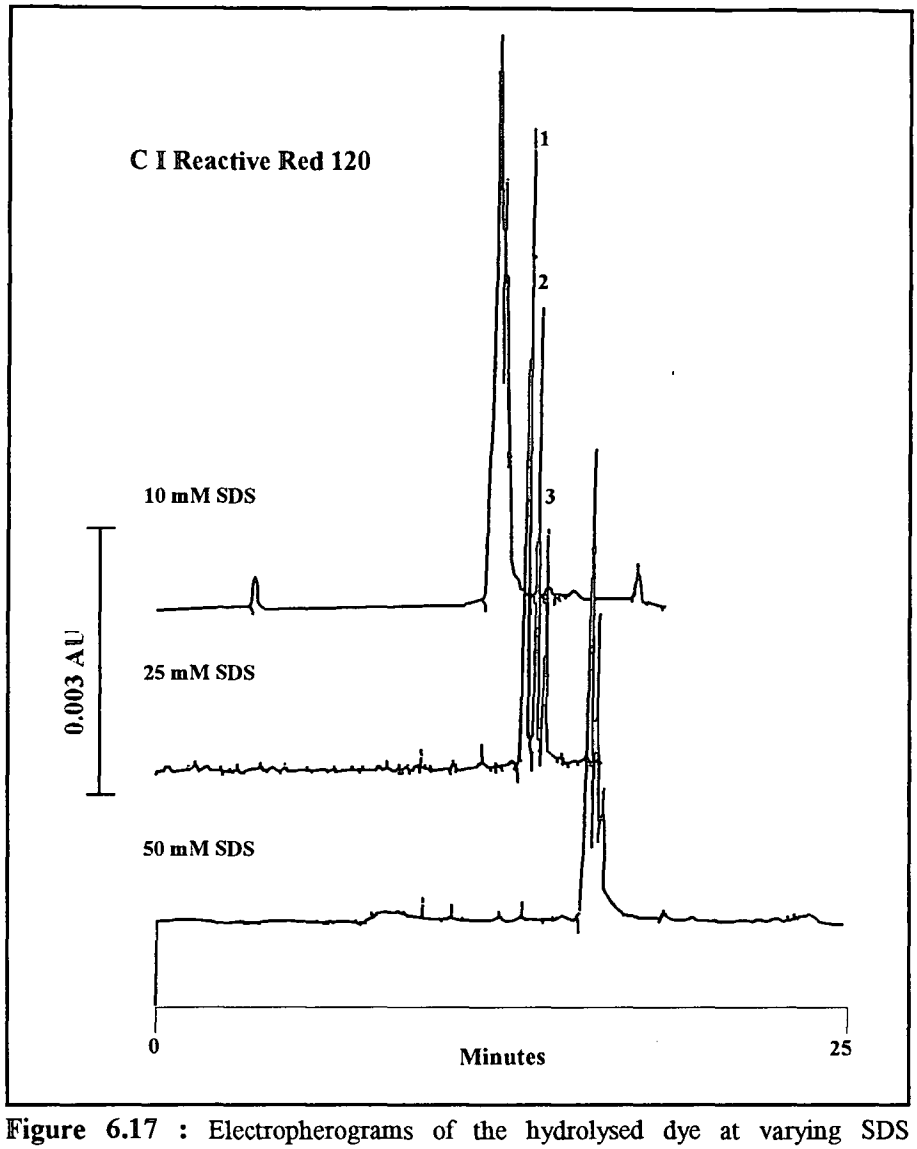

Figure 6.17 : Electropherograms of the hydrolysed dye at varying SDS concentrations in the MEKC buffer, where 1 = parent dye, 2 = the monohydroxy derivative and 3 = the dihydroxy derivative. Analysis conditions as described in **Appendix 3.**

From these three buffers, the 25 mM SDS buffer showed the best separation efficiency for both C I Reactive Red 141 and C I Reactive Red 120 and their hydrolysed derivatives. An increase in migration time with an increase in SDS concentration was observed for C I Reactive Red 120 only and no such change was observed for C I Reactive Red 141. Peak shape was affected by SDS concentration. At lower concentrations C I Reactive Red 120 gave broad poorly resolved peaks whereas at higher concentrations C I Reactive Red 141 gave a broad peak but its hydrolysis products were unaffected.

Generally, peak deformations can be explained by conductivity differences between the zone of the analyte and the carrier electrolyte (Suzuki et al., 1994). However, this explanation may not be applicable for these dyes since some dye peaks showed good peak shape whereas others did not. Furthermore, C I Reactive Red 120 and C I Reactive Red 141 have very similar structures but their differences in hydrolysis behaviour as noted previously, peak shape and separation efficiency at the buffers tested belie this fact. In addition, no significant differences were observed for these dyes on the HPLC system in terms of peak shape and degree of separation.

The origins of these effects are unknown, but perhaps the impurities present in the dyes have some effect. Further investigations into these effects will be necessary for future clarity. However, these observed effects could be used as an advantage to identify peaks in complex mixtures. This effect was used as an identifying mechanism in **Section 6.6.3.**

6.6.2 Methods to Reduce Analysis Time

The objective in this section was to reduce analysis time as follows:

1) Adjust the buffer pH.
2) Reverse the polarity such that anionic compounds have the shortest migration time.
3) Add an ion-pairing reagent to the buffer system to reduce the negative charge and thus reduce migration time.

The CE analysis time was approximately 20 to 25 min for both CZE and MEKC. Taking into account the need for rinsing the capillary between injections, **Appendix 3**, the total time for CE runs was 30 to 35 min. Such long analysis time are certainly not ideal, hence, methods to reduce analysis time were sought.

Methods to reduce analysis time were investigated using C I Reactive Red 141 as a representative of this class of dyes. From a practical point of view the greatest changes in the electroosmotic flow (EOF) can be made by altering the pH value of the buffer. In changing the pH value from 8.5 to 10, C I Reactive Red 141 showed a definite decrease in analysis time from 25 to 15 min and an increase in resolution. However, in analysing the dye mixture, some peaks disappeared or decreased as

reported in **Chapter 5**. The use of pH as a means of reducing analysis time was thus not investigated further.

Since these dyes are highly negative, they have an electrophoretic mobility towards the anode, opposite to the EOF and, hence, have long migration times compared to cations and neutral species. A study by Oxspring an co-workers (1995) reported reduced analysis time for anionic compounds by reversing the EOF. This was achieved by employing a buffer with a low pH value and applying a negative voltage to the capillary which in turn reverses the direction of the EOF. This procedure changes the order in which species migrate through the capillary to anions, neutrals and cations.

At the time of investigation the Beckman P/ACE system used, did not have the facility of applying a negative voltage, thus polarities had to be changed manually. However, an attempt at using this technique, with polyvinyl alcohol as the run buffer, resulted in the capillary being blocked and this technique was not investigated any further.

In further attempts at reducing analysis time, it was realized that if the negative charge on the dyes could be reduced then analysis time would decrease. With this view an ion-pairing reagent, tetrabutylammonium bromide (TBAB) used during the HPLC investigations, was added to the run buffer. Phosphate/borate buffers with 10 mM SDS and with either 1, 5 and 10 mM TBAB were prepared and tested. The separation of C I Reactive Red 141 and its hydrolysed products using an ion-pairing reagent resulted in excellent separation and a significant decrease in analysis time at 5 and 10 mM TBAB concentrations as shown in Fig 6.18. An increase in resolution is clearly evident in the buffer with 5 mM TBAB as shown in Fig 6.18 (b).

The increase in resolution was unexpected, however, upon closer examination it was realized that the addition of an ion-pairing reagent to reduce analysis was an oversimplified concept.

Since SDS acts as a *pseudo-stationary phase* (Heiger, 1992), there would be an increase in the interactions between the SDS micelle and the now less ionic dye - a mechanism similar to that of reversed phase ion-pair chromatography. Thus, the retention time should have increased with increased interactions.

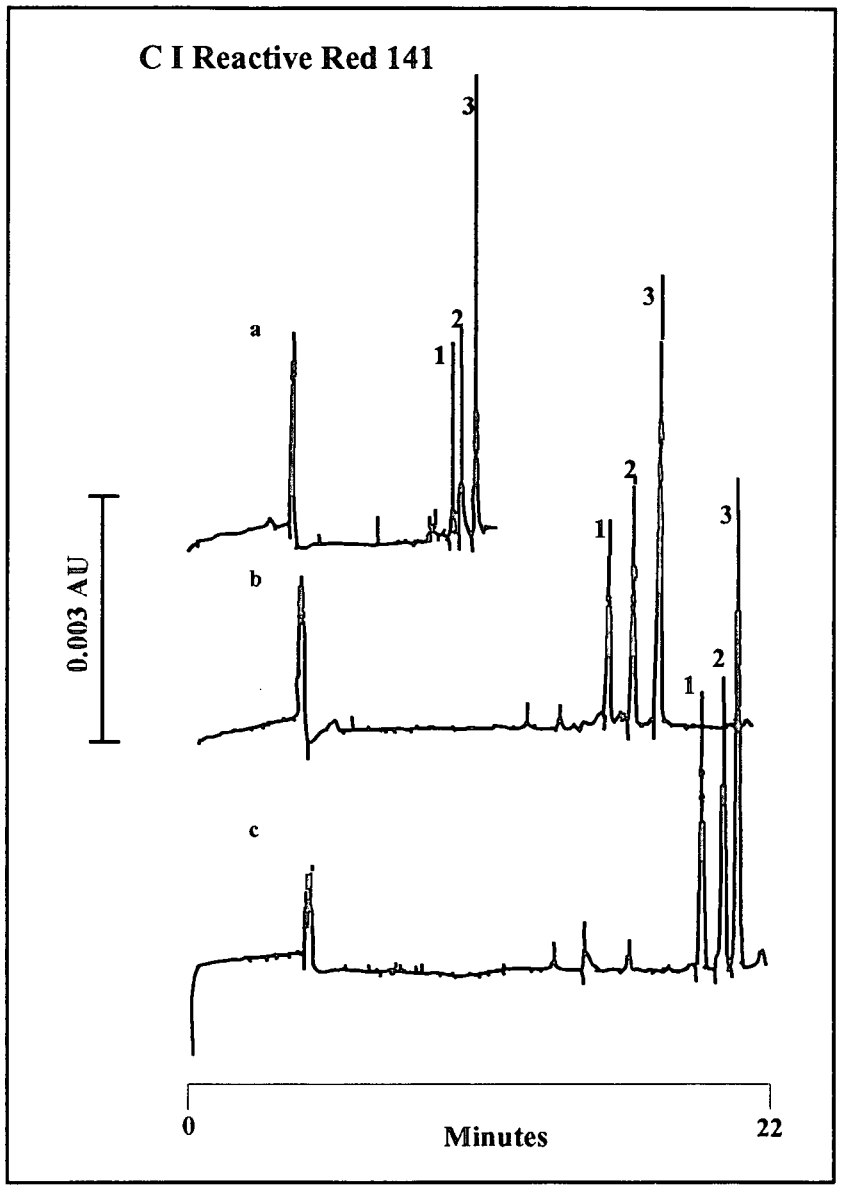

Figure 6.18 : Electropherograms of C I Reactive Red 141 and its hydrolysis products at (a) 10 mM SDS and 10 mM TBAB, (b) 10 mM SDS and 5 mM TBAB and (c) 10 mM SDS and 1 mM TBAB; where 1 = parent dye, 2 = the monohydroxy derivative and 3 = the dihydroxy derivative. Analysis conditions as described in **Appendix 3.**

A reasonable explanation for these results was sought in the literature. Research by Nishi and Tsumagari (1989) reported an increase in the analysis time with an increase in resolution for anionic compounds with the addition of an ion-pairing reagent to the buffer. The concentrations of SDS and

ion-pairing reagents used in the latter paper were 50 to 200 mM SDS and 20 to 40 mM tetramethylammonium bromide which where significantly higher than the buffers used in this study. An attempt at using higher SDS concentrations, 50 mM and 25 mM, together with the ion-pairing reagent resulted in very high currents being generated and no conclusive results achieved. At 20 mM SDS the addition of 10 mM TBAB to the buffer resulted in improved resolution and a decrease in analysis time as shown in Fig 6.19. However, the decrease was not as significant as the buffer with 10 mM SDS.

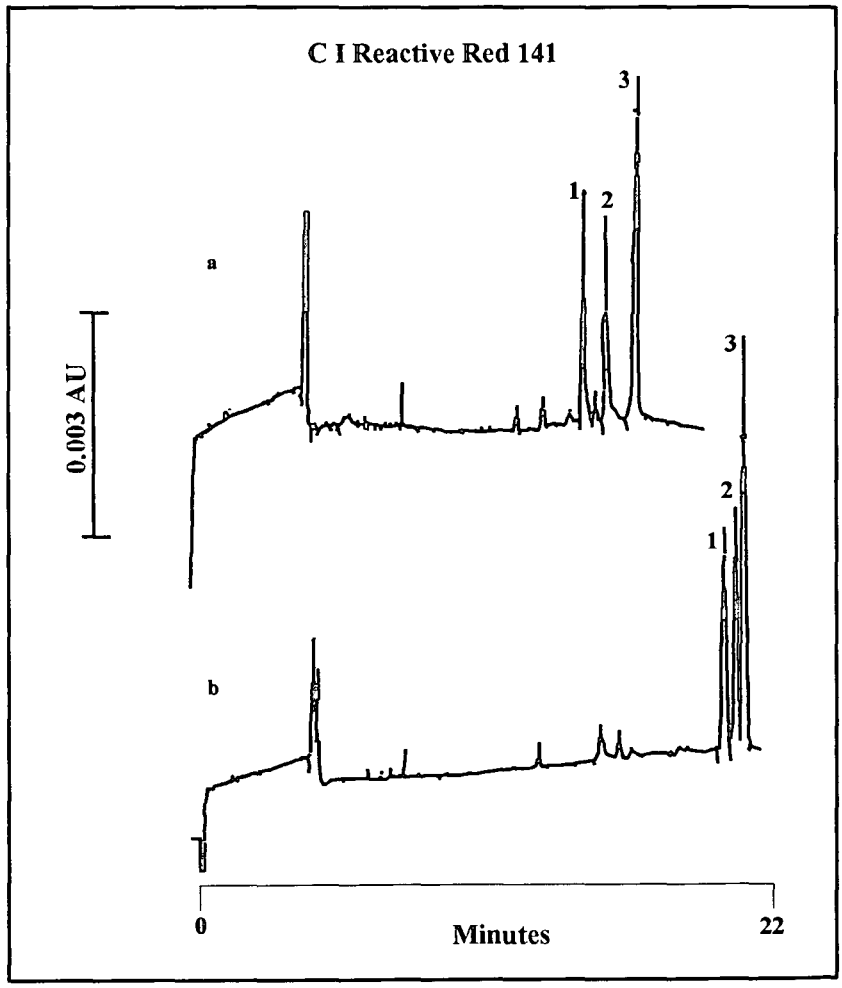

Figure 6.19 : Electropherograms of C I Reactive Red 141 and its hydrolysis products at (a) 20 mM SDS and 5 mM TBAB and (b) 20 mM SDS; where 1 = parent dye, 2 = the monohydroxy derivative and 3 = the dihydroxy derivative. Analysis conditions as described in **Appendix 3.**

A possible explanation is that the addition of the ion pairing reagent to the SDS buffer affected the micelle structure or reduced the micelle concentration or perhaps destroyed the micelle structure. The mixture of 5 dyes, analysed using the phosphate/borate buffer with 10 mM SDS at varying concentrations of TBAB, showed a significant loss of resolution at concentrations of 5 and 10 mM TBAB. However, the phosphate/borate buffer with 10 mM SDS and 1 mM TBAB showed improved peak shape for C I Reactive Red 120, shown in Fig 6.20, which was previously broad. There was no change in analysis time.

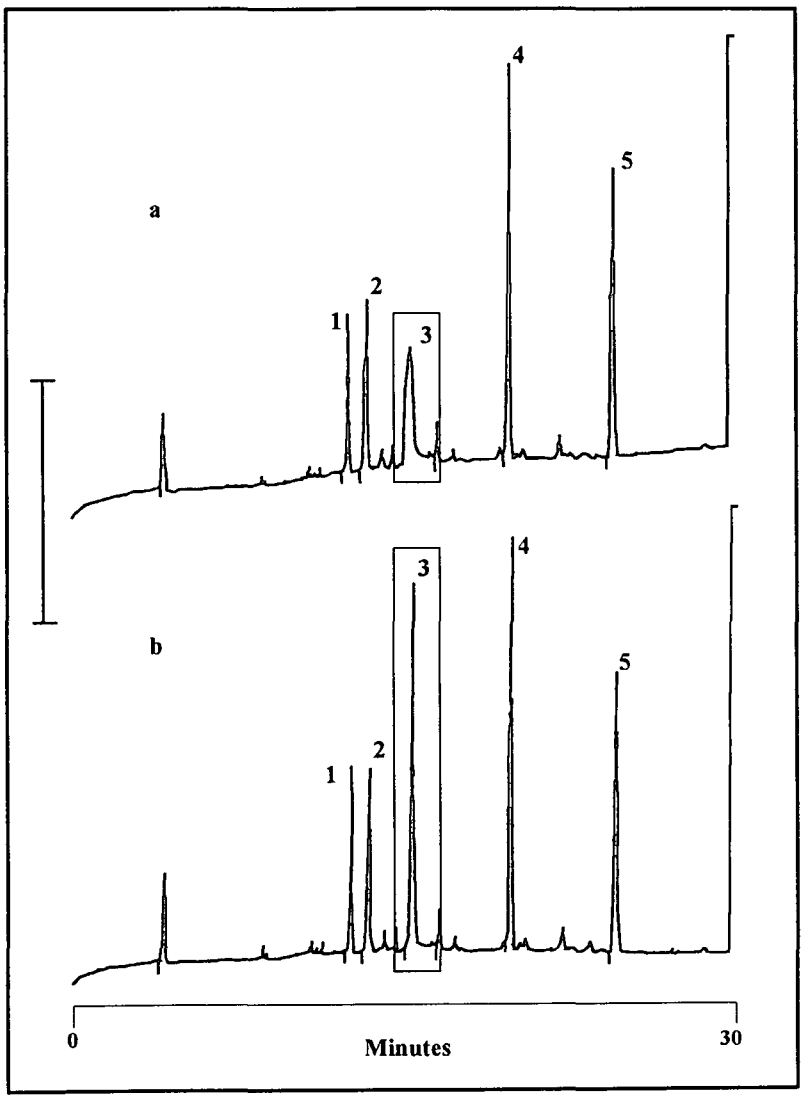

Figure 6.20 : Electropherograms of a mixture of 5 dyes using the phosphate/borate buffer at (a) 10 mM SDS and (b) 10 mM SDS with 1 mM TBAB. Peaks: 1 = C I Reactive Green 19; 2 = C I Reactive Blue 171; 3 = C I Reactive Red 120; 4 = C I Reactive Red 141 and 5 = C I Reactive Yellow 84. The highlighted area shows the effect of the ion-pairing reagent on peak shape. Analysis conditions as described in **Appendix 3.**

6.6.3 Analysis of Hydrolysed Dye Mixture

The objective in this section is to test the analytical techniques developed for the analysis of a complex mixture, i.e. a mixture of hydrolysed dyes. The rationale behind this was that in an industrial dyeing process the desired colours, for the C I Reactive HE range, are almost always achieved by using a mixture of 3 dyes.

An attempt was made at trying to resolve the hydrolysed mixture of the 5 dyes using both HPLC and CE. However, due to the number of products formed during hydrolysis, the HPLC method used showed poor separation. The organic concentration in the mobile phase was decreased in order to improve separation. This resulted in an improvement in separation however, significant band broadening and loss of sensitivity occurred due to the long analysis time of 35 min. This method did not seem to show much potential for analysing such a complex mixture.

Separation using capillary electrophoresis was investigated with the buffers used in the previous section. Using the phosphate/borate buffer with (a) 10 mM SDS and (b) 10 mM SDS with 1 mM TBAB showed the potential of using CE for complex mixture analysis as illustrated by the electropherograms in Fig 6.21. The buffer with the TBAB showed much better separation efficiency than the buffer with SDS only. Due to the very close migration times between peaks, and the changes in migration times between runs, the hydrolysed dye mixture had to be spiked with the parent dye to establish which peaks were due to the parent dye. The hydrolysed peaks were not positively identified since standards were unavailable.

The area blocked on the electropherograms in Fig 6.21, highlights the improved separation on addition of the ion pairing reagent. Between **Peaks 2** and **3,** four distinct peak are visible, the first 2 are most probably due to the hydrolysis products of C I Reactive Green 19 and the next 2 to the hydrolysis products of C I Reactive Blue 171. The peaks due to C I Reactive Red 120 and its hydrolysis products are easily identified from the peak shape, previously mentioned and observed in Fig 6.17. The addition of 1mM tetrabutyl ammonium bromide to the SDS buffer improved peak shape and resolution for C I Reactive Red 120. There are 3 peaks between C I Reactive Red 141 and C I Reactive Yellow 84; the first two of these peaks are most probably due to the hydrolysis products of C I Reactive Red 141 since the migration times are comparable to that obtained in Fig 6.16. The last of these 3 peaks is most probably due to one of the hydrolysis products of

C I Reactive Yellow 84, with the second product co-migrating with one of the hydrolysis products of C I Reactive Red 141. However, these assignments are not definite but illustrate the potential of MEKC with an ion pairing reagent to separate complex mixtures. Hence, its application in analysing complex residual dye-bath liquors, textile effluent and environmental samples.

An interesting observation is the improved separation and peak shape for some dyes with the addition of a low concentration of the ion-pairing reagent. Suzuki et al. (1994) in the analysis of some azo food dyes reported *no effect of pH, buffer, SDS or sample concentration was observed on the peculiar fronting of Allura Red AC*. From the results obtained in this investigation, it would seem that the addition of an ion pairing reagent to the SDS buffer could resolve the problem of poor peak shape. No such effects have been reported in the literature reviewed thus far. The reasons for these effects need to be investigated further and could be applied to similar problems in other areas of interest

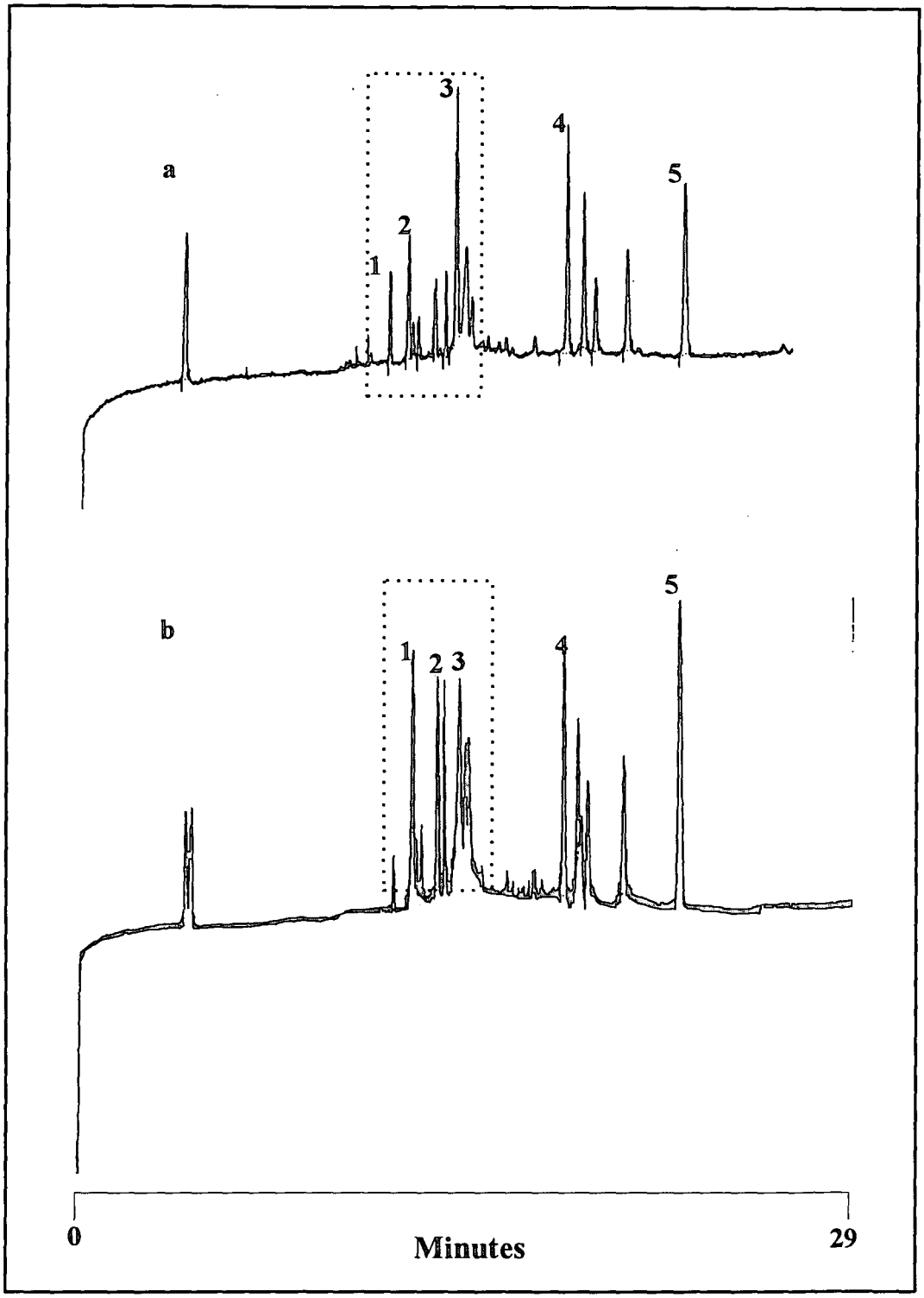

Figure 6.21 : Electropherograms of the hydrolysed mixture of 5 dyes using buffers (a) 10 mM SDS with 1 mM TBAB, and (b) 10 mM SDS. Peaks 1 = C I Reactive Green 19; 2 = C I Reactive Blue 171; 3 = C I Reactive Red 120; 4 = C I Reactive Red 141 and 5 = C I Reactive Yellow 84. Analysis conditions as described in Appendix 3.

6.7 CONCLUSIONS

Methods for the determination of reactive dyes and their hydrolysis products were investigated using high performance liquid chromatography (HPLC) with an ion pairing reagent, capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). Of the 5 selected dyes, all 5 dyes were well resolved from their hydrolysis products using the HPLC method developed in **Chapter 5**. However, the CZE method used was unsuccessful in resolving the parent dye from its hydrolysis derivatives for C I Reactive Red 141 and C I Reactive Red 120. Thus, an alternate capillary electrophoresis technique, MEKC, was investigated. Using this technique with 25 mM sodium dodecyl sulphate added to the CZE buffer, C I Reactive Red 141 and C I Reactive Red 120 were resolved from their hydrolysis products.

This chapter included a number of investigations not initially foreseen. In the hydrolysis of C I Reactive Red 141, it was observed that more than 2 hydrolysis products formed depending on the pH and temperature of the reaction. The third product was assigned to the product/s formed as a result of the imino bridge groups being hydrolysed. Further investigations would have to conducted to confirm this assignment, however, the work cited by Morita (1996) gives strong evidence that these are the most likely products.

An interesting observation was the non-retention of the dye-bath samples on the HPLC column, which was found to be related to the concentration of sodium sulphate in the dye-bath. This effect of non-retention may also be a reason for the lack of literature on the analysis of dye-bath effluent using HPLC. Due to this effect, all dye-bath samples had to be dialysed to remove the salts prior to analysis by HPLC. It was proposed that the non-retention of the dye-bath samples was due to dye aggregation in the presence of salts.

In order to overcome the necessity of dialysis, the dye-bath samples were also analysed by CE prior to dialysis. However, zone broadening was observed due to the high ionic strength of the dye-bath solution. Thus, all samples were dialysed prior to analysis.

In the analysis of the dye-bath effluent, using HPLC, there is evidence of the reactive form of the dye still present at the end of the dyeing cycle, indicating incomplete dye hydrolysis. This is of concern

in terms of its toxicity, and points strongly to more effort required in monitoring these dyes in the aquatic system. The methods presented in this chapter show potential for studying these pollutants.

The use of micellar electrokinetic chromatography for the separation of the hydrolysis products shows immense resolving power for closely related substances. Finally, the addition of an ion-pairing reagent to the MEKC buffer showed it capability of improving resolution, peak shape and reducing analysis time. Further research in this area is certainly needed, both in terms of dye analysis as well as other ionic analytes. Explanations for the effects observed will also require further research.

Chapter 7

DEVELOPMENT OF METHODS FOR THE EXTRACTION OF REACTIVE DYES FROM WATER

The aim in this chapter is to develop extraction methods for reactive dyes from water systems. In the analysis of environmental pollutants, important stages are sample clean-up, extraction and concentration. Current analytical methods of polar ionic compounds from water consist of acidification of the sample, followed by liquid-liquid extraction (LLE) (Soniassy et al., 1994). Currently, there is a tendency to change from LLE to solid phase extraction. In the analysis of dyes in wastewater the general trend has been the use of solid phase extraction methods as reported in **Chapter 3**. Using this technique, the recoveries of dyes with a number of sulphonic acid groups have generally been lower than their unsulphonated or monosulphonated derivatives. The reason for low recoveries for sulphonated dyes, is probably due to the high water solubility conferred by the sulphonic acid groups.

Schramm et al. (1988) reported recoveries of 90 to 95 % for C I Reactive Red 4, a dye with four sulphonic acid groups, from spiked water using liquid-liquid extraction with an ion-pairing reagent. Thus, LLE with an ion-pairing reagent was chosen for the investigation.

C I Reactive Red 141 was chosen as a representative of the selected dyes and its extraction efficiency evaluated from tap water and river water. The rationale behind this approach was to test if substances in river water such as humic substances have any effect on the extraction efficiency and if other interferants are co-extracted from river water. Although, it was acknowledged that the expected product in textile effluent and hence river water would be the hydrolysed derivative, the parent dye is regarded as being a greater health risk, since the reactive form is electrophilic and concerns about its possible ecotoxicity and mutagenecity have been raised (Richardson et al., 1993). Second, although the hydrolysed dye is slightly more polar than the parent dye, its extraction behaviour is expected to be similar to the parent dye.

Preliminary investigations into the extraction of reactive dyes from tap water spiked with C I Reactive Red 141 were conducted by B Tech student (D Pillay). Pillay (1997) reported that as

the pH value of the phosphate buffer increased, the extraction recoveries increased with recoveries ranging from 60 to 85 % . The reason for the lower recoveries obtained compared to the published values was concluded to be related to the much higher molecular mass and twice the number of sulphonic acid groups on C I Reactive Red 141 compared to C I Reactive Red 4.

However, upon examination of the chromatograms in Pillay's report it was observed that there were non-retained peaks in the chromatograms of the extracted samples, as seen in the chromatograms of the dye-bath sample reported in **Section 6.2**. In studying the extraction method used by Pillay, it was found that in the final extraction step, the concentration of the phosphate buffer used was 100 mM. It was speculated that the non-retained peak observed in the sample chromatograms was a result of the concentration of the phosphate buffer resulting in dye aggregation and *false* recoveries.

Hence, the objective of this investigation is to test recoveries of C I Reactive Red 141 from spiked tap and river water using a lower concentration of the extraction buffer.

Initially, the extraction recoveries were analysed by CE only, due to instrumental problems with the HPLC system at the time of the investigation. However, in order to test the recoveries by HPLC and confirm the elimination of dye aggregation, extractions were conducted on spiked tap water only at a later date.

Due to its short analysis time of 10 min, all CE analysis were performed using the phosphate/borate buffer with 10 mM sodium dodecyl sulphate (SDS) and 10 mM tetrabutylammonium bromide at a pH value of 8.5. The calibration of the CE system using this buffer was evaluated and the results presented in **Appendix 4**. The analysis by HPLC was on the Waters HPLC system. The analysis conditions for the CE and HPLC techniques used are as described in **Appendix 3**. All recoveries were calculated using a single point calibration and the data and results are presented in **Appendix 5**.

7.1 LIQUID-LIQUID EXTRACTION

The objective in this section is to evaluate recoveries of C I Reactive Red 141 from spiked tap water and river water using a lower concentration of the extraction buffer.

The method used by Schramm et al. (1988) was as follows:
A volume of 1 mℓ 25 % tetrabutylammonium bromide (TBAB) aqueous solution was added to 100 mℓ of spiked water sample. This mixture was shaken for 30 min with 10 mℓ cyclohexanol in a 250 mℓ separatory funnel. The dye-TBAB complex is extracted into the organic phase.The organic phase was extracted with 1 mℓ of phosphate buffer (pH 6) and 30 mℓ hexane by shaking vigorously for 30 s. The mixture separates into an organic phase (hexane and cyclohexanol) and an aqueous phase (phosphate buffer and cyclohexanol dissolved in water) into which the dye is extracted. The aqueous phase was collected and the organic phase re-extracted with 0.5 mℓ buffer. The aqueous phases were combined, the volumes measured and the extract analysed by HPLC.

The dye, C I Reactive Red 141, was dissolved in water to give a standard solution of 100 mg/ℓ. In the recovery experiments 100 mℓ of tap or river water was fortified with this solution to obtain a concentration of 1 mg/ℓ of the dye.

7.1.1 Extraction of C I Reactive Red 141 from Tap Water

The objective in this section was to evaluate recoveries of C I Reactive Red 141 from spiked tap water using a lower concentration of the extraction buffer at varying pH values.

The method described by Schramm was tested as described on 100 mℓ of tap water using a 20 mM phosphate buffer at pH values of 6; 7 and 8. The volume of TBAB was increased to 2 mℓ because incomplete extraction was noted (visual) with just 1 mℓ of the TBAB solution. The combined extracted volume was 2 mℓ. Extractions were done in triplicate and analysed by CE. The recoveries of C I Reactive Red 141 at the different buffer pH values showed that the extraction was essentially complete at all pH values tested, Table 7.1.

Table 7.1 : Recovery of a 1mg/ℓ solution of C I Reactive Red 141 from tap water, analysis by CE.

Buffer pH	% Recovery			Average % Recovery
	Ext 1	Ext 2	Ext 2	
6	110.99	94.27	98.47	101.24
7	101.58	105.71	102.55	103.28
8	97.27	93.06	101.72	97.35

From these results, the pH value of the buffer does not seem to have a significant effect on the recoveries. Thus, all subsequent extractions were carried out using an extraction buffer at a pH value of 6.

The extraction recoveries by HPLC were tested a few months later. Six extractions were prepared as above and analysed by HPLC, the results are presented in Table 7.2. From the chromatograms of the extracted samples, no evidence of a non-retained peak was observed. From the results presented below, it is clear that the use of an extraction buffer at a lower concentration of 10 mM compared to 100 mM (as used by D Pillay) eliminated the effect of dye aggregation and close to 100 % recoveries were achieved.

Table 7.2 : Recovery of a 1mg/ℓ solution of C I Reactive Red 141 from tap water, analysis by HPLC.

Number	% Recoveries
1	102.67
2	97.52
3	99.52
4	101.17
5	100.99
6	101.17
Average	**100.51**

In comparing the recoveries achieved by CE and HPLC, there were greater deviations in the CE analysis with recoveries ranging from 93 to 110 % whereas recoveries by HPLC were between 97 to 102 %. This variation was probably due to the negative peak observed in Fig 7.1, interfering with the analyte peak resulting in poor integration of the analyte peak. This peak was only evident in the sample runs and not the standards. The reason for the negative peak was not investigated but may be due to the cyclohexanol dissolved in the aqueous phase, this however, did not have any effect on the HPLC chromatograms, hence better recoveries were achieved.

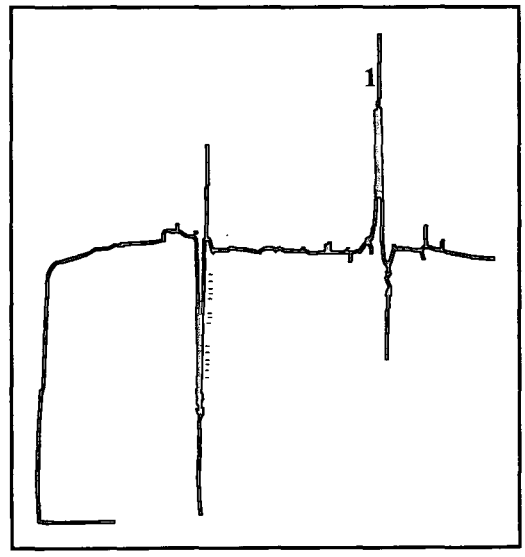

Figure 7.1 : Electropherogram for an extracted tap water sample where 1 = C I Reactive Red 141. Analysis conditions as given in **Appendix 3.**

7.1.2 Extraction of C I Reactive Red 141 from River Water

The objective in this section is to evaluate, the method established in the latter section, for the extraction of C I Reactive Red 141 from river water.

The Umbilo Wastewater Treatment Plant receives textile effluent from industries in the Pinetown region. This treatment plant discharges its effluent into the Umbilo River. This area was chosen for sampling river water for the extraction studies. Water samples were taken in early January 1998, at the time when the textile industries were closed. Water samples were taken from the river close to the wastewater treatment plant at the following points: up-stream from the plant (UP); down-stream from the plant (DS); the outlet of the old plant (OP); and the outlet of activated sludge plant (AS). Water samples were fortified with C I Reactive Red 141 and extracted as before. The results are tabulated in Table 7.3.

Table 7.3 : Recovery of a 1 mg/ℓ solution of C I Reactive Red 141 from river water, analysis by CE.

Ext No	% Recovery			
	UP	DS	OP	AS
1	80.45	50.36	86.07	82.13
2	32.83	73.17	92.8	49.11
Average	56.64	61.77	89.08	65.62

Recoveries were poor, but these results are not a true reflection of the amount extracted because integration of sample extracts was poor due to interfering peaks as shown in Fig 7.2, which may have been due to other organic compounds that were co-extracted from the river water.

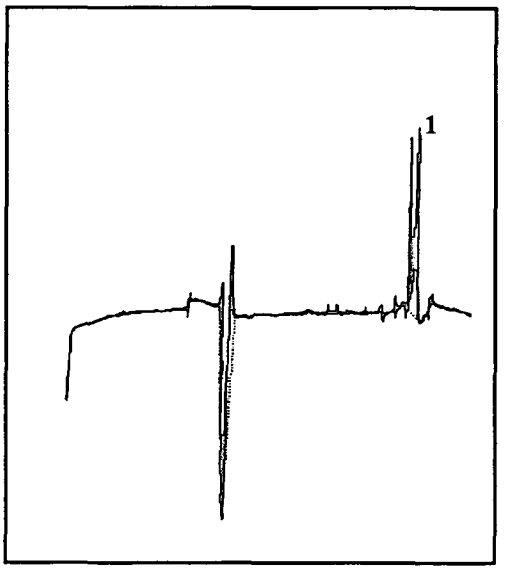

Figure 7.2 : Electropherogram of an extracted river water sample, where 1 = the dye peak. Analysis conditions as described in **Appendix 3.**

7.2 SOLID PHASE EXTRACTION

The objective in this section is to develop an extraction method to remove the interferants from river water observed in the previous section. Detection of water samples without clean-up appears not to be practical because many other substances in the environmental samples e.g. chlorophyll and humic substances interfere with the analytical measurements as seen above. An alternate extraction method was investigated, solid phase extraction (SPE), using C_{18} Sepak cartridges from Millipore.

Recoveries by solid phase extraction using ion suppression techniques were reported to be generally lower for the disulphonated azo dyes compared to the monosulphonated dyes as reported in **Chapter 3**. Since C I Reactive Red 141 is highly sulphonated, an alternate solid phase extraction technique, using ion pairing reagent, was evaluated for the extraction of this dye from river water. The use of ion pairing techniques according to Barcelo et al.(1997) have not been fully explored.

This method was formulated with the experienced gained in ion-pair reverse phase chromatography and the liquid-liquid extraction method used above.

7.2.1 Extraction of C I Reactive Red 141 from River Water

The cartridge was activated by sequentially passing 2 mℓ methanol, 2 mℓ 50% methanol and 2 mℓ water through the column. To the river water spiked with C I Reactive Red 141 at a concentration of 1 mg/ℓ (100 mℓ), 2 mℓ of the 25 % tetrabutylammonium bromide solution (used above) was added to the sample and the sample passed through the cartridge. The column was sequentially washed with 2 mℓ of water and 2 mℓ 25 % methanol. The dye was eluted with 2 mℓ 100 % methanol. The extracted volume was 2 mℓ. Extractions were done in duplicate and the methanolic extract analysed by CE. Using methanol as a solvent decreased migration time slightly, with a slight interference observed in the current but the current stabilised soon after injection. An example of an electropherogram obtained is given in Fig 7.3 (b). A blank extraction was carried out on the downstream water sample and no peak was detected at the migration time of C I Reactive Red 141 as shown in Fig 7.3 (a). Recoveries were calculated using a single point calibration with a 50 mg/ℓ standard of C I Reactive Red 141 in methanol. The recoveries of C I Reactive Red 141 from the different river water samples showed good recoveries, Table 7.4.

Table 7.4 : Recovery of a 1 mg/ℓ solution of C I Reactive Red 141 from river water, analysis by CE.

Ext No	% Recovery			
	UP	DS	OP	AS
1	96.26	102.94	104.21	97.2
2	93.91	96.66	96.26	93.12
Average	95.09	99.8	100.24	95.16

SPE, using the procedure outlined above, was successful in removing the interferants resulting in satisfactory recoveries of C Reactive Red 141 from river water. However, these results cannot be considered conclusive in itself. Further investigations using proper controls and blanks will be required and should be considered for further investigations.

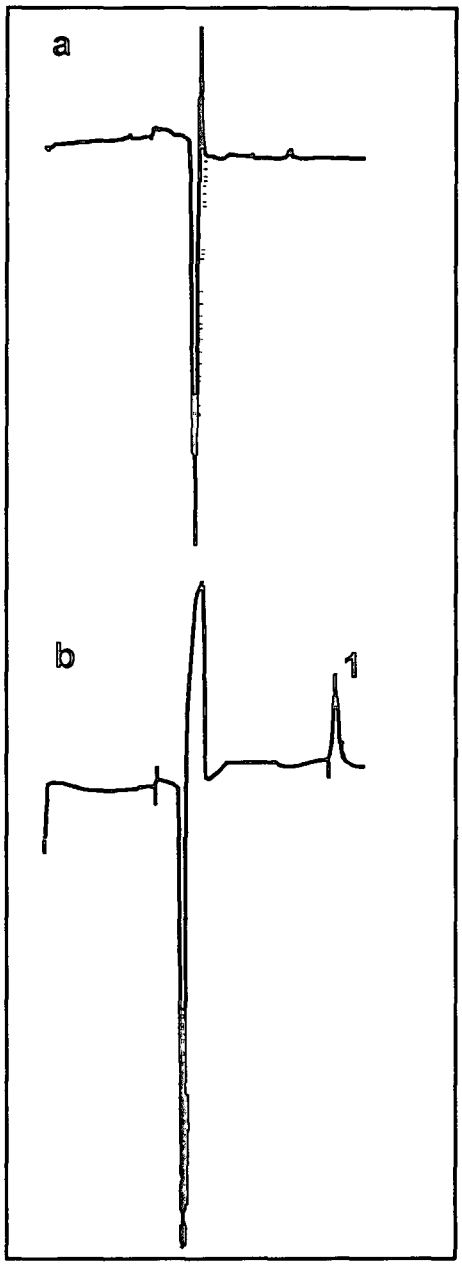

Figure 7.3 : Electropherogram for samples extracted by SPE; (a) blank extraction and (b) C I Reactive Red 141 extracted from river water. Analysis conditions as described in **Appendix 3.**

7.3 CONCLUSIONS

Methods for the extraction of reactive dyes from water systems were investigated using liquid-liquid extraction (LLE). Due to the non-removal of interferants present in river water an alternate method, solid phase extraction (SPE) was investigated.

The use of LLE for the extraction of C I Reactive Red 141 from tap water gave good recoveries by both HPLC and CE. No significant effect was observed in varying the pH value of the extraction buffer.

In the extraction of C I Reactive Red 141 from river water using LLE, low and inconsistent recoveries were obtained by CE. These low values may have been due to the interfering peak observed in the electropherogram of the extracted river water samples. These samples were not analysed by HPLC, it is possible that better recoveries could have been achieved if HPLC was used for the analysis on condition that the interfering compounds do not co-elute with the analyte peak on the HPLC column.

The use of SPE with an ion-pairing reagent for the extraction of C I Reactive Red 141 from river water, gave good recoveries and shows greater potential for removing contaminants from wastewater. The waste generated by SPE is significantly lower than that of LLE methods, hence less expensive and more in-keeping with environmental protection policies.

As reported earlier, the use of pH adjustments is the most direct approach for isolating dyes by LLE and solid phase extraction. However, ion-pairing techniques offer an alternate method that has not been fully explored (Brumley et al., 1994), reasons for this lack are not clear. But the preliminary results presented here, show that this technique should be considered for further investigations.

Chapter 8

CONCLUSION AND RECOMMENDATIONS

This study has concerned investigations into the analysis of reactive dyes and their hydrolysis products using high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). The HPLC technique used was reversed phase chromatography with an ion-pairing reagent. The CE techniques used were capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC).

The first phase of the investigation involved developing the analytical techniques chosen for the analysis of the 5 selected reactive dyes. All 5 dyes were well resolved with high efficiency by CZE using a phosphate-borate buffer. The HPLC method with an ion-pairing reagent showed limited separation efficiency with C I Reactive Green 19 and C I Reactive Blue 171 co-eluting, highlighting the problem of separating compounds with very similar structures and UV-visible absorbance properties by HPLC.

The dynamic linear range of the two techniques were comparable in the range 10 to 100 mg/ℓ, with HPLC giving better reproducibility. Although the CZE migration time reproducibility was poor (RSD = 5.89 %) its normalised peak area reproducibility was satisfactory (RSD = 2.76 %) implying that the technique is well suited for quantitative purposes. However, identification of compounds on migration time only could prove problematic. Although no special attention was paid to dye impurities, both techniques showed potential for evaluating or comparing individual dyes in this area.

The essence of this investigation was to develop analytical methods for the analysis of reactive dyes in dye-bath effluent. An important consideration, according to accepted literature, was that under normal conditions of use the hydrolysed derivative rather than the reactive form of the dye enters the textile waste stream. Thus, the next phase of the experimental investigation was to hydrolyse the dyes and develop appropriate techniques that will distinguish between the hydrolysed derivatives and the parent dye.

In analysing the individual dyes and their hydrolysis products it was found that all 5 dyes were well resolved from their hydrolysis products using HPLC, whereas CZE lacked selectivity in resolving

the hydrolysis products for C I Reactive Red 141and C I Reactive Red 120. Thus, an alternate CE technique, micellar electrokinetic chromatography (MEKC), was investigated. MEKC resolved the hydrolysis products from the parent dye with high efficiency.

The optimum conditions for the separation of a mixture of the 5 dyes or for the separation of the hydrolysed derivatives from the parent dye were achieved by using:

a) HPLC with a mobile phase of 58 % water and 42 % acetonitrile with $1x10^{-3}$ M tetrabutylammonium bromide and 1 mℓ 20 % v/v acetic acid and 1mℓ 5 % w/v potassium hydroxide. The chromatographic column was a Lichrosorb C_{18}, 5 µ, 4.6 x 300 mm.

b) CE with an electrophoresis buffer of 10 mM sodium tetraborate and 6 mM potassium dihydrogenphosphate adjusted to pH value 8.25 containing 25 mM sodium dodecyl sulphate (SDS).

The next stage was to analyse dye-bath effluent. From the HPLC chromatograms, it was evident that the parent dye, the monohydroxy and the dihydroxy derivatives were present in the residual dye-bath liquors. The results indicated that incomplete dye hydrolysis had occurred during the dyeing process. The presence of the parent dye and the partially hydrolysed dye raises concern in terms of ecotoxicity as well as with regard to dye wastage, indicating the need to optimise dyeing processes.

Multiple dye mixtures are generally required in achieving the desired shade in most reactive dyeing processes. Thus, the next stage of this phase was to test the applicability of the methods developed for the analysis of a mixture of the 5 dyes and their hydrolysis derivatives. The HPLC method showed low efficiency in resolving such a wide range of products. The MEKC method showed immense resolving power, with most peaks well separated (~15 peaks). The results achieved indicate that analysis of multiple dye mixtures in a reactive dye-bath can be carried out with high efficiency by MEKC.

The methods presented can be used to determine whether dyeing is complete as well as indicate the potential to recycle and/or reconstitute exhausted dye-baths to reduce processing costs by optimising the concentrations of the dyes, water, auxiliaries and energy used, whilst simultaneously reducing

effluent. Such potential economic benefits combined with increased awareness towards environmental issues should promote the use of the techniques developed to study dye hydrolysis behaviour during reactive dyeing processes.

One of the disadvantages of the CE techniques used, was the long analysis time (25 to 35 min) compared to HPLC (12 min). The addition of an ion-pairing reagent to the MEKC buffer showed its capability of reducing analysis time as well as improving resolution and peak shape.

It was speculated that the reasons for the decrease in analysis time was due to the breakdown of the micelle structure in the presence of the ion pairing reagent since the concentration of sodium dodecyl sulphate (SDS) used was close to it critical micelle concentration (8.27 mM). The results obtained indicate that the separation mechanism is more complicated than envisaged, and should be considered for further investigations.

From a practical point of view, it can be concluded that the identity of reactive dyes and their derivatives can be established using HPLC, CZE and MEKC, depending on the complexity of the sample. To avoid long analysis time, the identity of a single dye and its derivatives can be performed using MEKC with a phosphate/borate buffer containing an ion-pairing reagent in less than 10 min.

An interesting observation was the non-retention of the dye-bath samples on the HPLC column. The experiments carried out, with respect to this, gave strong evidence that the non-retention was due to the concentration of salts/electrolytes present in the dye-bath. It was proposed that this effect was due to dye aggregation in the presence of salts. Further research would be required to confirm this proposal. The non-retention of the dye-bath samples may be a reason for the lack of literature on the analysis of dye-bath effluent using HPLC.

A disadvantage of the methodology presented, is the need to dialyse all samples, to remove salts, prior to analysis. Perhaps further developments in the methodology could overcome this shortfall. In the analysis of the dye-bath samples by CZE prior to dialysis, zone broadening was observed, a phenomenon generally related to conductivity differences between the analyte zone and buffer. A possibility for future research is to consider using CE with buffers of much higher conductivity to overcome this disadvantage.

Potential applications of the methods presented include: checking the purity of the reactive dye sample, monitoring reactions as well as rates of reactions (kinetic studies) of reactive dyes with nucleophiles, analysing coloured effluents and investigating the breakdown of reactive dyes in the dyeing process and in effluent treatment processes.

In applying these techniques to environmental analysis, an important step is sample clean-up, extraction and pre-concentration.Thus, the final phase of the study was to develop extraction techniques for reactive dyes in water systems. Methods for the extraction of reactive dyes from water systems were investigated using liquid-liquid extraction (LLE). Due to the non-removal of interferents present in river water an alternate method, solid phase extraction (SPE) was investigated.

Using SPE with an ion-pairing reagent for the extraction of C I Reactive Red 141 from river water, gave good recoveries and shows greater potential for removing contaminants from wastewater than LLE. The waste generated by SPE is significantly lower than that of LLE methods, hence less expensive and more in-keeping with environmental protection policies.

The use of pH adjustments is the most direct approach for isolating dyes by LLE and solid phase extraction. However, ion-pairing offers an alternate method that has, for unknown reasons, not been fully explored (Brumley et al., 1994). The preliminary results presented here, showed that this technique should, however, be investigated further.

In summary, the methods developed provided a suitable methodology for analysing both residual dye-bath liquors and residual rinse liquors. HPLC was shown to be a very effective technique for monitoring individual dyes and their derivatives but lacked the selectivity and efficiency to deal with complex mixtures. The high separation efficiency achieved by MEKC indicates its potential as an alternative to HPLC for the determination of reactive dyes and their derivatives in complex mixtures.

Although HPLC shows poorer separation efficiency than CE methods, this technique is an old, well established technique that offers superiority in terms of reproducibility and reliability. By comparison, CE is still in the development stages with problems associated with reproducibility and reliability.

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Appendix 1

THE DYEING PROCESS

Appendix 1 discusses the industrial dyeing process. When cotton is dyed with reactive dyes there are usually three main steps / stages in the dyeing process, Fig A.1.1.

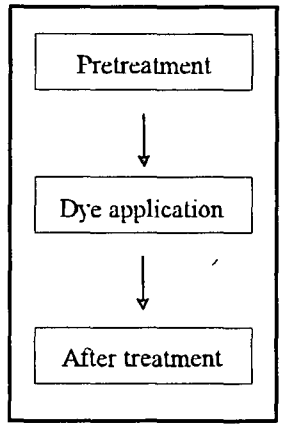


Figure A1.1 : The 3 stages in the dyeing process.

A1.1 PRETREATMENT

Preparation while varying from fibre to fibre, can be summed up very simply: scouring to remove impurities and bleaching to render the fibre as white as possible.

In the reactive dyeing process a coloured cellulosic fibre is synthesized by means of an ether or ester bond. Coloured cellulosic impurities as well as varying amounts of dye hydrolysates will form as by-products of this synthesis. Dye hydrolysates are formed by the reaction of the dye with the hydroxy group of the water instead of the hydroxy of the cellulose. The coloured cellulose can be considered as *colour fast* only, when all coloured by-products have been removed from the fibre. The pretreatment, such as washing, alkaline scouring or bleaching will remove a considerable amount of the cellulose impurities before dyeing. Pretreatment is essential for better fastness and for economic reasons, i.e. more dye onto the fibre.

A1.2 DYE APPLICATION

Fig A1.2 shows a typical exhaustion and fixation curve measured during dyeing of cellulosic fibres with reactive dyes. During the primary exhaustion stage, dye is taken up into the fibre in the presence of added salt. The adsorption of the dye by the fibre is due to the presence of electron-rich sites within the dye molecule capable of forming hydrogen bonds with the cellulose molecule, this property of the dye being termed *substantivity*.

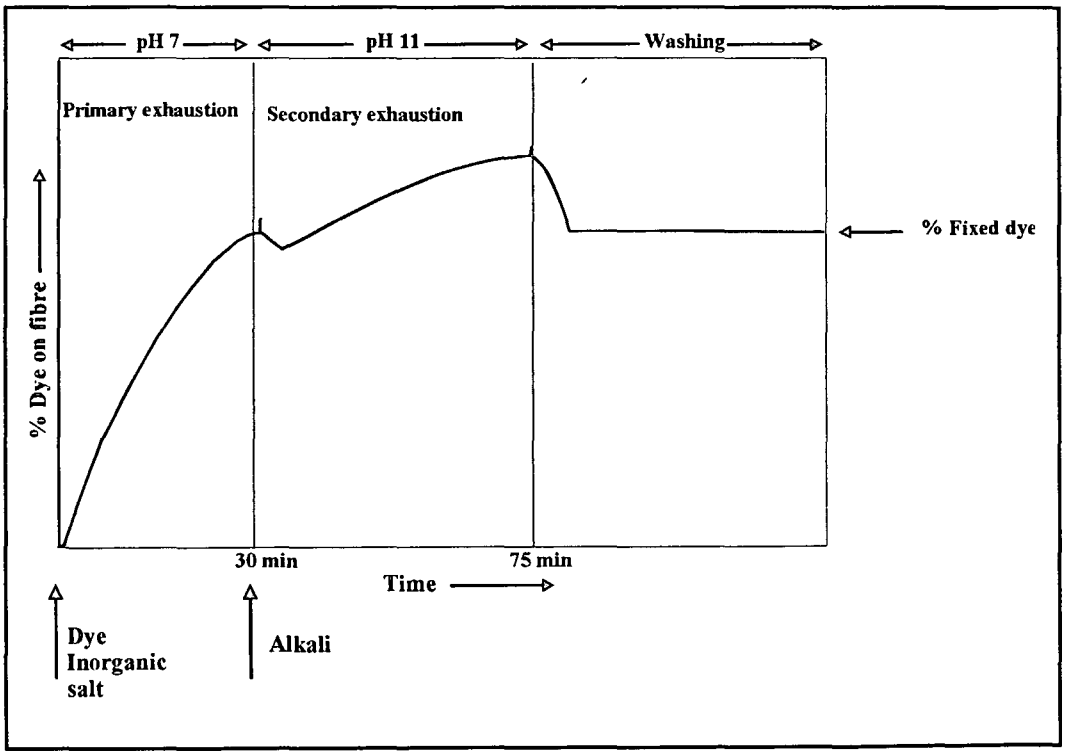


Figure A1.2 : Rate of exhaustion and fixation of reactive dyes.

Since reactive dyes have only low fibre substantivity, salt increases the dye uptake of the cellulose and prevents separation of the dye from the fibre by the pad liquor. The electrolyte screens the forces of repulsion between the negatively charged cellulose and the dye anions. In addition the salt will increase the internal pH value. If there are precipitations due to excessive electrolyte concentration, the rate of reaction will drop.

These processes are purely physical in nature, and at this stage no chemical reaction takes place, since the chlorotriazine is susceptible to attack only by nucleophiles and at near-neutral pH values these are not present to any sensible extent. When satisfactory adsorption has been achieved, usually after about 45 min, sodium carbonate solution or some other convenient alkali is added to raise the pH to about 10.5. Ionisation of both the water and cellulose occurs and the dye is attacked by either the cellulosate ion, resulting in fixation of the dye to the substrate, or by a hydroxide ion, resulting in hydrolysis and loss of the dye to the dyeing process.

The ratio between these two reactions is the all-important factor in determining the technical utility of the dye, since only that portion of the dye which becomes fixed (bonded) to the cellulose gives the desired result. When it is borne in mind that between 5 and 20 times as much water as cotton is present in the dyebath, it would be expected at first sight that the process would be an abject failure. Most dyes do, however, give fixation values of 60 % or better. Two factors are responsible for this. The first of these is that the dye has been physically adsorbed onto the cellulose fibre prior to the reaction stage and this results in about 60 to 80 % of the dye lying on the cellulose and thus being ideally sited for fibre reaction. The second is that the internal pH value within the cellulose fibre is maintained primarily by cellulosate ions, which in the fibre phase outnumber hydroxyl ions by about 25:1. These two effects swing the balance of the reaction in favour of fixation and ensure success.

Auxiliaries are added to the dye-bath to reduce the risk of off-shading. *Wetting agents* provide even wetting as well as a uniform distribution of the liquor and thus have levelling effect. *Lubricants* prevent the formation of running creases. *Sequestering agents* are also added to soften the water and prevent the precipitation of alkaline earth metals during dye fixation and their deposition on the dyed material. An excess of inorganic sequesterent can reduce the colour yield. Organic sequesterents are avoided as they decopperize metal-complex reactive dyes and affect the light fastness and shade. Alternatively, the dye-house can use soft water or demineralised water from a water softening treatment plant.

A1.3 AFTER TREATMENT

After dyeing or printing, the fibres are cleaned by rinsing, soaping and rinsing again. This is a process of paramount importance. At the end of dyeing stage the fibre contains unfixed hydrolysed dye and possibly some residual active dye. Before a reactive dye can show true fastness such loose colour must be removed or rendered insignificant in amount.

These rinsing and soaping processes cause most of the processing costs involved in reactive dyeing. Soaping is carried out at high temperatures and takes a long time thus rendering the reactive dyeing process quite costly.

A1.3.1. Cationic after treatment

It is not always possible to remove all dye hydrolysates from the fibre by soaping and rinsing only. The residual hydrolysates are bound to the fibre by the cationic after treatment agent in order to obtain a good level of wet fastness. This applies not only to the hydrolysates which are present on the fibre from the dye application process but also to the ones that are formed during subsequent dye hydrolyses. A cationic after treatment cannot prevent a subsequent hydrolysis, but since it induces a fixation of the dyes prior to the subsequent hydrolysis, the hydrolysates then formed cannot bleed and impair the wet fastness.

Dye which is bound to the cellulose is separated from the fibre for various reasons:

i) High temperatures (during drying for example) can separate the dye bound to the fibre by the reaction, thermohydrolysis.

ii) Acid hydrolysis takes place quite often after the softening process since softeners have to be applied in an acid medium to improve exhaustion. Since there is no rinsing after softening the acid remains on the goods and may seriously damage the dye-fibre link before being removed during drying. Acid exhaust gases or a humid and warm climate may have the same effect on goods in storage.

Machine:		**Colour:**	GREEN 6594C (M)	**Job Card No:**	0
Quality:		**Client:**		**Weight:**	100
Volume:	1000 litres			**Date:**	06-Mar-97
Time In:		**Time Out:**		**Time:**	12:37:45

Qty gpl	Description	Qty gpl	Add gpl	Add gpl	Add gpl	Cost R's
	Fill Machine to Level Raise to 50°					
1.00	Revatol SD	1000				5.36
1.00	Rucolin JES	1000				4.50
1.00	Verolan NBI	1000				5.30
	50°run 10mins add over 30mins					
60.00	Glauber Salt	60000				57.00
	At 50°run 10mins Dose over 30mins,					
0.30%	Evercion Yellow HE4R	300				12.00
0.13%	Remazol Red H-7B	130				4.84
0.92%	Evercion Green HE4BD	920				57.89
	Raise to 95°at 1.5per mins-Run 20mins Cool to 80°					
	At 80°add over 30mins					
15.00	Soda Ash	15000				21.75
	80°run 60mins Cool to 60° Sample Rinse 10mins Drain					
	Fill water only 60°run 10mins Drain Fill Add					
0.60	Verolan NBI	600				4.24
	Raise to 95°run 20mins Cool to 70° Rinse 10mins Drain					
	Fill water only 60°run 10mins Rinse 10mins					
	Take final Sample Drain Fill Add					
0.10	Acetic Acid	100				0.43
1.00%	Levogen WRD	1000				13.26
	40°15mins Add					
2.00%	Persoftal OE	2000				26.02
	40°20mins Off Load					

Appendix 2

CAPILLARY ELECTROPHORESIS

Appendix 2 gives a brief introduction to capillary electrophoresis (CE), its basic principles of operation with emphasis on the two modes used in the current investigation, viz. capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC).

Electrophoresis is the separation of charged molecules in an applied potential field. Electrophoresis within a capillary tube (generally a fused silica capillary), referred to as capillary electrophoresis (CE) was developed in the early 1980's. CE has gained popularity due to its ability to perform very fast and efficient separations of ionic species; both large and small molecules.

A schematic diagram of a capillary electrophoresis system is shown in Fig A2.1. It comprises a high voltage supply, two buffer reservoirs joined by a capillary and a detector. In CE detection is conventionally performed *on-line* by creating a window in the capillary wall.

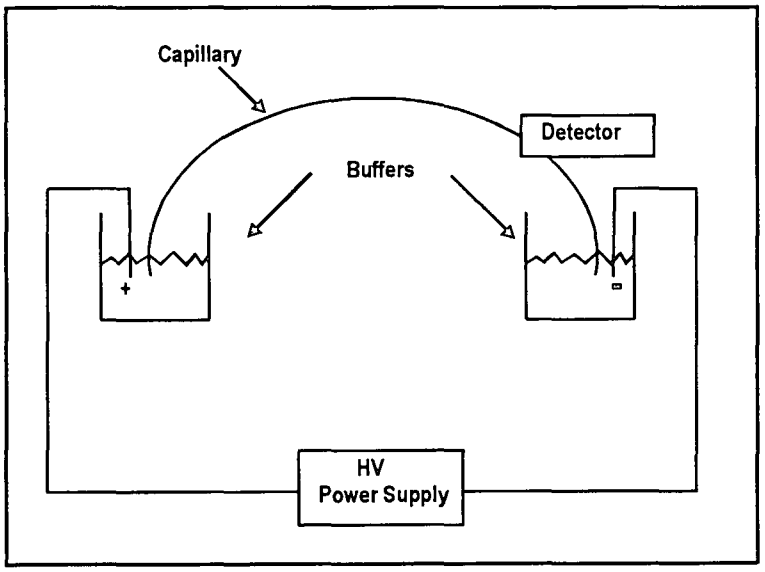


Figure A2.1 : Schematic diagram of a basic capillary electrophoresis instrumentation.

A2.1 ELECTRO-OSMOTIC FLOW

One of the fundamental processes that drives CE is electro-osmosis. This phenomenon is a consequence of the surface charge on the wall of the capillary. The inner wall of the silica capillary is coated with silanol groups. These groups under certain conditions will become ionised which results in the walls having a net negative charge. Positively charged cations in the buffer will associate with the anionic species on the capillary wall. When a voltage is applied the cationic species migrate towards the cathode and because they are solvated this has the effect of dragging the solvent molecules along with the cations. This causes the bulk flow of the liquid towards the cathode. This is termed electroosmotic (EOF) or electroendo-osmotic flow (Heiger, 1992).

A unique feature of EOF in the capillary is the flat profile of the flow, as depicted in Fig A2.2 (a). Since the driving force of the flow is uniformly distributed along the capillary wall, there is no pressure drop within the capillary, and the flow is nearly uniform throughout the capillary. This is in contrast to that generated by an external pump which yields a laminar flow or parabolic flow, Fig A2.2 (b), due to the shear force at the wall.

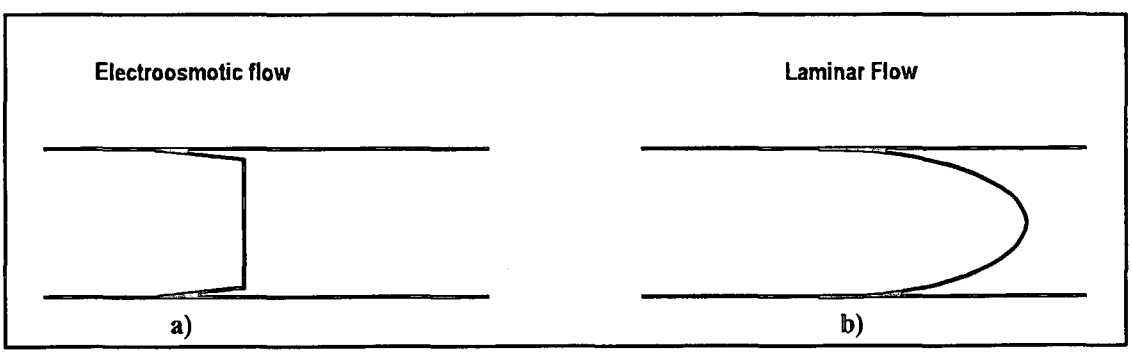


Figure A2.2 : Flow profiles in (a) capillary electrophoresis and (b) liquid chromatography.

Any charged molecule in the buffer has an electrophoretic mobility. This is the speed at which it migrates. The speed will depend on the applied voltage, and the direction will depend on the net charge on the analyte, if it is negative it will migrate towards the anode and if it is positive it will migrate towards the cathode.

However, the actual mobility of the analyte will be the sum of its electrophoretic mobility and the electroosmotic flow. Under normal circumstances the electroosmotic flow is greater than the electrophoretic mobility. This results in all analytes migrating towards the cathode, even the anionic ones. Thus, in a mixture of anions, cations and neutral compounds, cations elute past the on-line detector first as their electrophoretic mobility is in the same direction as the bulk flow. Neutral species, with zero electrophoretic mobility, would elute second, traveling at approximately the same speed as the buffer anions. Finally anions would elute as they resist the electroosmotic flow. This process is illustrated in Fig A2.3.

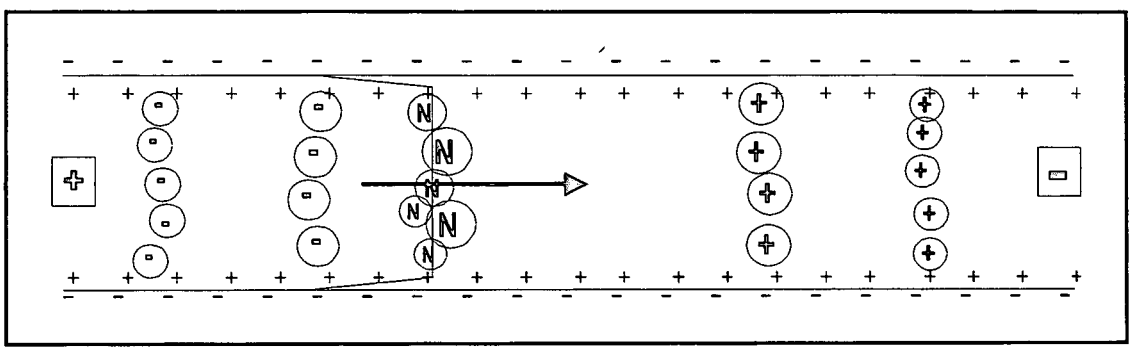


Figure A2.3 : Differential flow migration superimposed on electroosmotic flow in capillary zone electrophoresis.

A2.1.1 Factors that Affect the Electroosmotic Flow

Since EOF is a result of the charge on the capillary wall, factors that affect this charge will also affect the flow. In order for CE separations to be performed reproducibly in the presence of EOF, the EOF must be consistent from run to run. The control of EOF often requires extensive capillary conditioning before and between analysis (Terabe, 1991).

Some factors that affect the EOF are:

Buffer pH : The pH of the buffer can be used to influence the degree of ionisation of the silanol groups and so affect the velocity of the EOF, as the pH of the buffer increases the EOF will increase. However, pH can also be used as a selective variable to separate molecules by affecting solute charge and mobility.

Buffer concentration : EOF can also be affected by the concentration and ionic strength of the buffer. Increasing the buffer concentration can also increase the separation efficiency, however, this is limited by heating within the capillary.

Capillary Modifications : EOF can be controlled by modifications of the capillary wall, by means of buffer additives (dynamic coatings) or by covalent bonding. These coatings can increase, decrease or reverse the surface charge and thus EOF. Buffer additives can alter, among other things, electrophoretic mobilities, i.e. can change the selectivity of the separation.

A2.2 MODES OF CAPILLARY ELECTROPHORESIS

Capillary electrophoresis comprises a family of techniques that have different operative and separative characteristics. The techniques are:

i) capillary zone electrophoresis
ii) micellar electrokinetic capillary chromatography
iii) isoelectric focusing
iv) capillary gel electrophoresis
v) isotachophoresis

A2.2.1 Capillary Zone Electrophoresis

Capillary zone electrophoresis (CZE) is the simplest and most commonly used technique in CE; this technique employs a single buffer system in free solution. Fundamental to CZE are homogeneity of the buffer solution and constant field strength throughout the capillary.

The separation mechanism is based on differences in the mass to charge ratio. The components in a sample mixture separate into discrete zones as shown in Fig A2.3. Separation of both anionic and cationic solutes is possible by CZE due to the electroosmotic flow (EOF). Neutral species will be carried by the EOF and will not be resolved. In CZE, selectivity can most readily be altered through

changes in the pH value of the run buffer or by use of buffer additives such as surfactant or chiral selectors. While these factors results in changes in the EOF, the EOF is itself not responsible for the changes in selectivity, only in migration time and resolution (Heiger, 1992). Many of the theoretical aspects of CZE have been considered in **Section A2.1**.

A2.2.2 Micellar Electrokinetic Chromatography

Micellar electrokinetic chromatography (MEKC) has been developed to extend the applicability of CE to neutral as well as charged molecules (Terabe et al., 1984). When a surfactant is added to water or a buffer in concentrations greater than its *critical micelle concentration* (CMC), micelles are formed. A micelle is like a tiny droplet of oil with a highly polar outer surface and a hydrophobic inner core. The most commonly used surfactant in CE is sodium dodecyl sulphate (SDS) which has a CMC of 8.27 mM (Heiger, 1992).

Micelles are usually charged and migrate either with or against the EOF depending on the charge. Anionic surfactants such as SDS have an electrophoretic mobility toward the anode, i.e. in the opposite direction of the EOF. Since the EOF is generally faster than the electrophoretic mobility of the micelles at neutral or basic pH, the net movement is in the direction of the EOF. As a result, analytes that have a greater affinity for the micelle have a slower migration velocity compared to analytes that spend most of their time in the bulk phase.

The surfactant micelles constitute a *pseudo-stationary phase* (Heiger, 1992) into which analyte molecules partition themselves between the micellar phase and the aqueous phase in a fashion analogous to reversed phase HPLC, during the course of electrophoresis. For neutral species, it is only partitioning that effects separation. The more the solute interacts with the micelle the longer is its migration time. When a solute is not in contact with the micelle, it undergoes normal electrophoretic separation. Compared to reversed phase HPLC, MEKC separations exhibit high efficiency due to two factors.

i) The flat flow profile of EOF compared to laminar flow profiles resulting from an external pump in HPLC, thus eliminating band broadening, Fig A2.2.

ii) The rapid exchange of the surfactant between monomer and micelle phases results in high mobile phase - micelle phase mass transfer kinetics (Terabe, 1989).

The overall MEKC separation is depicted schematically in Fig A2.4.

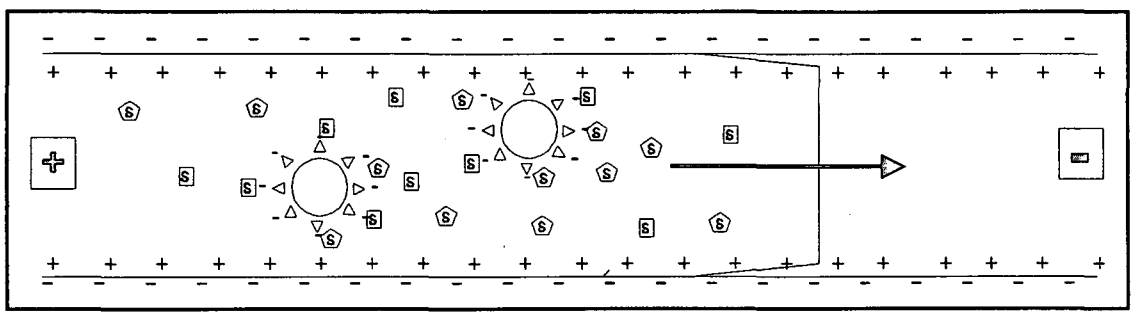


Figure A2.4 : Schematic diagram of the overall MEKC separation process.

The resolving power of MEKC can be enhanced by a number of variations. Organic modifiers can be added to help resolve very hydrophobic analytes which otherwise would co-migrate at the velocity of the micellar phase. Cyclodextrins added to the buffer can act as chiral selectors to resolve neutral enantiomers (Janini and Isaaq, 1992).

A2.3 QUANTITATIVE ANALYSIS

An interesting aspect of quantitative analysis, is the peak area differences due to different migration velocities of the solutes. This is contrast to chromatographic techniques in which all solutes travel at the same rate when in the mobile phase. It is therefore necessary to correct for velocities since residence time in the detector window artificially affect peak area. Solutes of low mobility remain in the detector window for a longer period of time than those with a higher mobility and, thus, have increased area. This phenomenon can be corrected simply by dividing the integrated peak area by the migration time; called the normalised area (Heiger, 1992).

Appendix 3

EXPERIMENTAL TECHNIQUES

Appendix 3 gives the details of all chemicals and instruments used during this investigation. Included in this section are the instrumental conditions employed together with the preparation of the mobile phases and buffers required for the high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) techniques, respectively.

Two analytical techniques, high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) were investigated during the course of this research project. For CE, two techniques were investigated, capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC). In CZE a phosphate bórate buffer was used and in MEKC, sodium dodecyl sulphate (SDS) was added to the CZE buffer at various concentrations in order to improve resolution between the hydrolyzed dyes and the parent dye. To the MEKC buffer an ion-pairing reagent was added to reduce analysis time. The various buffers tested used and procedures for their preparation are described.

For the HPLC investigation 2 different instruments were employed. The mobile phase used on both systems were the same except variations in the organic composition. Procedure for the preparation of the mobile phase is described together with the conditions used on the different systems. Details for equilibrating the HPLC and CE system as well as procedures for flushing these system are also given.

A3.1 Chemical and Reagents

HPLC grade acetonitrile and disodium hydrogen orthophosphate were obtained from BDH lab supplies. Tetrabutylammonium bromide (TBAB) was obtained from Sigma. Sodium tetraborate and *n*-hexane were obtained from Riedel-de Haen. Sodium dihydrogen phosphate, orthophosphoric acid 85 %, acetic acid, potassium hydroxide, sodium hydroxide and hydrochloric acid were obtained from Saarchem. Cyclohexanol 98 % was obtained from Fluka. All chemicals used were analytical-reagent grade except the acetonitrile.

The five commercial dyes were supplied by Ninian and Lester. These dyes are marketed under the trade name Evercion which are equivalent to the Procion dyes manufactured by Zeneca. These dyes were used without further purification.

All solutions were made in Milli-Q water (Millipore Corp). All HPLC mobile phases and CE buffers as well as samples were filtered through a 0.45 μm pore size membrane filter (Millipore).

All samples were dialysed using standard cellulose dialysis tubing from Spectrapor with a molecular weight cut-off of 12 000 to 14 000.

A3.2 Instruments and Methods

The following equipment mentioned below were used during the course of this research project unless otherwise stated in the text.

A3.2.1 pH Measurements

All pH measurements were made using a Model 691 pH meter (Metrohm) calibrated daily with buffers at pH values of 4 and 7 unless otherwise stated in the text.

A3.2.2 Ultraviolet and Visible Absorbance Measurements

The absorbance spectra of the dye solutions were measured on a Varian DMS 100 double beam UV-VIS spectrophotometer. The spectra were recorded between 350 and 900 nm using a pair of matched quartz curvettes with a pathlength of 10 mm. Milli-Q water was used as the reference solution.

A3.2.3 High Performance Liquid Chromatography

During the course of this study two different HPLC systems were used. Initial investigations were on the Varian 5000 HPLC system connected to a UV-50 variable wavelength detector and a Spectraphysics 4290 intergrator. Later the Waters 600E system controller with a Gilson 401C autosampler and a Waters photodiode array detector connected to a computer with the Waters 991computer package. Tables A3-1and A3-2 lists the instrumental parameters used, any changes to columns and mobile phases from these conditions are indicted on the respective chromatograms.

Table A3.1 : Instrumental conditions used for the Varian HPLC system..

Column	Novapak C_{18}, length 100 mm, 4.6 mm ID; from Waters
Mobile phase	55 % water/45 % acetonitrile, containing 1 x 10^{-3} mol/ℓ tetrabutylammonium bromide and 1mℓ/ℓ 20% v/v acetic acid and 1mℓ/ℓ 5% w/v potassium hydroxide.
Flow rate	1mℓ/min
Column temperature	ambient
Injection volume	10 μℓ
Wavelength	520; 406; 605 nm

Table A3.1 : Instrumental conditions used for the Waters HPLC system.

Column	Lichrosorb C_{18}, length 300 mm, 4.6 mm ID; from Merck
Mobile phase	58 % water/42 % acetonitrile, containing 1 x 10^{-3} mol/ℓ tetrabutylammonium bromide and 1mℓ/ℓ 20% v/v acetic acid and 1mℓ/ℓ 5% w/v potassium hydroxide.
Flow rate	1mℓ/min
Column temperature	35°C
Injection volume	10 µℓ
Detector	290 nm

The HPLC system was equilibrated with the mobile phase for 1 h at a flowrate of 0.5 mℓ/min. C I Reactive Red 141 was then injected 3 times to test the system for retention time repeatability. If changes in the retention time were noted, the system was allowed to equilibrate for a further 30 min. At the end of the day, the system was flushed with acetonitrile : water (50 : 50) for 30 min a 1 mℓ/min. However, if the system was to be used the next day, flushing was omitted, in order to reduce equilibration time. In the latter case, the system was allowed to equilibrate for 15 min.

HPLC mobile phase preparation

The mobile phase was prepared by dissolving 0.322 g tetrabutyl ammonium bromide (two separate weighings) into beakers containing 5 mℓ of Milli-Q water and 1 mℓ of 20 % v/v solution of acetic acid and 1 mℓ of a 5 % w/v solution of potassium hydroxide. The ultrasonic bath was used to aid dissolution. These solutions were transferred to 2 volumetric flasks, one was diluted to volume with Milli-Q water (Solution A) the other with acetonitrile (Solution B). The mobile phase was prepared by mixing the required quantities of solutions A and B. The mobile phase was filtered daily through a 0.45 µm filter prior to use. Therefore, the ratio of acetonitrile : water given in the above tables and else where in the text, refers to the ratio of solutions **A** and **B**.

A3.2.4 Capillary Electrophoresis

The capillary zone electrophoresis and micellar electrokinetic chromatography were carried out on a Beckman P/ACE 5000 capillary electrophoresis system connected to a photodiode array detector. Data was analysed with the System Gold software. Separations were performed in a 57 cm (50 cm effective length) x 75 0.45 μm i.d. fused silica capillary (Beckman Instruments).

Table A3.3 : Instrumental conditions used for all capillary electrophoresis analysis.

Potential	20kV
Injection	Pressure for 5s
Detection	290 nm

The capillary was equilibrated with 0.1 M NaOH (5 min), water (5 min) followed by buffer (5min) at the beginning of each day. The capillary was regenerated with 0.1 M NaOH (2 min), water (2min) followed by the buffer (2 min) between each analysis. The temperature of the capillary was set at 25°C. After a pressure injection of 5 s, a voltage of 20 kV was applied across the capillary.

At the end of each day, the capillary was flushed with 0.1 M NaOH (3 min), 1 M NaOH (3 min), 0.1 M NaOH (3 min), water (5min) followed by air (5min).

CE Buffer Preparations

A number of separation buffers were used during this investigation. The buffers were prepared by dissolving the required quantity of sodium dihydrogen phosphate, sodium tetraborate, sodium dodecyl sulphate (SDS) and tetrabutyl ammonium bromide (TBAB) in Milli-Q water to obtain the concentrations given in Table A3.4. In the buffers with SDS, dissolution was aided by using an ultrasonic bath and extra care was taken in transferring these buffers to the volumetric flask so as to avoid bubble formation. The pH value of the buffer solutions was adjusted with either 0.1 M sodium hydroxide or 0.1 M hydrochloric acid to the required pH value. Buffers were stored in the

fridge and allowed to reach room temperature prior to use. All buffers were degassed for 5 min in an ultrasonic bath and passed through a 0.45 μm syringe filter prior to use.

Table A3.4 : CZE and MEKC buffers systems used, pH values adjusted with 0.1 M HCℓ or 0.1 M NaOH.

	Sodium dihydrogen phosphate (mM)	**Sodium tetraborate (mM)**	**Sodium dodecyl lauryl sulphate (mM)**	**Tetrabutyl ammonium bromide (mM)**	**pH**
CZE	10	6	0	0	8.0
	10	6	0	0	9.0
	10	6	0	0	10.0
	10	6	0	0	8.5
MEKC	10	6	10	0	8.5
	10	6	10	1	8.5
	10	6	10	5	8.5
	10	6	10	10	8.5
	10	6	20	0	8.5
	10	6	20	10	8.5
	10	6	25	0	8.5
	10	6	50	0	8.5

Appendix 4

CALIBRATION AND REPRODUCIBILITY

Appendix 4 presents the data and results for the reproducibility and calibration studies for both the high performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) methods referred to in **Chapter 5**. The calibration for the capillary electrophoresis method, micellar electrokinetic chromatography (MEKC), used for quantitation in **Chapter 7** is also included here.

A4.1 CALIBRATION DATA FOR THE HPLC SYSTEM

HPLC calibration data for C I Reactive Red 141 at 290 nm. The data was obtained on the Waters HPLC system. Analysis conditions as described in **Appendix 3**.

Table A4.1 : HPLC calibration data for C I Reactive Red 141 at 290 nm.

Concentration mg /ℓ	Peak Area at 290 nm
10	0.001998
20	0.00449
30	0.00696
40	0.00899
50	0.01175
60	0.01423
70	0.01717
80	0.01963
90	0.02161
100	0.02322

Calibration graph, Fig A4.1, using the above data given above Table A4.1.

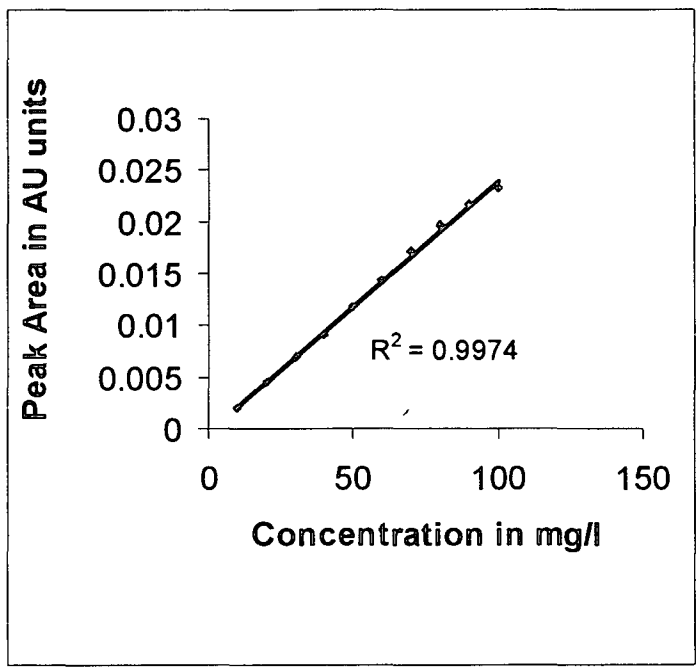


Figure A4.1 : HPLC calibration graph for C I Reactive Red 141 at 290 nm.

A4.2 Reproducibility Data and Results for HPLC System

HPLC reproducibility data for C I Reactive Red 141 at 290 nm. The data was obtained on the Waters HPLC system. Analysis conditions as described in **Appendix 3**.

Table A4.2 : Reproducibility data at 290 nm for the HPLC method performed on the Waters HPLC system. Analysis conditions as described in **Appendix 3**.

Run No.	Retention time (min)	Peak Area (AU)
1	10.62	0.01028
2	10.62	0.01079
3	10.63	0.01020
4	10.63	0.01040
5	10.63	0.01042
6	10.63	0.01036
7	10.63	0.01043
8	10.62	0.01033
9	10.62	0.01026
10	10.61	0.01021
11	10.62	0.01025
12	10.62	0.01022
13	10.63	0.01084
14	10.62	0.01046
15	10.62	0.01049
16	10.62	0.01040
17	10.61	0.01035
18	10.62	0.01031
19	10.61	0.01031
Average	**10.62**	**0.01038**
RSD (%)	**0.06**	**1.67**

A4.3 CZE CALIBRATION

Capillary zone electrophoresis (CZE) calibration data for C I Reactive Red 141 at 290 nm using 10 mM sodium dihydrogen phosphate and 6 mM sodium tetraborate buffer system at a pH value of 8.5. Analysis conditions as described in **Appendix 3**. The reason for using a normalised peak area is given in **Appendix 2**.

Table A4.3 : CZE calibration data for C I Reactive Red 141 at 290 nm.

Concentration mg/ℓ	Migration Time (min)	Peak Area (AU)	Norm. Area
10	19.831	0.1011	0.00510
20	19.923	0.4333	0.02175
30	19.937	0.6354	0.03187
40	19.946	0.8457	0.04240
50	20.107	1.0536	0.05240
60	20.133	1.2873	0.06394
70	20.108	1.4910	0.07415
80	20.011	1.6839	0.08415
90	19.998	1.8964	0.09483
100	20.105	1.9993	0.09944

Calibration graph, Fig A4.2, using the data given above in Table A4.3.

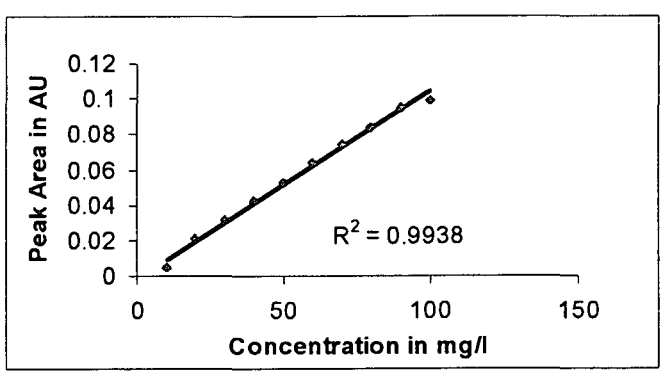


Figure A4.2 : CZE calibration graph for C I Reactive Red 141 at 290 nm. Buffer used : 10 mM sodium dihydrogen phosphate and 6 mM sodium tetraborate, pH 8.5.

A4.4 Reproducibility Data and Results for the CZE System

Capillary zone electrophoresis (CZE) reproducibility data for C I Reactive Red 141 at 290 nm using 10 mM sodium dihydrogen phosphate and 6 mM sodium tetraborate buffer system at a pH value of 8.5. Analysis conditions as described in **Appendix 3**. The reason for using a normalised peak area is given in **Appendix 2**.

Table A4.4 : Reproducibility data of the CZE method, using the 10 mM sodium dihydrogen phosphate and 6 mM sodium tetraborate buffer system. Analysis conditions as described in **Appendix 3**.

Run No	Migration Time (min)	Peak Area (AU)	Norm Area
1	19.825	1.0264	0.05177
2	19.882	0.9845	0.04952
3	20.924	1.0393	0.04967
4	20.904	1.0549	0.05046
5	19.837	0.9935	0.05008
6	19.546	0.9669	0.04947
7	19.031	0.9541	0.05013
8	17.058	0.8438	0.04947
9	18.108	0.8563	0.04729
10	19.133	0.9372	0.04898
11	18.736	0.8794	0.04694
Average	**19.362**	**0.9578**	**0.04944**
RSD (%)	**5.89**	**7.58**	**2.76**

A4.5 MICELLAR ELECTROKINETIC CHROMATOGRAPHIC CALIBRATION

The following calibration was performed to test the linearity of the buffer used for quantitation in **Chapter 7**. Analysis conditions as described in **Appendix 3**.

Table A4.5 : MEKC calibration data for C I Reactive Red 141 at 290 nm, buffer used contained 10 mM sodium dihydrogen phosphate, 6 mM tetraborate, 10 mM sodium dodecyl sulphate (SDS) and 10 mM tetrabuytlammonium bromide.

Concentration mg/ℓ	Migration Time (min)	Peak Area (AU)	Norm Area
10	9.23	0.0406	0.00440
20	9.18	0.1847	0.02012
30	9.13	0.2832	0.03102
40	9.03	0.4031	0.04464
50	9.08	0.4825	0.05314
100	9.01	0.9399	0.1044

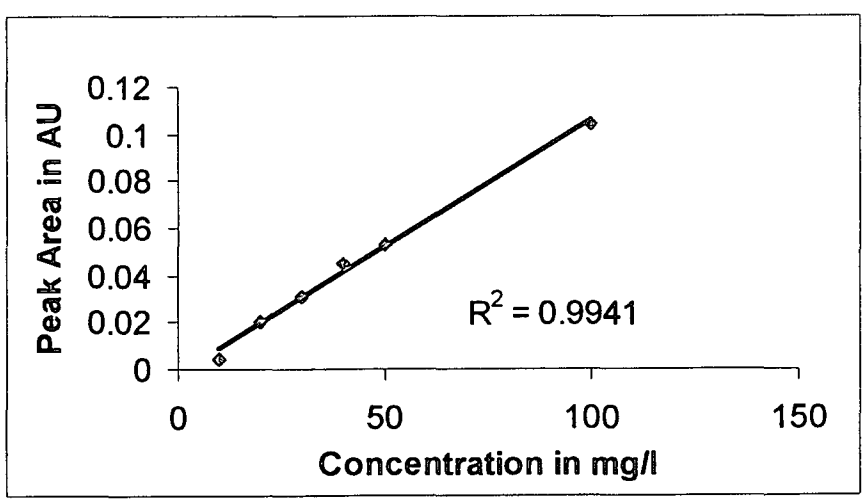


Figure A4.3 : Calibration graph for the buffer used in **Chapter 7**, viz. 10 mM sodium dihydrogen phosphate, 6 mM tetraborate, 10 mM sodium dodecyl sulphate (SDS) and 10 mM tetrabuytlammonium bromide.

Appendix 5

EXTRACTION DATA

Appendix 5 presents the data and results of the extraction of C I Reactive Red 141 from fortified tap and river water. River water samples were taken from the Umbilo River near the Umbilo Wastewater Treatment Works. Two extraction methods were employed for the extractions from river water analysis viz. liquid-liquid extraction (LLE) and solid phase extraction (SPE). Results were calculated using a single point calibration based on the knowledge that both the CE and HPLC methods used where linear in the concentration range used, 50 mg/ℓ (see **Appendix 4** for calibration). The reason for using normalised peak areas for CE is given in **Appendix 2.**

Table A1 : Data and results of the LLE extractions of C I Reactive Red 141 from tap water and subsequent HPLC analysis.

Standard		**Sample**			
Run No	**Area AU**	**Ext No.**	**Area**	**Average**	**% Recovery**
1	0.008259	1	0.008494		
2	0.008203		0.008465	0.008480	102.7
3	0.008145	2	0.008056		
4	0.008392		0.008051	0.008054	97.52
5	0.008135	3	0.008192		
6	0.008314		0.008246	0.008219	99.52
7	0.008291	4	0.008407		
8	0.008301		0.008303	0.008355	101.17
9	0.008288	5	0.008337		
Average	0.008259		0.008344	0.008341	100.99
% Recovery =		6	0.008374		
(Sample area / standard area) x 100			0.008336	0.008355	101.17
Average Recovery					**100.51 %**
Standard Deviation					**1.77 %**

Table A2 : Data and results of the LLE extractions of C I Reactive Red 141 from tap water and analysis by CE.

Standard				Sample					
Run No	**Mig Time min**	**Area AU**	**Norm Area**	**Buffer pH**	**Ext No.**	**Mig Time min**	**Area**	**Norm Area**	**% Recovery**
1	8.70	0.46567	0.05353	6	1	8.87	0.52207	0.05886	110.99
2	8.75	0.46538	0.05319	6	2	8.85	0.44246	0.04999	94.27
3	8.91	0.45998	0.05163	6	3	8.87	0.46320	0.05222	98.47
4	9.02	0.48507	0.05378	% average recovery at pH 6					**101.24**
Average			0.05303	7	1	8.94	0.48159	0.05387	101.58
% Recovery =				7	2	8.88	0.49785	0.05606	105.71
				7	3	8.85	0.48130	0.05438	102.55
(Sample area / standard area) x 100				% average recovery at pH 7					**103.28**
				8	1	8.80	0.45394	0.05158	97.27
				8	2	8.75	0.4318	0.04935	93.06
				8	3	8.78	0.47363	0.05394	101.72
				% average recovery at pH 8					**97.35**

Table A3 : Data and results of the LLE extractions of C I Reactive Red 141 from river water, analysis by CE.

Standard				Sample					
Run No	**Mig Time min**	**Area AU**	**Norm Area**		**Ext No.**	**Mig Time min**	**Area**	**Norm Area**	**% Recovery**
1	9.30	0.52259	0.05619	Up stream	1	9.38	0.4301	0.04585	80.45
2	9.48	0.54396	0.05738		2	9.40	0.1759	0.01871	32.83
3	9.42	0.53356	0.05664	% average recovery					**56.64**
4	9.35	0.53985	0.05774	Down stream	1	9.35	0.2683	0.02870	50.36
Average			0.05699		2	9.37	0.3907	0.04170	73.17
% Recovery =				% average recovery					**61.77**
				Activated Sludge outlet	1	9.36	0.4591	0.04905	86.07
(Sample area / standard area) x 100					2	9.39	0.4966	0.05289	92.80
				% average recovery					**89.08**
				Old Plant	1	9.32	0.4363	0.04681	82.13
					2	9.29	0.2601	0.02799	49.11
				% average recovery					**65.62**

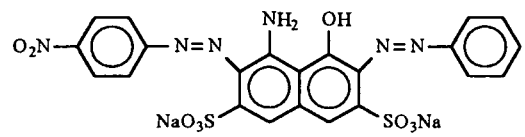
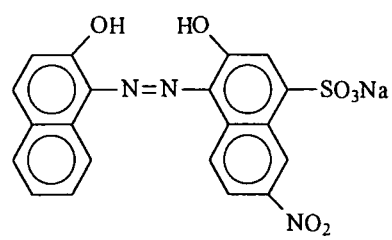
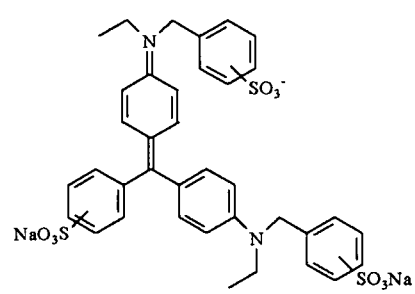
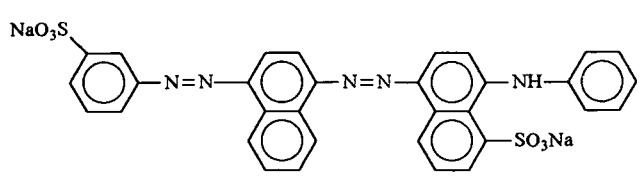
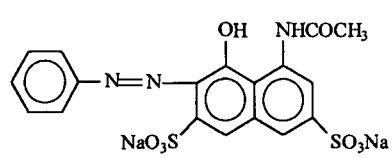
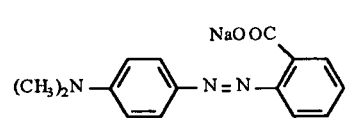
Table A3 : Data and results of the SPE extractions of C I Reactive Red 141 from river water, analysis by CE.

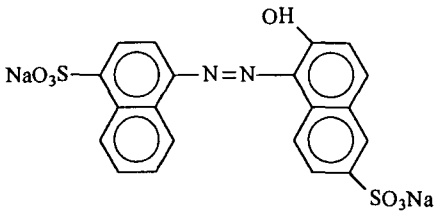
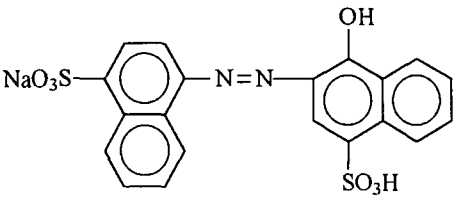
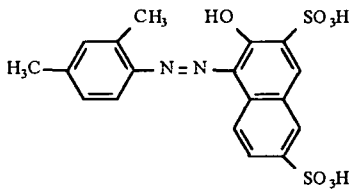
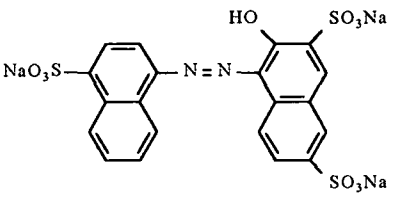
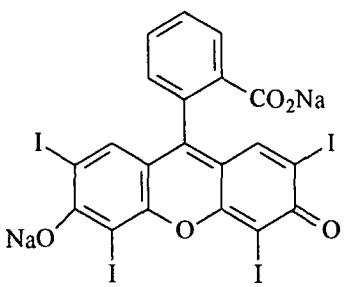
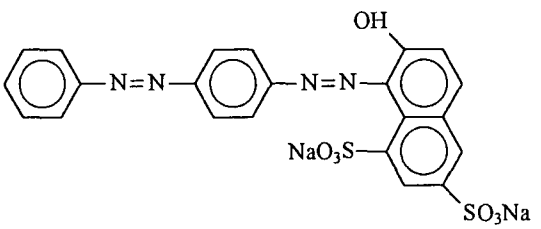
Standard				Sample					
Run No	**Mig Time min**	**Area AU**	**Norm Area**		**Ext No.**	**Mig Time min**	**Area**	**Norm Area**	**% Recovery**
1	8.75	0.44127	0.05043	Up stream	1	8.74	0.42934	0.04912	96.26
2	8.72	0.46512	0.05333		2	8.75	0.41932	0.04792	93.91
3	8.75	0.43564	0.04979	% average recovery					**95.09**
4	8.73	0.44159	0.05058	Down stream	1	8.75	0.4599	0.05253	102.94
Average			0.05103		2	8.76	0.4321	0.04933	96.66
% Recovery =				% average recovery					**99.80**
				Activated Sludge outlet	1	8.80	0.4680	0.05318	104.21
(Sample area / standard area) x 100					2	8.82	0.4332	0.4912	96.26
				% average recovery					**100.24**
				Old Plant	1	8.89	0.4409	0.04960	97.20
					2	8.87	0.4215	0.04752	93.12
				% average recovery					**95.16**

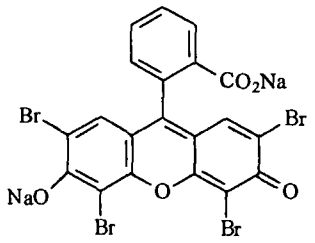
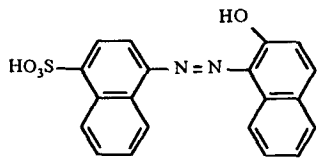
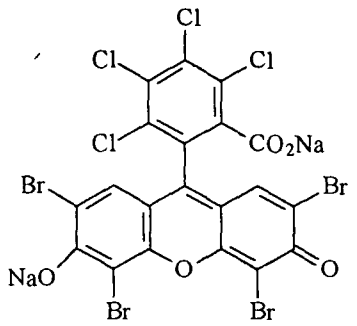
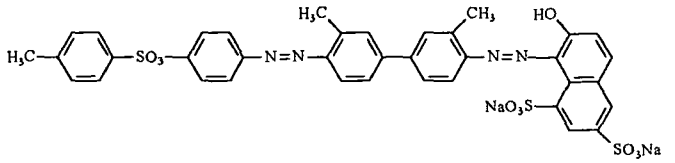
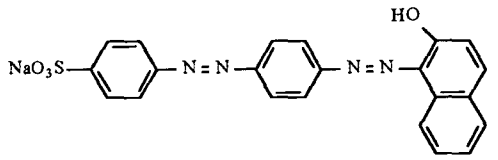
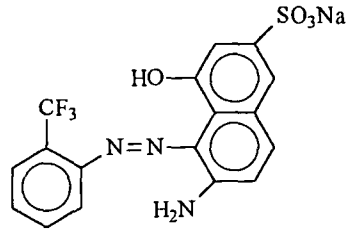
Appendix 6

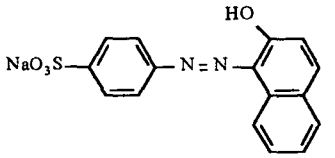
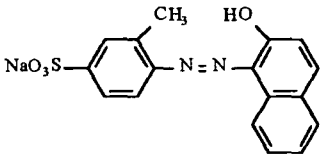
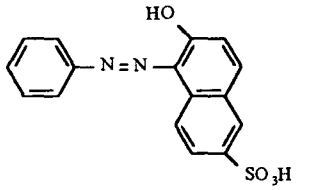
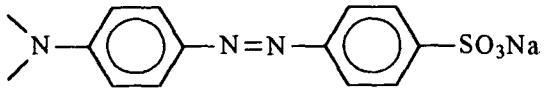
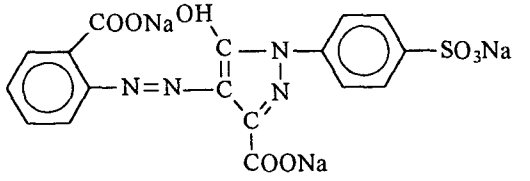
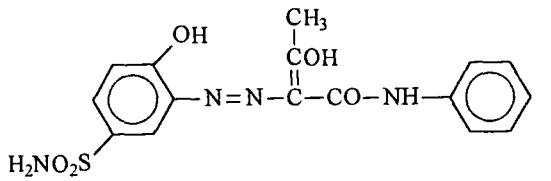
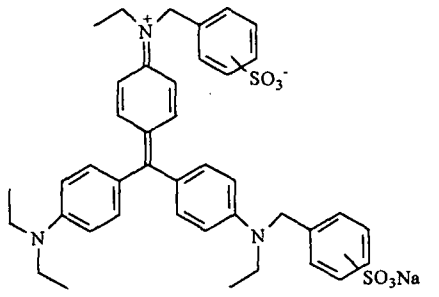
DYE STRUCTURES

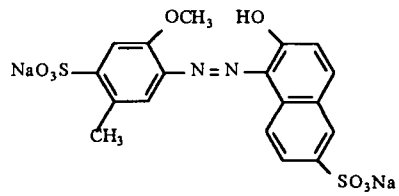
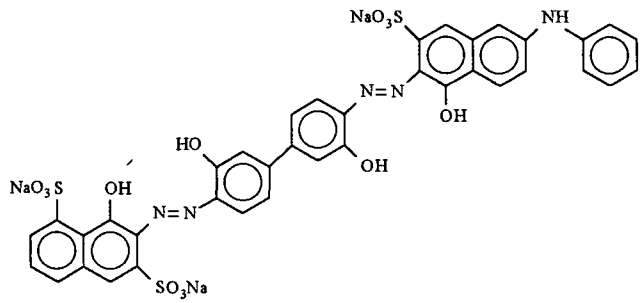
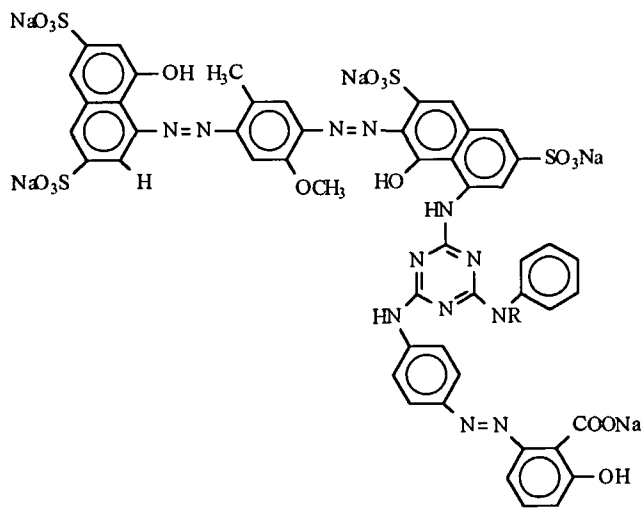
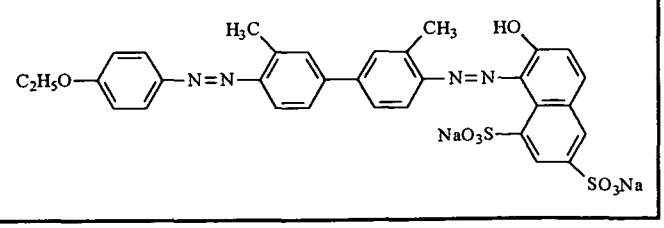
Appendix 6 lists the dyes referred to in the literature review, **Chapter 3**. The dyes are arranged alphabetically.

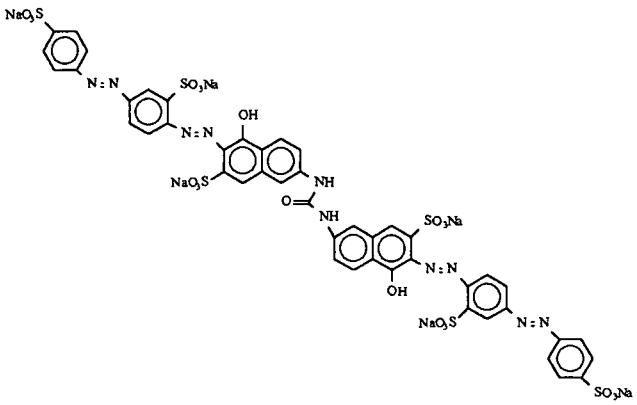
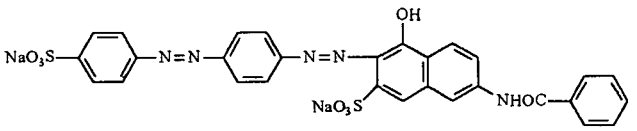
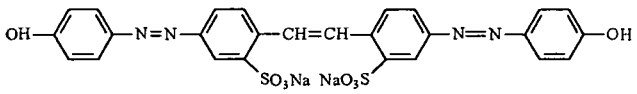
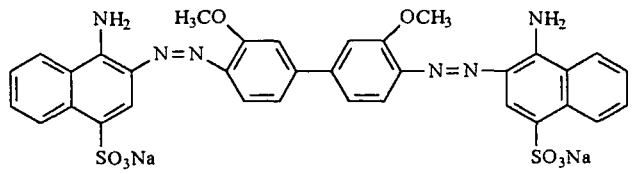
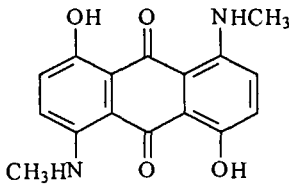
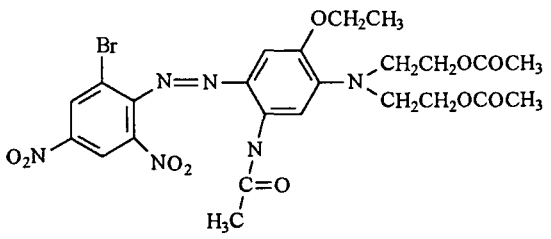
Dye Name	Structure
Acid Black 1 MW 616	
Acid Black 52 MW 461	 complexed with chromium
Acid Blue 9 MW 921	
Acid Blue 113 MW 681	
Acid Red 1 MW 509	
Acid Red 2 (Methyl Red) MW 291	

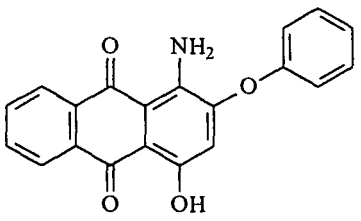
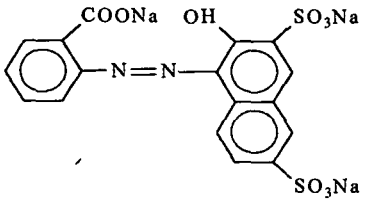
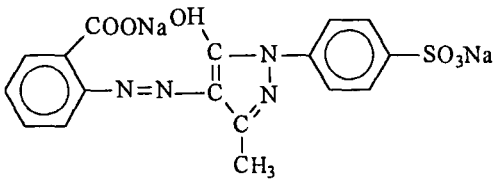
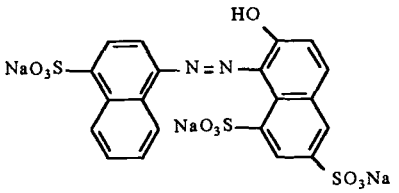
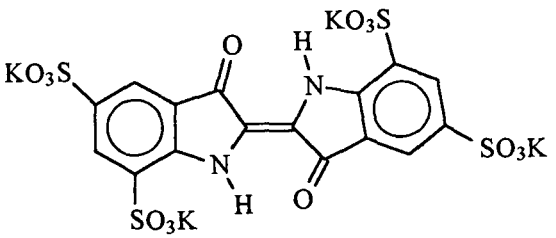
Acid Red 13 MW 502	
Acid Red 14 MW 502	
Acid Red 26 (Ponceau 2R) MW 436	
Acid Red 27 (Amaranth) MW 604	
Acid Red 51 MW 942	
Acid Red 73 MW 556	

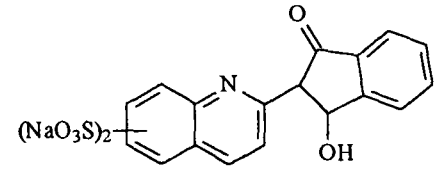
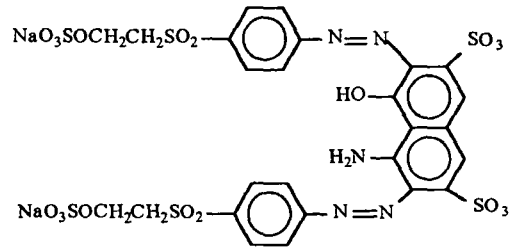
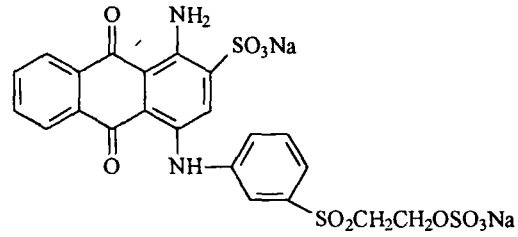
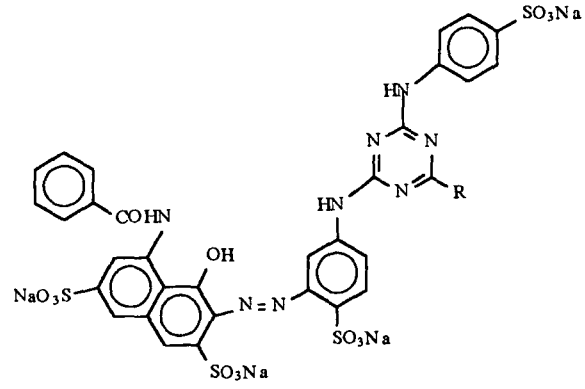
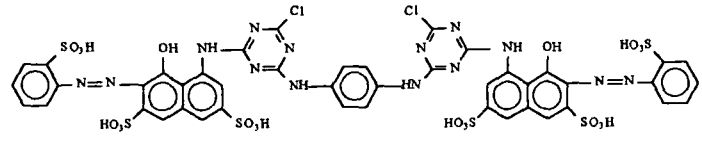
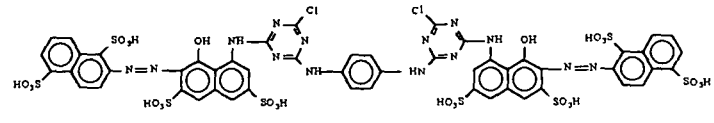
Acid Red 87 MW 754	
Acid Red 88 MW 378	
Acid Red 92 MW 892	
Acid Red 114 MW 846	
Acid Red 151 MW 454	
Acid Red 337 MW 433	

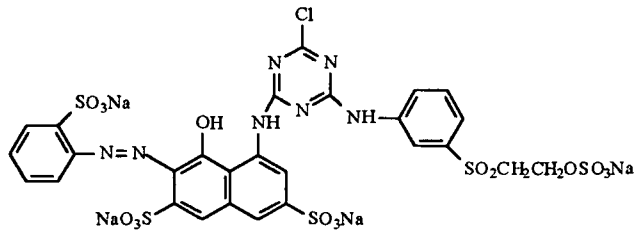
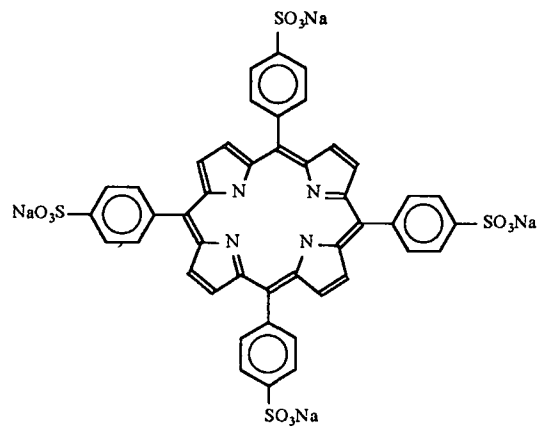
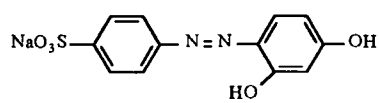
Acid Orange 7 (Orange II) MW 350	
Acid Orange 8 MW 364	
Acid Orange 12 (Crocein Orange G) MW 328	
Acid Orange 52 MW 327	
Acid Yellow 23 MW 534	
Acid Yellow 151 MW 376	
Acid Violet MW 874	

Allura Red AC MW 527	
Amaranth	see Acid Red 27
Crocein Orange G	see Acid Orange 12
Direct Blue 98 MW 1049	
Direct Green 26 MW 1332	
Direct Red 39 MW 705	

Direct Red 80 MW 1373	
Direct Red 81 MW 690	
Direct Yellow 4 MW 638	
Direct Violet 32 MW 757	
Disperse Blue 26 MW 298	
Disperse Blue 79 MW 652	

Disperse Red 60 MW 331	
Orange II	see Acid Orange 7
Methyl Red	see Acid Red 2
Mordant Red 9 MW 518	
Mordant Yellow 8 MW 446	
New Coccine MW 620	
Ponceau 2R	see Acid Red 26
Potassium Indigo Tetrasulphonate MW 735	

Quinoline Yellow MW 322	
Reactive Black 5 MW 988	
Reactive Blue 19 MW 626	
Reactive Red 4 MW 959	
Reactive Red 120 MW 1504	
Reactive Red 141 MW 1764	

Reactive Red 194 MW 999	
Tetrasodium-meso-tetra-(4-sulphonatophenyl) Porphine MW 1045	
Tropaeolin O MW 316	

Appendix 7

PURIFICATION OF DYES

Appendix 7 describes the procedures used for the attempted purification of the commercial dyes referred to in **Chapter 4**.

A7.1 Precipitation Purification Method

Nesynova et al. (1984) purified Active Bright Red 5 Skh by the precipitation method described by Venkataraman (1977). The technical dye obtained was dissolved in dimethyformimide (DMF) to remove the salts and precipitated by the addition of acetone or chloroform.

Using this method no precipitate was obtained with the addition of acetone or chloroform for the 5 selected dyes investigated. From the study conducted at Georgia Institute of Technology (Tichner, 1988), some acid and direct dyes were purified by the precipitation method. The precipitate was achieved by the addition of isopropanol. Hence, the addition of isopropanol was attempted to precipitate the selected dyes, but this as well did not yield a precipitate.

A7.2 Resin Adsorption Technique

Tichner (1988) used the resin adsorption technique to purify a number of acid, direct and reactive dyes. In this method the solution of dye being purified was prepared in distilled water, filtered, and mixed with Amberlite XAD-2 resin beads which had previously been extracted with methanol and water. The beads were recovered by filtration and thoroughly washed with water. The adsorbed dye was then removed from the beads by methanol extraction in a Soxhlet unit. The dye extracted from the resin beads was then recovered by evaporation of the methanol.

This above method was applied for the purification of C I Reactive Red 120 and C I Reactive Red 141, however, the removal of the dyes from the resin beads by Soxhlet extraction was unsuccessful despite changing the extraction solvent. The very large sizes of the reactive dyes used may have resulted in the dye being irreversibly bound to the resin due to strong resin-dye interaction.

In the Tichner report Reactive dyes; Orange 4, Red 2 and Blue 19, were purified by the resin adsorption method. However, concentration of acid, disperse, direct and reactive dyes from wastewater based on the resin adsorption method was reported to work well only for acid and disperse dyes but was not satisfactory for direct and reactive dyes. The reasons given were that either the larger sizes of the reactive and direct dyes were not compatible with the pore sizes of the resin or the resin-dye interaction was too strong for quantitative dye removal.

It should be noted that the reactive dyes used in this study were much larger than the reactive dyes used by Tincher. This may explain why the attempt at dye purification by the resin adsorption was unsuccessful for the present study.

A7.3 COLUMN CHROMATOGRAPHY

Dyes have traditionally been analysed by thin layer chromatography (TLC) using TLC plates DC-Alufolien Kieselgel 60F254. Forensic analysis of reactive dye adducts from wool were analysed by TLC (Crabtree et al, 1995). Using the latter paper as a guide, various combinations of eluents were attempted for developing the TLC plates for the dyes in this study. The eluent that gave the least tailing was found to be: n-butanol : ethanol : ammonia : pyridine : water at a composition of 8:3:4:4:6. Using this eluent composition on a column packed with silica gel for the purification of C I Reactive Red 141, resulted in the dye being irreversibly adsorbed onto the silica gel. Changing the compositions of mobile phase was unsuccessful in removing the dye.

N Naidoo (1995) in his MSc study used flash column chromatography with silica gel and 30 : 70 methanol / methylene chloride in an attempt to purify C I Reactive Red 141. He reported that the dye adsorbed to the silica gel and separation was not possible.

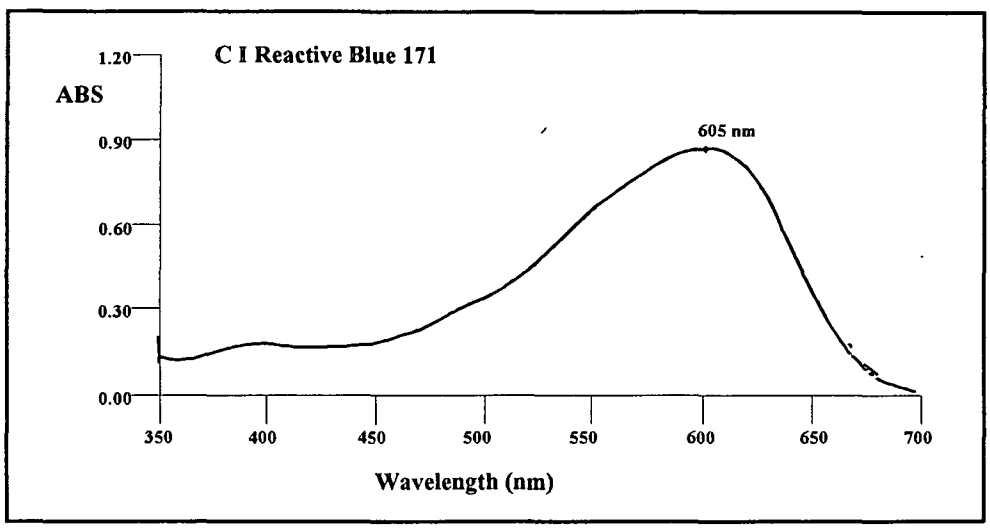
No further attempts were made at purifying the dyes.

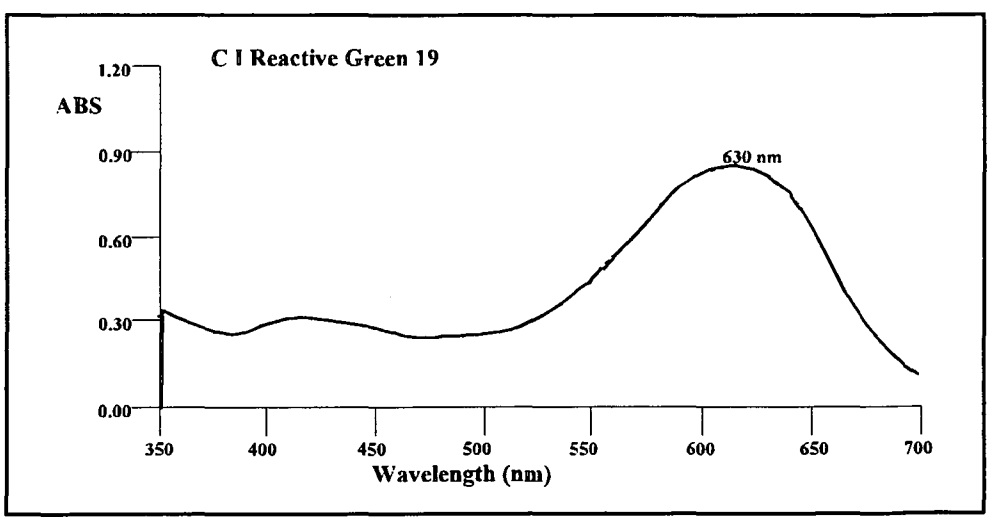
Appendix 8

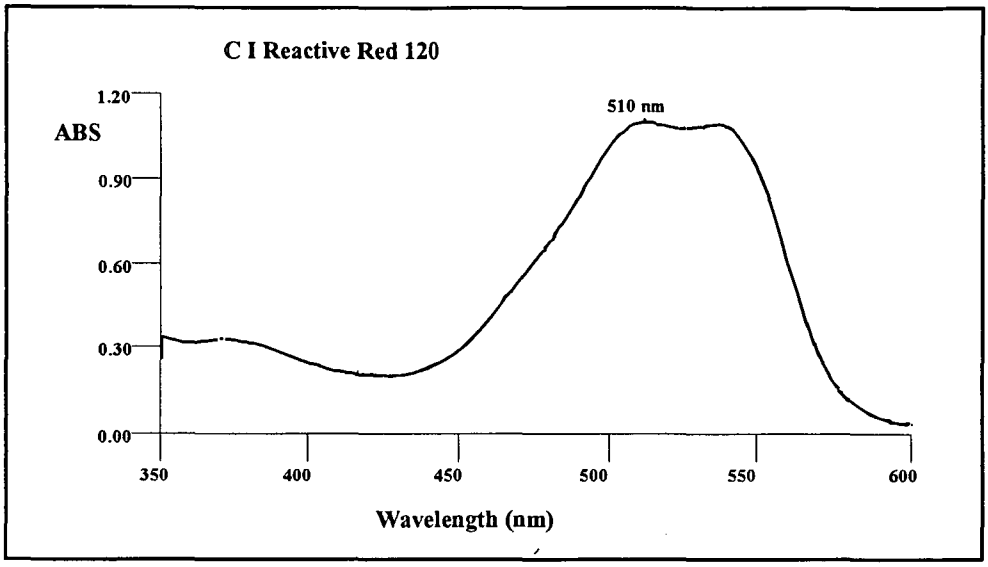
ABSORBANCE SPECTRA

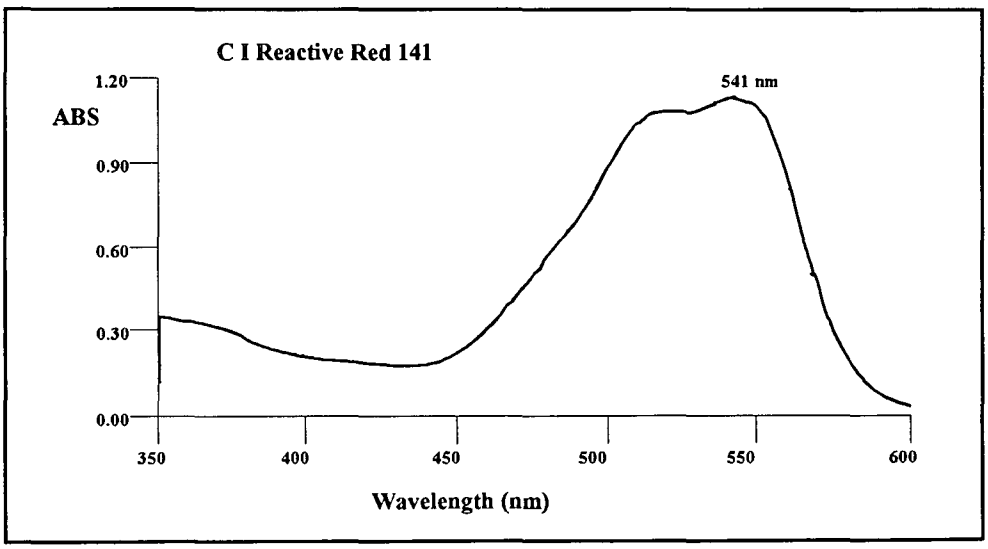
Appendix 8 contains the absorbance spectra of the 5 selected dyes.

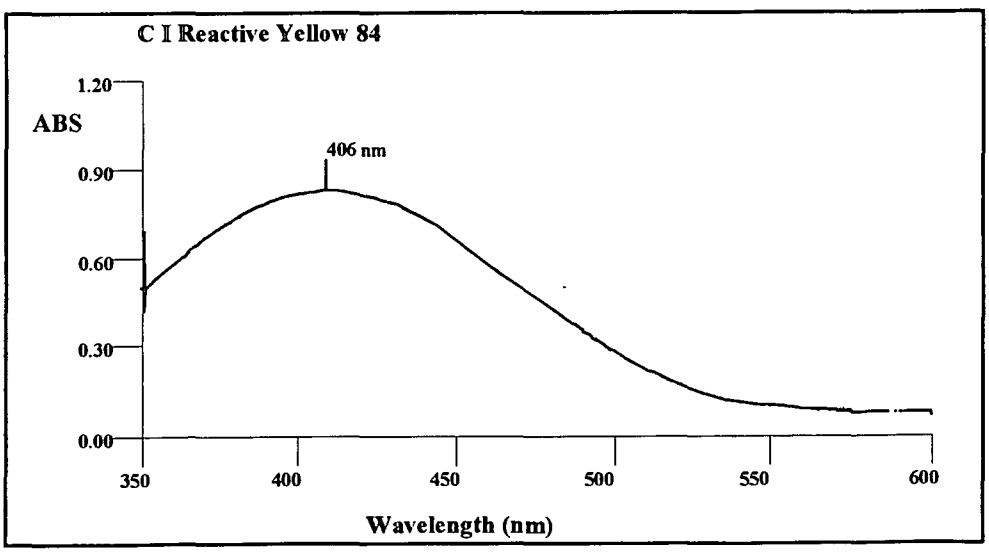
I. Absorbance Spectra recorded on ultraviolet-visible absorbance spectrophotometer. The spectra were recorded in the visible range using dye solutions at a concentration of 50 mg/ℓ. Analysis conditions as described in **Appendix 3**.



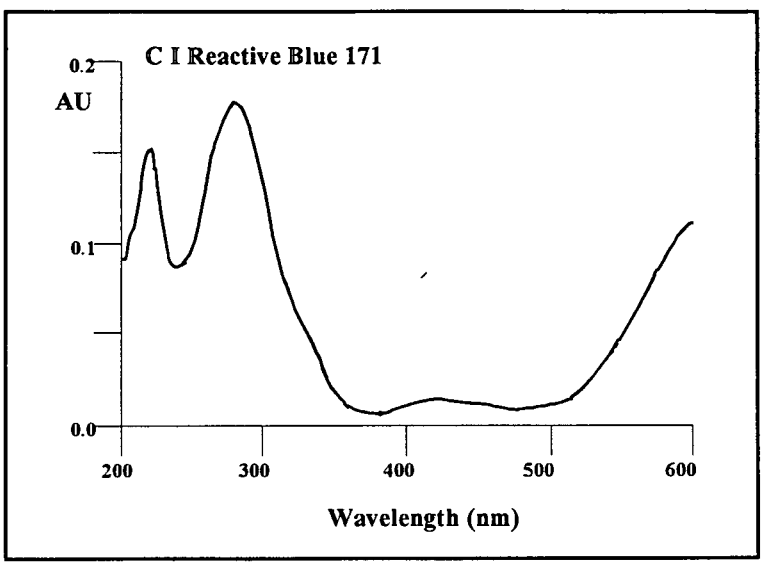


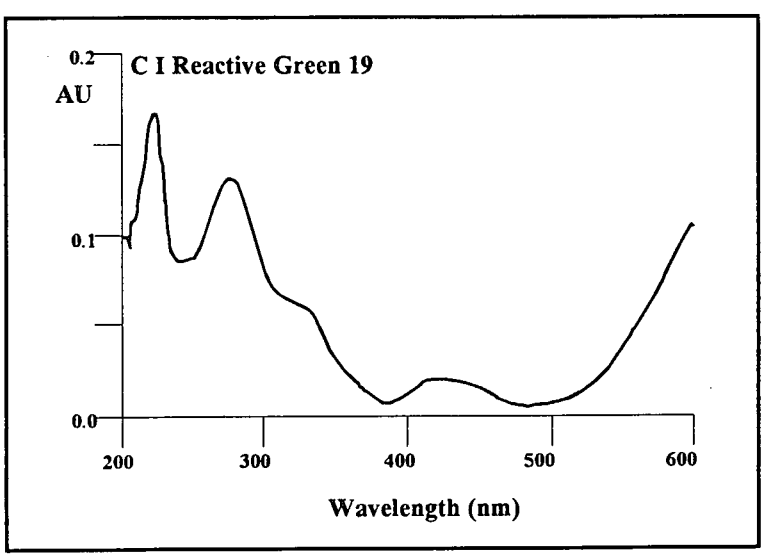

C I Reactive Red 120
ABS
1.20
0.90
0.60
0.30
0.00
510 nm
350
400
450
500
550
600
Wavelength (nm)

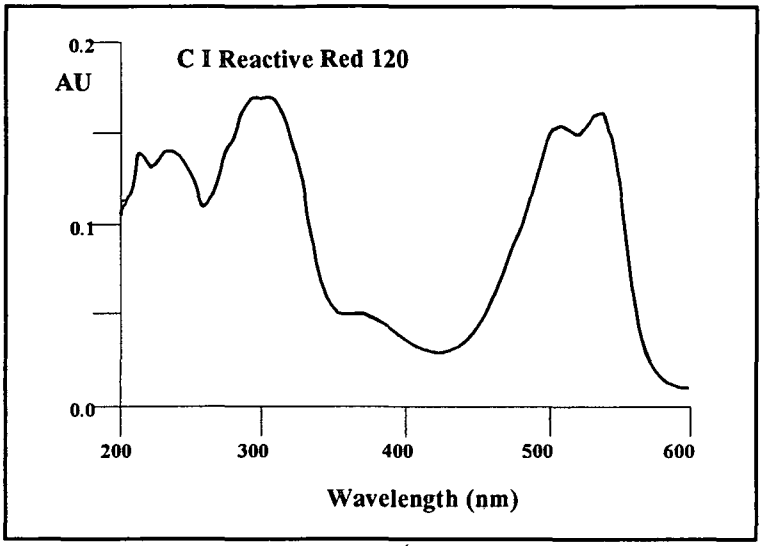

C I Reactive Red 141
ABS
1.20
0.90
0.60
0.30
0.00
541 nm
350
400
450
500
550
600
Wavelength (nm)

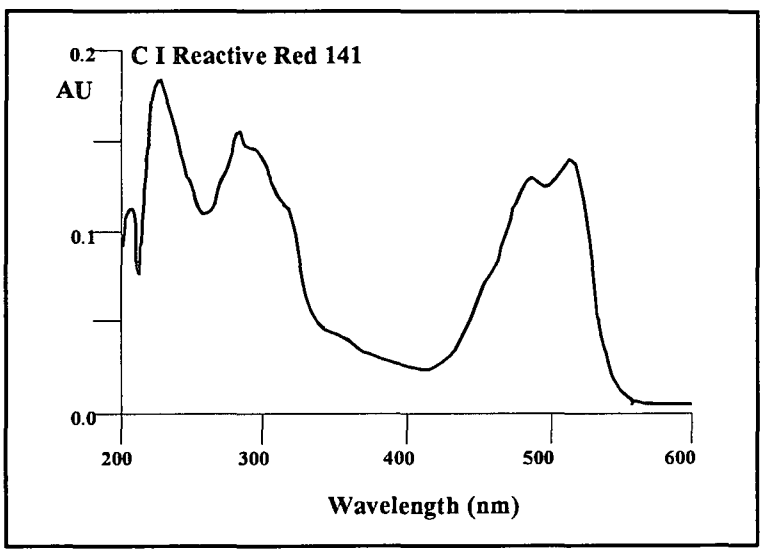

C I Reactive Yellow 84
ABS
1.20
0.90
0.60
0.30
0.00
406 nm
350
400
450
500
550
600
Wavelength (nm)

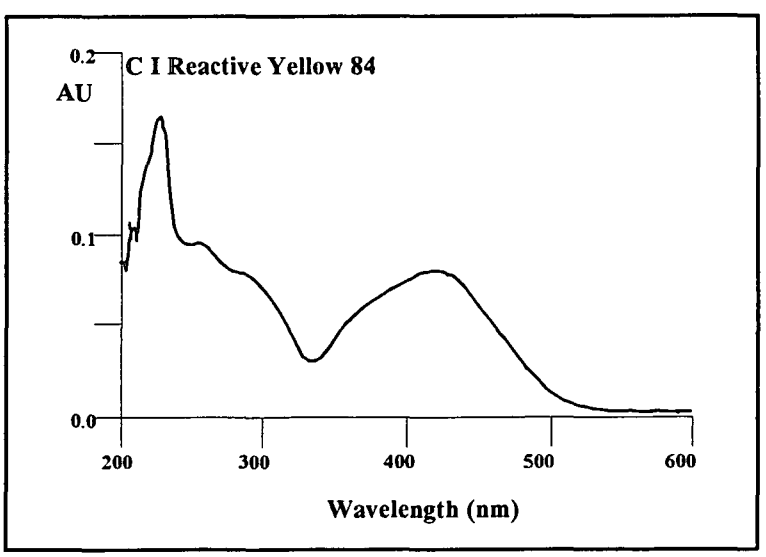
II. Absorbance spectra were recorded using the photodiode array detector (PDA) after injecting the dye standards on the Waters HPLC system. The spectra were recorded in the region 200 to 600 nm using dye solutions at a concentration of 50 mg/ℓ. Analysis conditions as described in **Appendix 3** for the Waters HPLC system.






C I Reactive Red 120
0.2
AU
0.1
0.0
200
300
400
500
600
Wavelength (nm)


C I Reactive Red 141
0.2
AU
0.1
0.0
200
300
400
500
600
Wavelength (nm)


C I Reactive Yellow 84
0.2
AU
0.1
0.0
200
300
400
500
600
Wavelength (nm)