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DYE ADSORPTION STUDIES ON CHITIN AND CHITOSAN

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A thesis submitted in partial fulfilment of the requirements of The Nottingham Trent University for the degree of Master of Philosophy

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Abstract

Title: Dye Adsorption Studies on Chitin and Chitosan

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This thesis reports on the mechanism of adsorption of metallised and non-metallised dyes by chitin and chitosan at neutral pH. This was achieved by examining the build up of various pre-metallised and non-metallised acid and direct dyes on powdered chitin and the diffusion characteristics of these dyes through chitosan films. The effects of varying temperature and electrolyte concentration were also examined to assess any effects these may have on the adsorption characteristics of chitin and chitosan. The stability of the dye-fibre interaction was examined using Soxhlet extractions and colour-fastness tests developed for textile quality assessment. Comparison has been made between the adsorption mechanisms of cellulose, wool and nylon under normal dyeing conditions, and the adsorption at neutral pH.

It was found that the adsorption mechanism of 1:1 pre-metallised acid dyes was different at neutral pH to that at acid pH. It was also found that both the adsorption mechanism and diffusion characteristics of coppered direct dyes were different to that of uncoppered direct dyes, the coppered directs being adsorbed by a site specific mechanism whereas the uncoppered direct dyes were not. This led to the conclusion that a metal complex was formed between the central metal ion in the dye molecule and the chitin or chitosan chain, the amine groups acting as ligands for the available co-ordination sites. The diffusion coefficient for C.I. Direct Blue 15 on chitosan film was determined.

The amine groups available within the polymer chains of wool and nylon were proposed as possible sites for metal complex formation and this was also found to be the case for these polymers.

In memory of

Collin Owen Cobb

&

Marjorie Cobb

Who now will sing me lullabies

Acknowledgements

The work for this thesis was carried out in the labs of the Design of Materials Group in the department of Fashion and Textiles at The Nottingham Trent University.

I would like to express my sincere gratitude to Dr G. A. F. Roberts for the guidance, patience and friendship I have received whilst studying under his supervision. I would also like to thank Mrs F. A. Wood for her support and advice, which I have received from her during my time within the department.

I would also like to thank Dr C. C. Perry of the Department of Chemistry and Physics, Nottingham Trent University, Miss Rossmarie Dauth, I could not of asked for a better friend and colleague, and also Dr B. A. Austin of M.I.T Ltd. Belfast for making the Sekaido roll apparatus.

Thanks must also go to my family, particularly Lisa, for the vast amount of patience she has shown me whilst preparing this document.

As to the colour usually found in beautiful bodies, it may be somewhat difficult to ascertain them, because, in the several parts of nature, there is an infinite variety

Edmund Burke (1729–1797). On the Sublime and Beautiful.

The Harvard Classics. 1909–14.

Contents

1 Introduction

Chitin and its deacetylated derivative chitosan are naturally occurring polysaccharides obtained primarily from the exoskeletons of crustacea, molluscs and insects. Chitin is the second most abundant biopolymer, after cellulose; the material used for this study came from the 1000's of tons of waste material produced by the seafood industry.

Chitin is poly[β-(1-4)-2-acetamido-2-deoxy-**D**-glucopyranose] and its idealised structure is given in Fig.1.1. Chitosan is poly[β-(1-4)-2-amino-2-deoxy-**D**-glucopyranose] and its idealised structure is given in Fig. 1.2. However in reality these are two extremes of a continuous series of copolymers of the two monomer residue units, anhydro *N*-acetyl-**D**-glucosamine and anhydro **D**-glucosamine, chitosan being defined as the range of these copolymers soluble in dilute acid.

In recent years interest has grown in naturally occurring polymers, and the rise of environmental issues regarding the recycling of waste products generated by industrial processors has led to an increase in investigations into the potential use of chitin and chitosan in various applications.

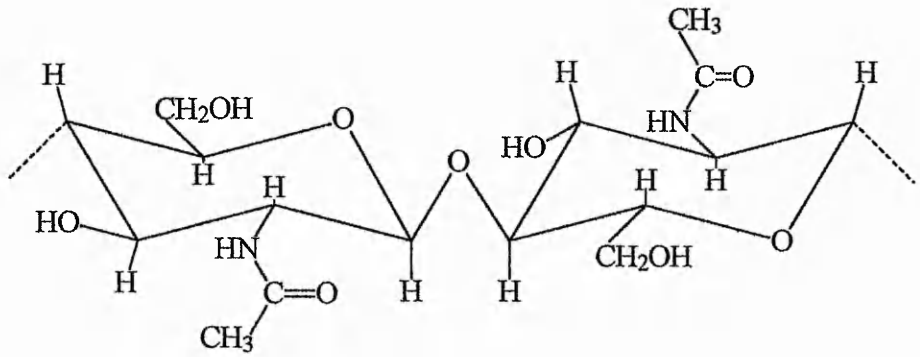


Fig 1.1 Idealised structure of Chitin

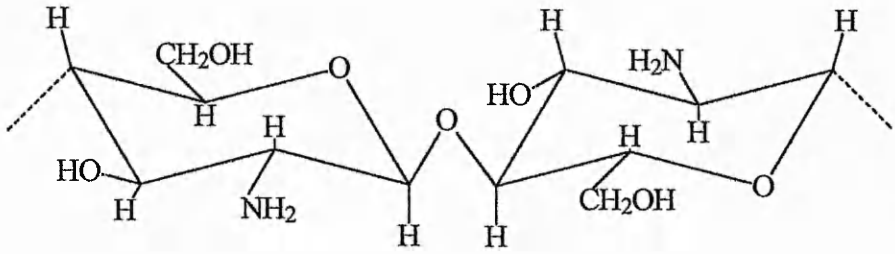


Fig 1.2 Idealised Structure of Chitosan

2 Historical Review

2.1 Discovery of Chitin and Chitosan

Chitin was first described in 1811 by Braconnot[1], when he isolated a product from fungi, which he named fungine. In 1823, Odier[2] established the relationship between plant tissue and insect cuticle and it was he who named the isolated product chitin. The term chitin was derived from the Greek for tunic or envelope. The presence of nitrogen in chitin was first demonstrated by Lassaigne[3] in 1843 in his work with silkworms. Ledderhose[4] proposed that chitin was composed of glucosamine and acetic acid and the presence of glucosamine was confirmed in 1894 by Gilson.[5]

Chitosan, which is now known to be the primary derivative of chitin, was first prepared in 1859 by Rouget[6], when he treated chitin with concentrated potassium hydroxide. This "modified Chitin" was named chitosan in 1894 by Hoppe-Seyler[7].

Research continued into the twentieth century concerning the occurrence of chitin in living organisms and into its chemical constitution. In 1946 after much controversy surrounding the chemical form of the nitrogen, Purchase and Braun[8] described chitin as a polymer of *N*-acetyl-**D**-glucosamine.

2.2 Occurrence of Chitin and Chitosan in Nature

Chitin abounds in nature. It is the major structural polysaccharide used by most invertebrates, arthropods being the most significant group. However chitin is also found in less evolved groups as well as in lower plants such as

fungi. Both fungal and animal chitin has been shown to have the same identity[9, 10, 11, 12, 13, 14, 15, 16]

2.2.1 Chitin in Lower Plants

Chitin is present as a major structural component in the cell wall of plants such as fungi and moulds. These plants metabolise considerable amounts of nitrogen. Wisselingh[17] showed that the cell wall of fungi consists of either cellulose or chitin and that they are responsible for the shape and rigidity of the cells.

2.2.2 Chitin in Animals

Chitin is the major structural polysaccharide in most invertebrates, but whereas in plants the chitin is associated with other polysaccharides, in animals it is associated with proteins, particularly collagen[18] as well as mineral salts. Chitin is found in all major classes of arthropods both in their exoskeletons as well as in internal organs. The dry organic matter found in their cuticle can contain up to 80% chitin. This abundance has led to the arthropods being a major source of chitin for industrial use. Chitin is also found in the shells of various mollusc species, however the shells of molluscs only contain between 1-5% chitin, the rest being calcium carbonate. Although mollusc shells are available in large quantities the low level of chitin in them means that they are not a suitable source for chitin on an industrial scale.

2.3 Morphology of Chitin

Studies using X-ray diffraction[19, 20] have shown that chitin exists in three polymorphic forms, α, β, and γ chitin. The three forms differ in the arrangement of polymer chains within the unit cell. In α chitin the chains have an anti-parallel arrangement, which makes this the most stable and compact form of chitin. In β chitin, which exists as a crystalline hydrate[21, 22], the chains are arranged in parallel, the lower stability of this form being attributed to the relative ease with which water can penetrate the lattice. γ chitin is the least stable form of chitin and in this form the chains are in a two chains "up" to every one chain "down" arrangement.

The reason for the existence of these three polymorphic forms has been attributed to function[23]; α chitin is associated where extreme hardness is required, such as the exoskeletons of crustacea. β and γ chitin are associated with collagen type proteins and are found where toughness and flexibility are required. The three forms have been found within the same organism; in the squid *Loligo* the beak is formed from α chitin, the pen is formed from β chitin, and the lining of the stomach from γ chitin. This appears to confirm the link between morphology and function as opposed to taxonomic grouping.

In recent years doubt has been cast as to the existence of γ chitin since its first description by Rudall[19]. It is now suggested that what Rudall thought to be γ chitin is a distorted form of either α or β and not a true polymorphic form.

2.4 Isolation of Chitin

Chitin is usually found in conjunction with other substances. In crustacea, which is the most abundant source for chitin, it is closely associated with calcium carbonate and proteins. The isolation of chitin generally involves harsh chemical treatment, typically decalcification with acid followed by deproteination with hot alkali, but not necessarily in that order. These harsh treatments generally lead to a certain amount of degradation of the polymer, loss of molecular weight due to acid hydrolysis and deacetylation in the alkali. Several methods of isolating chitin have been proposed and these have been reviewed by Roberts[24].

2.5 Preparation of Chitosan

Chitosan has been found in the cell wall of certain fungi[25, 26], but this has not so far been used as a source for chitosan. Chitosan used for commercial purposes is generally produced by the deacetylation of chitin from crustacea.

The cleavage of acetamido groups adjacent to *trans*-related hydroxyl groups, as is the case with chitin, requires much harsher conditions than the cleavage of *cis*-related analogues. Therefore the deacetylation of chitin is carried out in concentrated alkali.

A comprehensive review of the techniques used in the deacetylation of chitin has been carried out by Roberts[24].

2.6 Degree of N-acetylation in Chitin and Chitosan

2.6.1 Introduction

As has already been discussed chitin and chitosan exist as a series of copolymers, chitosan being described as the members of this series which are soluble in dilute organic acid. This implies that the distribution and amount of the minor structural components, anhydro-**D**-glucosamine in chitin and anhydro *N*-acetyl-**D**-glucosamine in chitosan, have a considerable effect on the properties of the polymer. Studies have shown that the solubility of chitin in organic solvents however is inversely dependent on the degree of deacetylation[27]. Further studies were carried out on the relationship between solubility and degree of *N*-acetylation by Sannan et al[28], who found that samples deacetylated to about 50% residual *N*-acetyl content under homogenous conditions were water soluble. Samples of greater or lesser degrees of residual *N*-acetyl content were either gel forming or insoluble. It can be seen therefore that it is of vital importance that in any study carried out on chitin and chitosan, that the degree of *N*-acetylation of the materials under study should be determined.

2.6.2 Methods for Determining the Degree of N-Acetylation of Chitin and Chitosan

2.6.2.1 Colorimetric Techniques

2.6.2.1.1 Dye Adsorption

This is a relatively simple technique developed by Roberts[29], and is based on the fact that at equilibrium there is a 1:1 stoichiometry[30] for the interaction of the sulphonic acid groups within anionic dyes with protonated amine groups along the polymer chain. A fuller description of this technique can be found in the experimental section of this work (section 5.6).

2.6.2.1.2 Metachromatic Titration

Metachromatic titration involves polyelectrolyte-induced metachromasy in suitable dyes. The absorbance at λ_{max} of a dye showing polyelectrolyte-induced metachromasy, decreases with increasing added polyelectrolyte concentration, until a minimum is reached. After this point further addition of polyelectrolyte has no further effect. Using a plot of absorbance versus volume of added polyeletrolyte solution, the intersection represents the point at which the system contains an equivalent number of dye ions and charged groups on the polyelectrolyte.

Gummow and Roberts[31] observed chitosan induced metachromasy in C.I. Acid Red 88 and C.I. Acid Orange 7.

2.6.2.1.3 Residual Salicylaldehyde

Domszy and Roberts[32] determined the amine group content of chitosan by utilising the reaction between the free amine groups within the chitosan chain and salicylaldehyde. The reaction forms the yellow Schiffs base, *N*-Salicylidenechitosan. The basis for this technique involves reacting chitosan in excess salicylaldehyde and subsequent spectroscopic analysis of the remaining salicylaldehyde at the end of the reaction period.

2.6.2.2 Titrimetric Techniques

The measurement of the primary amine groups of a sample shows significant advantages over the measurement of residual *N*-acetyl content[33]. It is a direct measure of the functional group of the polymer, it is readily measured by titration and is more sensitive than nitrogen analysis[28, 31, 34, 35].

2.6.2.3 Spectrophometric Techniques

2.6.2.3.1 UV Spectroscopy

The first attempt to use UV spectroscopy to determine the degree of *N*-acetylation of chitin or chitosan was carried out by Castle et. al.[36], but the technique was found to be unsuitable for quantitative analysis.

Muzzarelli and Rochetti[37] reported a method for determining the degree of *N*-acetylation of chitosan using first derivative UV spectroscopy and *N*-acetyl-**D**-glucosamine solutions as calibration.

2.6.2.3.2 IR Spectroscopy

The IR spectrum of α chitin shows two adsorption bands at approximately 1655 and 1625 cm^{-1}. These two bands are characteristic of hydrogen bonded amide groups. Darmen and Rudall[38] noted the disappearance of these bands during de-acetylation. It was however several years before their use in determining the degree of *N*-acetylation was proposed[39]. Several other workers have described infrared techniques utilising either the amide I or the amide II band[40, 41, 42].

2.6.2.4 Gas Chromatographic Techniques

Radhakrishnamurthy[43] determined the quantity of *N*-acetyl groups in mucopolysacharides by chromatographic techniques and Holan[44] proposed a similar method for determining chitin in yeast cell walls. Both methods involve hydrolysis of the polymer followed by chromatographic determination of the amount of released acetic acid.

Muzzarelli[45] used a gas chromatographic method with chitin and chitosan. In this method the degree of *N*-acetylation was determined by the retention time of methanol eluted through a column of polymer, the retention time increasing with increasing degree of *N*-acetylation.

2.6.2.5 NMR Spectroscopy

The determination of the degree of *N*-acetylation of chitin by NMR was first carried out by Hirano and Yamaguchi[46] in their work on *N*-acetylchitosan

gels. This method is most accurate for samples with high levels of *N*-acetylation. It has also been reported that the extent of deacetylation of chitin may be determined by using solid state ^{13}C CP/MAS NMR spectroscopy[47].

2.7 Adsorption of Metal Ions by Chitin and Chitosan

The chelation of metal ions with chitin and chitosan was first described by Muzzarelli[48]. Hauer[49] also studied the ability of chitosan to form chelated metal ion complexes, attributing the adsorption of metal ions to the NH_2 groups. Other work carried out by Yaku and Koshijima[50] involved the preparation of a **D**-glucosamine/Cu (II) complex from a water soluble glucosamine oligomer as a model for the chitosan/Cu (II) complex. This work concluded that one mole of cupric ions was co-ordinated with four moles of **D**-glucosamine. This result is however in conflict with those obtained by Blair and Ho[51] who claimed that in chitosan film two moles of **D**-glucosamine were complexed with one mole of cupric ions, and those of Muzzarelli et al[52] who stated that at a pH of 4.0-5.0 one or two nitrogen atoms per cupric ion were involved, and that at higher pH the co-ordination number increased with OH groups becoming involved also.There are however considerable differences between complex formation of Cu (II) ions with water soluble oligomers on the one hand and with chitosan polymer chains in the solid state on the other. It is therefore not surprising that differences in the number of amine groups complexed with a Cu (II) ion was found.

The exact mechanism for the adsorption of metal ions by chitin and chitosan has yet to be fully determined. Muzzarelli[53] has speculated that chelation, sorption and ion exchange may all be involved, and Yoshinara and

Subramanian[54] suggest that all three processes have varying degrees of importance depending on the metal ion.

Although chitosan has a high affinity for the first row transition metals it remains relatively inert to alkali metal and alkali earth ions[55].

2.8 The dyeing of Chitin and Chitosan

Few studies on the dyeing of chitin and chitosan have been carried out. The adsorption of sulphonated azo dyes has been studied by Giles et al[56, 57], who examined the adsorption of various azo dyes over a range of pH values.The bulk of the work was carried out on chitin isolated from the shell of the Norwegian lobster (*Nephrops Norwegicus).* Other material studied came from the common crab, the edible lobster, shrimps, prawns and the shell lining of the common lobster. The chitin was purified using the Thor method[58].This material was not thoroughly characterised, but was estimated to be about 13% deacetylated. The study showed there to be an increase in affinity as the number of aromatic nuclei within the dye molecule increased, but fell with increase in degree of sulphonation. The increase in the number of aromatic nuclei increases the planar area of the anion, and the increase in sulphonic acid groups increases the affinity of the anion for the aqueous phase resulting in a tendency for the anion to desorb from the substrate. Giles concluded that the adsorption of anionic dyes could be regarded as an ion exchange mechanism involving the acetamide groups, and can be represented by the equation shown below.

$$ChNH_2^+COCH_3\}^1/_2SO_4^= + NaDye \rightleftharpoons ChNH_2^+COCH_3\}Dye^- + {}^1/_2Na_2SO_4$$

Giles and Hassan[59] also carried out studies with Congo Red (C.I. Direct Red 28), and showed that the concentration of adsorbed dye at equilibrium at 50^0C with a $5*10^{-4}$ M dye solution was very pH dependent for chitin but not for cellulose. They also found that the concentration of adsorbed "half dye" was similar, if slightly less than that of C.I. Direct Red 28 on chitin under the same conditions but was significantly less in the case of cellulose. Giles and Hassan concluded that Van Der Waals forces were not as important in the case of chitin as in the case of cellulose, but that hydrogen bonding was more important in the dyeing of chitin than in the dyeing of cellulose.

McKay, Blair and Gardner[60, 61, 62, 63] have also carried out studies on the adsorption of dyes by chitin. Their series of studies was carried out using commercially supplied chitin from Sigma, and the dyestuffs used were also commercially supplied samples. The dyes under study were C.I. Acid Blue25 (anthroquinone acid dye), C.I. Acid Blue 158 (pre-metallised acid dye), C.I. Mordant Yellow 5 and C.I. Direct Red 84. Film mass transfer coefficients and intraparticle diffusion of these dyes were examined over a range of varying parameters such as temperature, initial dye concentration, solution pH, agitation and particle size. A model for mass transfer of acid dye onto chitin in fixed beds was also developed using C.I. Acid Blue 25[64].

Guthrie, Blair and O'Donnell[65] determined the diffusion coefficient of C.I. Acid Orange 10 (monoazo acid dye) on chitosan prepared from the shell of *Nephrops norvegicus)* as well as modified chitosan copper complexes. The material was studied in film form and the diffusion coefficient calculated using a modified Sekaido roll technique. They concluded that the diffusion coefficient of C.I. Acid Orange 10 was non-Fickian, and that it decreased with

increasing dye adsorption. This result is unusual in that in all other dye-substrate systems studied to date the diffusion coefficient is either independent of dye concentration or else it increases with increase in dye concentration. They also found that the presence of copper, which was introduced into the chitosan film prior to dyeing with C.I. Acid Orange 10, greatly reduced the rate of diffusion. This was thought to be caused by the cross-linking effect of copper in chitosan.

2.9 Metal Complex Dyes

2.9.1 Introduction

Metal complex dyes cover several different application classes, however they are most commonly associated with acid dyes used for dying wool and nylon. These dyes can be split into two groups, these being 1:1 metal complex dyes and 1:2 metal complex dyes. The 1:1 metal complex dyes have one dye molecule co-ordinated to the metal ion and the 1:2 metal complex dyes have two dye molecules co-ordinated to the metal ion.

The earliest metal complex dyes were produced directly on the fibre by reacting a metallisable dye with a chromium ion *in situ*. This application method is known as mordanting. The first metal complex dyes synthesised in substance were proposed by Bohn[66] of BASF in 1912. The application of these dyes requires a strongly acidic dyebath using sulphuric acid.[67]. These early synthetic dyes were 1:1 chromium complexes.

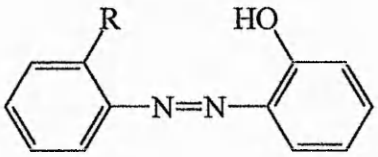
Development continued and in the 1950's 1:2 metal complex dyes were introduced. These dyes utilised non-ionic substituents to confer solubility, and

could be applied from a mildly acidic or neutral dyebath. Further developments led to the introduction of 1:2 metal complex dyes containing sulphonic acid groups thus increasing the hydrophilic properties of the dyes[68]. Metal complex acid dyes generally contain chromium as the metal ion but cobalt has also seen significant use in acid dye synthesis.

The other area where metal complex dyes are of significance is that of direct dyes for cellulose. Within the direct dye range there are a number of Cu complexes. These differ from the acid dye Cr and Co complexes in that the Cu(II) ion co-ordinates with only four ligands as opposed to the six ligands in Co(III) and Cr(III) complexes. This means that only 1:2 complexes are able to be formed. Indeed in symmetrical dis-azo dyes having two tridentate sites capable of complexing with a metal ion, a 2:1 complex may be formed in which two Cu (II) ions are complexed with the dye molecule.These direct dyes are applied to cellulose in the normal manner but are then subsequently aftertreated with suitable compounds to improve fastness properties.

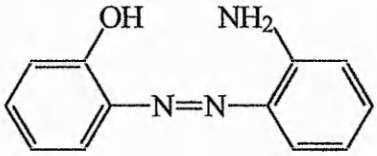
2.9.2 Structural Characteristics of Metal Complex Dyes

Generally metal complex dyes consist of tri-dentate, metallisable monoazo dye ligands, that form an annelated ring system. The most notable of these systems are o,o'-dihydroxyazo, o-carboxy-o'-hydroxyazo, o,o'-hydroxy-aminoazo, o,o'-dihydroxyazomethine and o-hydroxyarylazopyrazolone. Fig. 2.1 shows these structures.

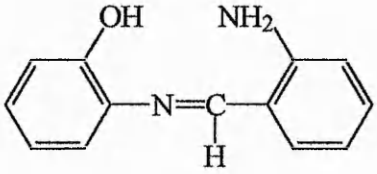


R=OH o,o'-dihydroxyazo

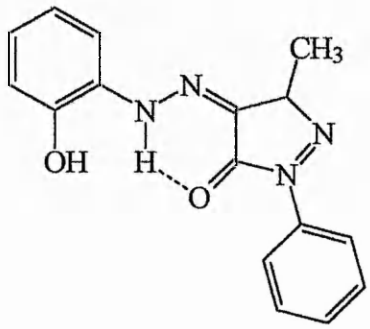
R=COOH o-carboxy-o'-hydroxyazo



o,o'-hydroxyaminoazo



o,o'-dihydroxyaminoazomethine



o-hydroxyarylazopyrazolone

Fig. 2.1 Structures found in metal complex dyes.

In a 1:1 metal complex containing either Cr, or Co the remaining three available co-ordination sites are occupied by three mono-dentate ligands such as water, in the case of a Cu complex there is only one remaining co-ordination site. In the case of a 1:2 metal complex the co-ordination sites are occupied by two tri-dentate dye molecules. Figs. 2.2 and 2.3 show the orientation of the ligands in a 1:1 metal complex and a 2:1 metal complex

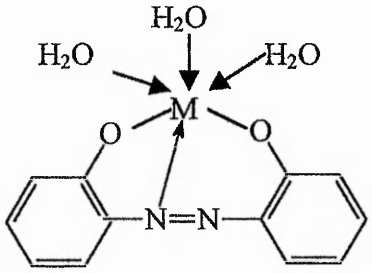


Fig. 2.2 1:1 Pre-Metallised Dye

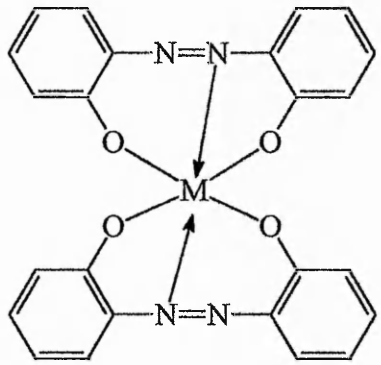


Fig. 2.3 1:2 Pre-metallised Dye

2.9.3 Metal Complex Formation

. The formation of a metal complex in which a nitrogen atom of the azo, azomethine or azopyrazalone group takes part involves the loss of a proton from the two o-substituted groups

The theory behind metal complex formation is known as ligand field theory. This is an amalgamation of two theoretical models proposed previously. The models are Crystal Field Theory and Molecular Orbital Theory. Crystal field theory is based on electrostatic interaction between the dye ion and the ligand whereas molecular orbital theory is based on ionic interaction

If we examine the first row of the transition elements in which Co, Cr, and Cu lie, each element has a partially filled 3d orbital. The 3d orbital can contain up to 10 electrons arranged in five sub-orbitals of 2 electrons each. Vacant orbitals are capable of accommodating donated electrons from either a negatively charged ligand or the lone pair from a neutral ligand. The five sub-orbitals are split into two sets known as t_{2g} and e_g. The e_g orbitals lie along the

x, y, and z axis and the t_{2g} sub-orbitals lie between them. Fig. 2.4 shows the orientation of the 3d sub-orbitals.

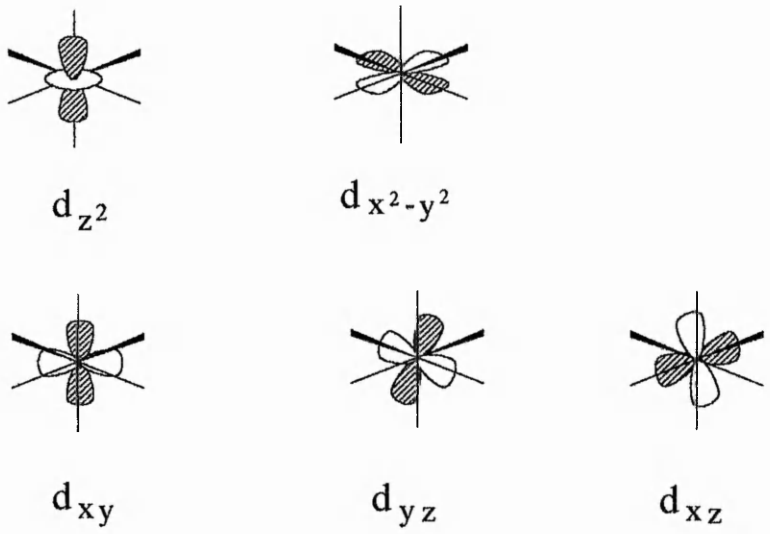


Fig. 2.4 The five sub-orbitals of the 3d shell

As the ligand atoms approach the central metal ion hybridisation between the available ligand electrons and metal electrons takes place with bond formation. It is this and the strength of the ligand field that determines the arrangement of the ligand entities around the central metal ion.

In the case of Cr^{3+}, and Co^{3+} which both have a CN of 6, the ligands approach the metal cation along the x, y, and z axis. As the ligand co-ordinates via a lone pair of electrons or the negative end of a dipole, it represents an area of negative charge directed towards the central metal cation. As the ligand approaches along the x, y, and z axis the electrons in the e_g orbitals are in a higher negative field than those within the t_{2g} orbitals. Therefore the e_g orbitals are of higher energy than the t_{2g} orbitals and the degeneracy of the five 3d

orbitals is split into two groups of different energy. This difference in energy is known as the crystal field stabilisation energy, and is represented by ΔA.

Other arrangements than the octahedral arrangement are also possible. The most familiar of these is the tetrahedral arrangement. The formation of a tetrahedral as opposed to a octahedral arrangement is dependant on the ligand field strength and the number of ligand systems that can be accommodated. In the tetrahedral arrangement the t_{2g} orbitals are in the higher negative field as the ligand systems approach the central metal ion. This means that the e_g orbitals are more stable i.e. the splitting of the orbitals is the reverse of that found in an octahedral arrangement.

The square planar arrangement can be considered as an octahedral arrangement in which two electrons (e.g. both z axis electrons) are completely removed from the central metal ions sphere of influence. If two ligand groups on the z axis are moved away from the central metal atom a distorted octahedral (tetragonal) structure is formed. This movement allows the xy equatorial groups to move in closer. As a consequence of this the orbitals in this plane experience a greater repulsion than in a regular octahedral structure. The d_{x2-y2} and the d_{xy} orbitals increase in energy. At the same time the repulsion effects on the orbitals on the z axis and in the xy and yz planes are reduced. Thus the energy of the d_{z2} orbital is reduced and the energies of the d_{xz} orbital and the d_{yz} orbital are reduced compared with their associated energies in a regular octahedral arrangement. When the two electrons of the z axis are completely removed to give a square planar arrangement, then these effects are greater still.

2.10 The Similarities of Chitin and Chitosan With Cellulose, Nylon And Wool

2.10.1 Introduction

To gain an understanding of how chitin and chitosan may behave under dyeing conditions it is useful to examine other substrates with similar structural characteristics. Both chitin and chitosan are similar to cellulose, all of which contain a backbone of β-(1→4)-**D**-glucose residues; Fig. 2.5 shows the structure of cellulose. The obvious similarity would suggest that both chitin and chitosan could be dyed using dyes suitable for cellulose i.e. direct dyes, reactive dyes etc.

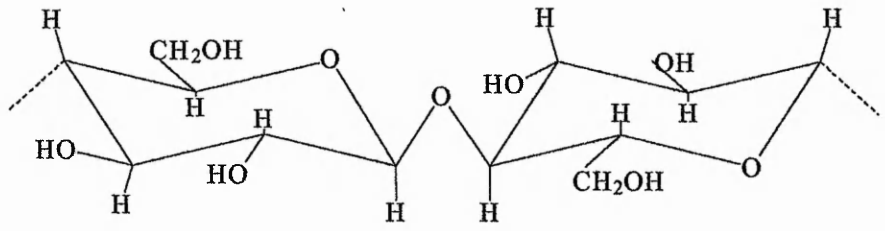


Fig 2.5 Structure of cellulose

Chitin and chitosan also contain amine groups which may act as dye sites for ionic dyes such as acid dyes, suitable for dyeing both wool and nylon. This would appear to mean that as well as dyes suitable for cellulose, dyes suitable for dyeing wool and nylon may be applied to chitin or chitosan. However the number of available NH_2 groups in chitin is significantly greater than in nylon. In 1% de-acetylated chitin there are approximately 50 meq kg^{-1} of NH_2 groups as opposed to approximately 40 meq kg^{-1} in regular nylon, therefore 2% de-acetylated chitin contains as many free NH_2 groups as ultra deep dyeing nylon, and chitosan contains many more still. This would suggest that dye adsorption

by chitin and chitosan would be greater than that of wool or nylon, allowing for a much greater depth of shade to be achieved.

These amino groups may also be able to act as ligands in metal complex formation. The main aspect of this work is to examine the potential of applying metallised dyes to chitin and chitosan, and to examine the mechanism by which adsorption of these dyes takes place.

2.10.2 Dying of Cellulose, Nylon and Wool

2.10.3 Mechanism of Dyeing Cellulose with Direct Dyes

The dyeing of cellulose with direct dyes is probably the most important process in terms of the quantity of material produced, but is possibly the least well understood as far as the mechanism of dye adsorption is concerned. Many theories have been proposed to account for the strong substantivity of direct dyes for cellulose, even though there are few reactive groups within the polymer chain. The early theories[69] proposed a purely mechanical mechanism, whereby the pores within the fibre were swollen by the hot dyebath sufficiently to allow dye molecules to enter and which are then trapped inside the fibre after cooling. Hodgson[70] made the first real advance stating that the dye molecule must be planar for the dye to have any significant affinity for cellulose. This led to several workers proposing that the mechanism is one of hydrogen bonding[71] brought about by the close proximity of the dye and polymer molecules caused by strong Van der Waals forces.

Wegmann[72] proposed attraction between dipoles in the cellulose ether groups and ionic groups on the dye molecule, but no extensive research has yet been carried out to investigate this theory.

Yoshida *et al*[73] suggested the formation of a hydrogen π bond and found that sucrose, glucose and cellobiose form a 1:1 complex with simple aromatic sulphonate anions but not with aliphatic ones. Evidence was in favour of this type of bond formation in the dyeing of cellulose until it was found that Chlorozol Sky Blue FF (C.I. Direct Blue 1) does not form such a complex with the water soluble model of cellulose, β-**D**-glucopyranoside, and so this mechanism was ruled out.

Agnbotri and Giles[74] produced evidence of a possible acid-base bond between cellulose and free amine groups on dyes. Refractometer tests have shown that with Chlorozol Sky Blue FF dissolved in a solution of cellulose in alkaline Cadoxen solvent a 1:1 complex between the dye and cellulose is formed.

Monolayer experiments have led to a further understanding of the dyeing of cellulose[74]. Evidence from these experiments are in favour of an attraction between the cellulose chain and the dye molecule and not just a chance juxtaposition of the two.

All the evidence appears to suggest that the mechanism between cellulose and direct dyes is therefore an amalgamation of several weak forces namely Van der Waals forces, acid-base bonds, possibly ion-dipole interactions and maybe a form of hydrophobic bonding.

2.10.4 Mechanism of Dyeing with Wool Acid Dyes

The chemical structure of wool differs from that of cellulose in terms of dyeing mechanisms in the fact that there are a considerable number of ionic groups, both acidic and basic, distributed along the polymer chain. This led to the natural conclusion that adsorption of dye ions was directly concerned with the presence of these groups, which led in turn to the idea that ions are adsorbed by wool onto specific sites along the polymer chain. The groups involved in this site specific mechanism are the $-NH_2$ and the –COOH groups located on the side chains of the wool chain; there will also be a contribution from other ionisable groups such as hydroxyl but this will only be small. The ionisation of these groups to $-NH_3^+$ and $-COO^-$ is pH dependent and hence the concentration of these groups and the overall charge on the polymer chain is also pH dependent; this will in turn effect the ability of the polymer to adsorb ions including dye ions.

The above mechanism is applicable to both non-metallised and 1:1 pre-metallised acid dyes, but in the case of the 1:2 pre-metallised acid dyes, where dyeing takes place at nearly neutral pH, the number of ionic sites is limited and hence the adsorption of these dye relies on other forces other than ionic interactions. The major forces involved in the adsorption of 1:2 metal complex dyes are Van der Waals forces and at the same time the affinity of these dyes for water is very small.

The presence of $-NH_2$ groups along the chain opens the possibility for metal complex formation as these groups are capable of acting as ligands in such a system, however no investigation into the possibility of producing a

complex between the amine groups in wool and the chelated metal ions in a 1:1 metal complex dye has been carried out so far.

2.10.5 Mechanism of Dyeing Nylon with Acid Dyes

The mechanism of dyeing nylon with anionic dyes is similar to that involved in the dyeing of wool, however the quantity of available basic groups is much smaller in nylon, typically 30-50 mmol kg^{-1} in the case of nylon compared to 800-900 mmol kg^{-1} in the case of wool.

A typical titration curve for an acid dye on nylon 6.6 is shown in Fig. 2.6. It can be seen that the curve can be split into three distinct regions. Region A is typical of anionic titration and its position on the pH axis is dependent on the affinity of the anion. As the pH decreases a plateau is reached, region B, which extends over several pH values. This region is not always well defined but it essentially corresponds to the number of amine end groups present in the polymer; this has been demonstrated with nylons containing different amounts of amine end groups[71], when the quantity of dye adsorbed was in a linear relationship with the number of end groups present[75]. At lower pH values, region C, dye is adsorbed in excess of the normal "saturation value" and adsorption continues to increase as pH decreases, apparently without limit. The change from region B to region C is also dye dependent; with certain dyes this transition can occur at relatively high pH values. This effect of dyeing in excess of the amine end group concentration is known as overdyeing.

Again no investigation into forming a complex with 1:1 pre-metallised acid dyes under neutral conditions has been carried out.

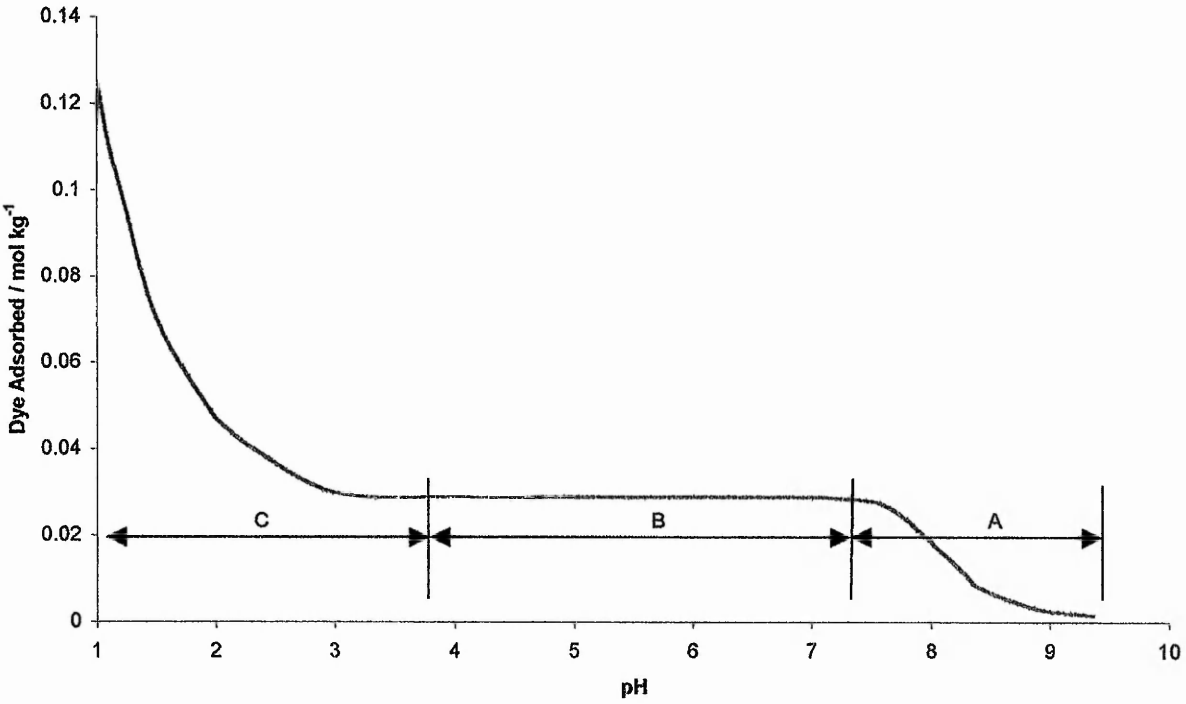


Fig. 2.6 Adsorption of acid dyes by nylon at different pH values.[76]

3 Results and Discussion

3.1 Dyeing Studies with Acid Dyes on Chitin

3.1.1 Introduction

As has previously discussed in Section 2.10.4 and 2.10.5, the mechanism of adsorption of acid dyes onto textile substrates is site specific. Within the group of dyes known as acid dyes there are three distinct chemical compositions, these being non-metallised acid dyes, 1:1 pre-metallised acid dyes and 1:2 pre-metallised acid dyes.

Non-metallised acid dyes can be further split into three application groups. These groups are based on the pH conditions required and the method of application is dependent more on the dyeing behaviour and wet fastness properties of the dye rather than chemical constitution, however dyeing behaviour is determined to a great extent by the relative molecular mass of the dyestuff. The normal way of classifying acid dyes is:

1. Level dyeing or equalising acid dyes
2. Fast acid or half-milling acid dyes
3. Milling acid dyes

Level dyeing acid dyes are normally applied at pH 2.5-3.5 and have an RMM of 300-500, fast acid dyes are normally applied at pH 3.5-5.5 and have an RMM of 500-600 and milling acid dyes are applied at pH 5.0-7.5 and have an RMM of 600-900.

1:1 pre-metallised acid dyes are normally applied to wool and nylon under strongly acid conditions, typically using between 4% and 8% sulphuric acid (H_2SO_4) on weight of fibre (owf)[77, 78]. When dyeing nylon under these conditions protonation of the amine groups within the polymer substrate occurs and an ionic bond formed between the protonated amine group and a sulphonic acid group on the dye molecule. The same mechanism of dye adsorption has been proposed for chitin and chitosan under acid conditions[60]. In the case of wool, although the substrate also contains amine groups, it is the carboxylic acid groups that are protonated. Under neutral pH the amine groups are not protonated therefore any dyeing that takes place must be via a different mechanism. Given that a 1:1 metal complex acid dye has three co-ordination sites occupied by water molecules, and given that chitin and chitosan are both capable of chelating with metal ions to form metal complexes, it is proposed that dyeing may take place by the displacement of one or more of the water molecules from the co-ordination sphere of the dye, and a complex formed between the dye and the substrate. A preliminary investigation has shown that chitin is indeed readily dyed with 1:1 pre-metallised acid dyes under neutral conditions, but a detailed investigation was not carried out[79].

1:2 pre-metallised acid dyes are generally applied to textile substrates from a weakly acid (pH 5-6) or neutral dyebath. As all the co-ordination sites of the central metal ion are full, being occupied by two tri-dentate dye molecules, there are no available sites for metal complex formation between the dye and chitin or chitosan.

Studies were carried out on chitin powder using a range of 1:1 pre-metallised acid dyes and comparisons made with 1:2 pre-metallised and non-metallised acid dyes.

3.1.2.1 Acid Dyes on Chitin at Acid and Neutral pH

Dyeings were carried out on chitin powder using C.I. Acid Violet 90 (1:2 pre-metallised acid dye) and C.I. Acid Orange 7 (non-metallised acid dye) under both acid and neutral pH.

It was observed that both dyes showed greater exhaustion when dyed using acid pH. In the case of C.I. Acid Orange 7 there was negligible dye uptake at neutral pH, and a very unlevel dyeing produced. This would seem to suggest that any colour on the chitin was due to staining by the dye rather than physical dyeing. In the case of C.I. Acid Violet 90 there appeared to be a significant decrease in dye adsorption at neutral pH, but that a small amount of true dyeing had in fact occurred.

Chitin powder was dyed with several 1:1 pre-metallised acid dyes. The dyes used were, C.I. Acid Yellow 99, C.I. Acid Orange 74, C.I. Acid Blue 158 and C.I. Acid Green 12, all of which were applied at both acid and neutral pH.

It was observed that the dyeings carried out under neutral pH showed a higher degree of exhaustion and, in some cases, showed a noticeable difference in hue. This phenomena was seen most clearly with C.I. Acid Green 12, which produced a very flat weak dyeing in acid pH, but gave a very strong, and bright dyeing when dyed in neutral conditions.

These results strongly suggest that the high affinity of the 1:1 pre-metallised acid dyes for chitin at a neutral pH is due to a complex being

formed between the metal ion in the dye molecule and the amine groups within the chitin.

3.1.2.3 Effect of Electrolyte on the Uptake of C.I. Acid Blue 158 by Chitin Under Neutral Conditions

In the general methods for the dyeing of wool and nylon with acid dyes, electrolyte is used to slow the rate of dye uptake to improve the levelling properties of the dyes. This retarding effect is due to competition between the dye anion and the electrolyte anion for the available dye sites within the polymer. It has been shown however that at higher pH values this effect is reversed and a similar effect of "salting on" as in the case of direct dyes on cellulose is achieved.

The effect of increasing amounts of electrolyte was studied using C.I. Acid Blue 158 on chitin powder at neutral pH. The electrolyte most commonly associated with acid dyeing is sodium sulphate and so this was used in the study.

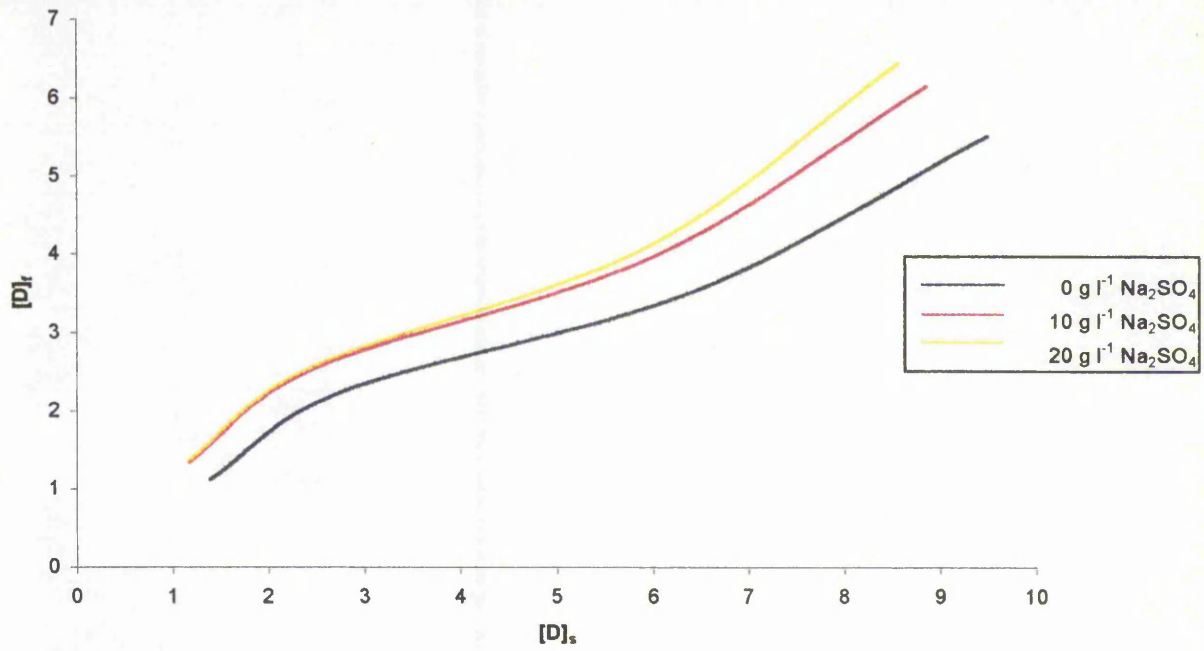


Fig. 3.1 The effect of electrolyte on the adsorption of C.I. Acid Blue 158 by chitin at neutral pH

From the plot it can be seen that at neutral pH the effect of adding electrolyte to the dyebath does show the "salting on" effect, suggesting that electrolyte reduces electrical repulsive forces on the substrate surface allowing the dye to approach closer to the surface, thereby aiding adsorption of the dye by the substrate. The effect of increasing the level of electrolyte from 10 g l^{-1} to 20 g l^{-1} does not seem to have as dramatic effect as increasing the level of electrolyte from 0 g l^{-1} to 10 g l^{-1}, which is reasonable as there is a minimum level to which the surface charge can be reduced.

3.1.3 Studies on the Build Up of Acid Dyes on Chitin

3.1.3.1 Introduction

Observations on the way in which dyes build up on a particular substrate are useful in that they highlight the rate at which dye is adsorbed onto the substrate surface. Study of build up curves can also show if saturation of the substrate occurs and at what level of dye uptake saturation is reached. Build up experiments are also a way of studying the effect on the rate of dye uptake due to altering dyebath conditions such as time, temperature and electrolyte concentration.

Build up curves can generally be broken down into two distinct portions. The first portion is concerned with the uptake of dye onto the substrate surface and usually produces a plot showing a rapid increase of dye on the substrate. The second portion of the plot represents the rate at which dye is adsorbed into the substrate from the surface. Unlike diffusion experiments (see section 3.2) they do not give any indication of the mechanism of diffusion, only the rate at which dye that has diffused into the substrate from the surface is replaced at the surface with dye from the dyebath.

3.1.3.2 Build Up at Neutral pH

Build up experiments were carried out on powdered chitin using a range of acid dyes. The dyes studied were C.I. Acid Orange 74 and C.I. Acid Blue 158 (1:1 pre-metallised), C.I. Acid Violet 90 and C.I. Acid Red 308 (1:2 pre-metallised) and C.I. Acid Orange 7 and C.I. Acid Blue 1 (non-metallised).

The results obtained show that for 1:1 pre-metallised acid dyes the initial strike rate was very rapid with 50% of dye adsorbed within the first five minutes, the rate then slowing until a plateau is reached. The point at which the rate begins to slow would suggest that the surface of the substrate is saturated with dye. The plateau portion of the plot suggests that equilibrium has been reached. The increase in adsorption from when the surface becomes saturated to the final equilibrium value is quite small.

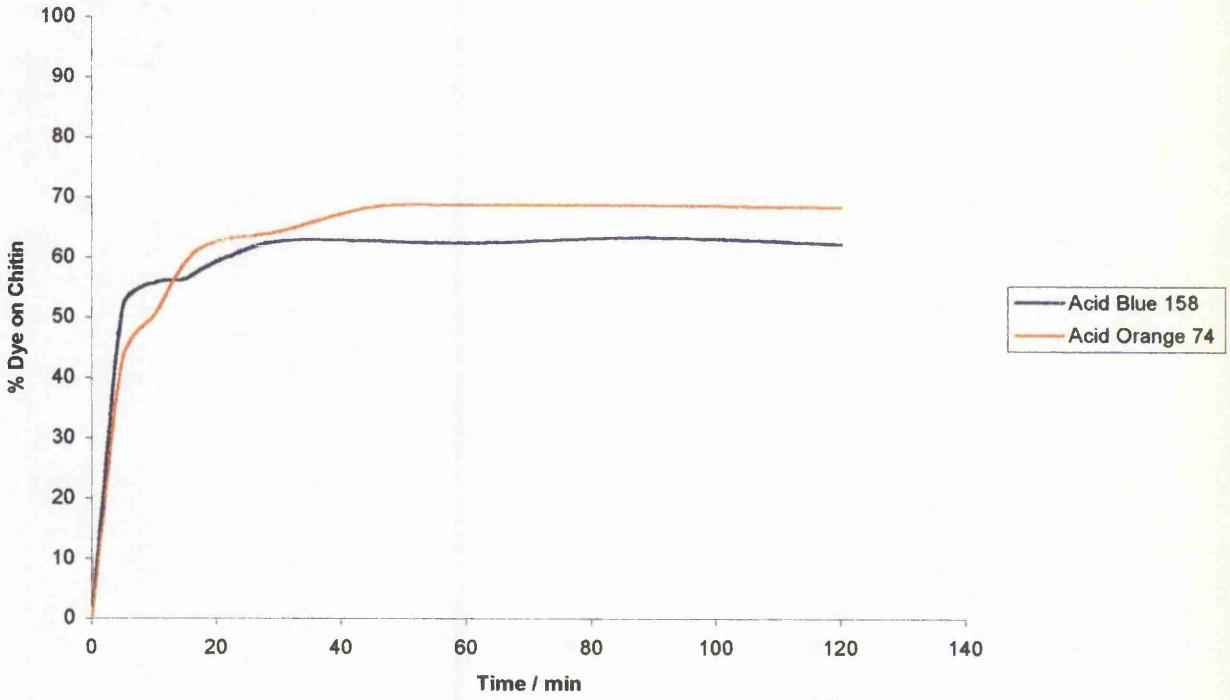


Fig. 3.2 Plot of the build up of 1:1 pre-metallised acid dyes on chitin at neutral pH

In the case of the 1:2 pre-metallised dyes there appears to be quite a large difference in the final level of adsorption. Again both dyes show a very rapid initial strike rate and both reach equilibrium very rapidly, in the case of C.I. Acid Violet 90 equilibrium is reached after only five minutes. The final level of adsorption of C.I. Acid Violet 90 appears to correlate with the initial dyeing studies (section 3.1.2.1) in that a certain amount of dyeing has taken place but

not to the level of the 1:1 pre-metallised dyes. The level of adsorption of C.I. Acid Red 308 does however appear to be more consistent with that of a 1:1 metal complex dye. Unfortunately the structure of C.I. Acid Red 308 has not been disclosed, and it is therefore not possible to offer any explanation as to why its adsorption is considerably higher than that of C.I. Acid Violet 90.

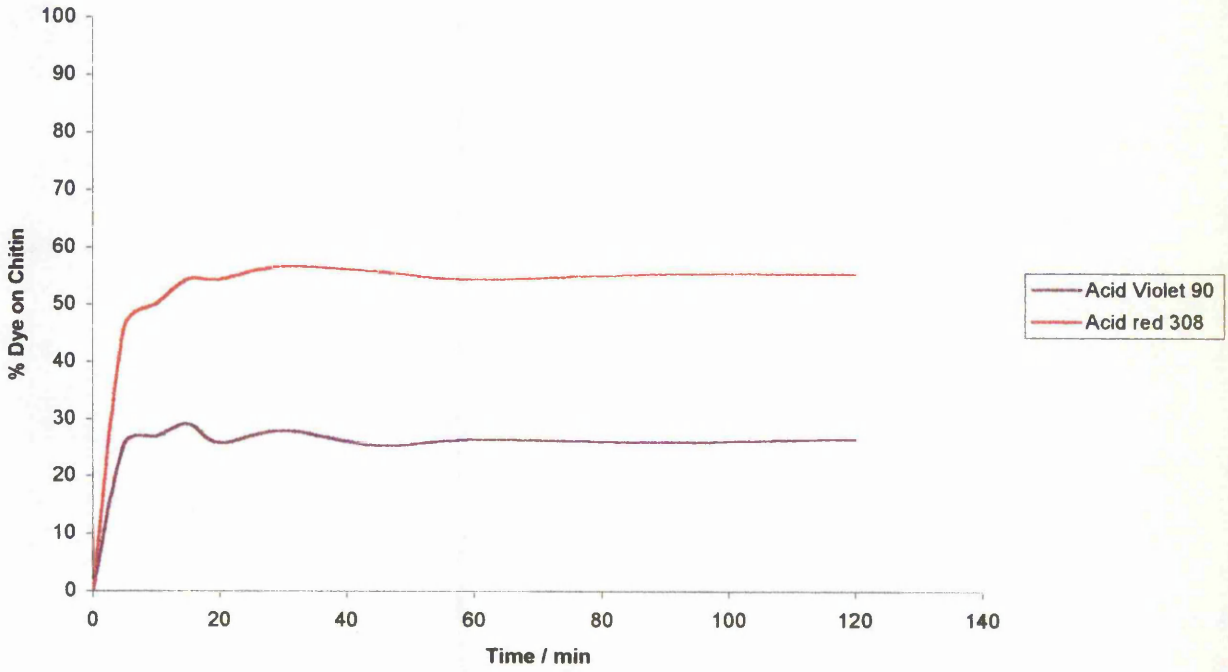


Fig. 3.3 Plot of the build up of 1:2 pre-metallised acid dyes on chitin at neutral pH

In the case of the non-metallised dyes it is quite clear that no real dyeing has occurred, particularly in the case of C.I. Acid Blue 1. This would seem to suggest that there is no affinity for the substrate with this type of dye under these conditions, possibly because there are no dye sites available for ionic interaction and that any Van der Waals forces between dye and substrate are very weak.

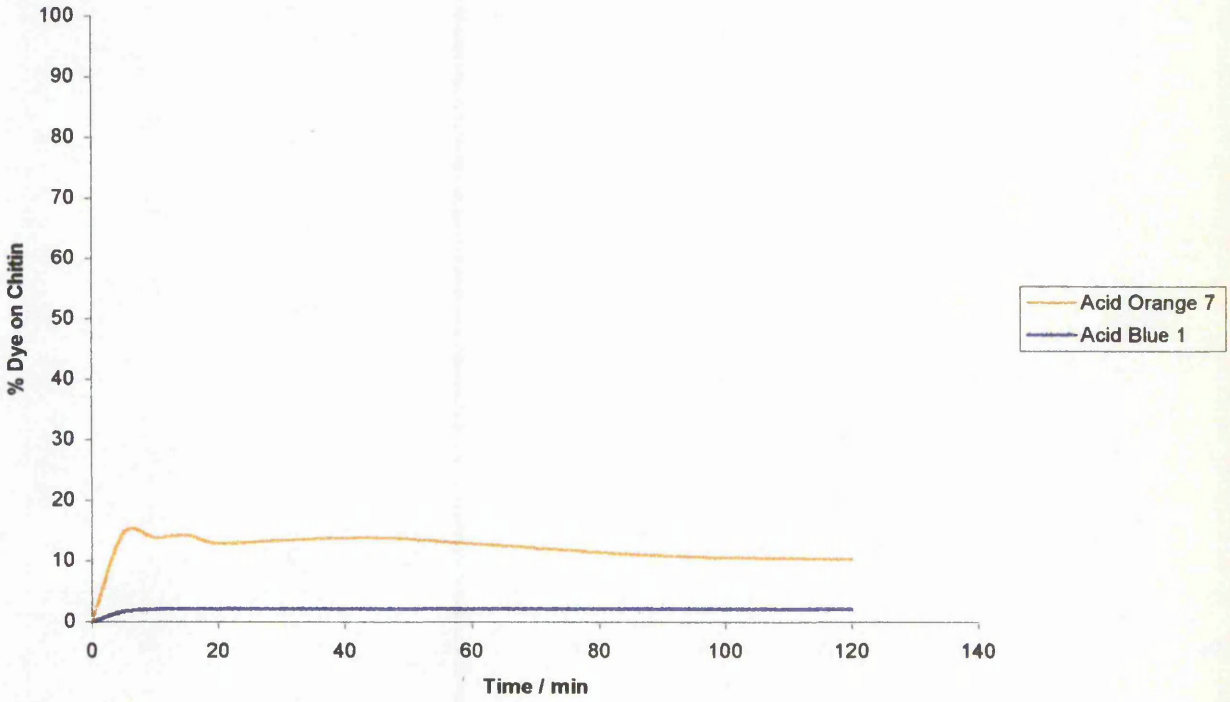


Fig. 3.4 Plot of the build up of non-metallised acid dyes on chitin at neutral pH

3.1.3.3 Effect of Temperature on the Build Up of C.I. Acid Blue 158 at Neutral pH on Chitin

The effect of temperature on the rate at which dyes are adsorbed by a substrate can be of great importance. In most dye-fibre systems the rate of dye adsorption increases as the temperature increases, and the final level of exhaustion decreases because dye adsorption is an exothermic process.

Build up experiments were carried out on chitin powder using C.I. Acid Blue 158 at a series of temperatures to assess what effect this may have on the rate of dye uptake and also the final level of dye adsorption.

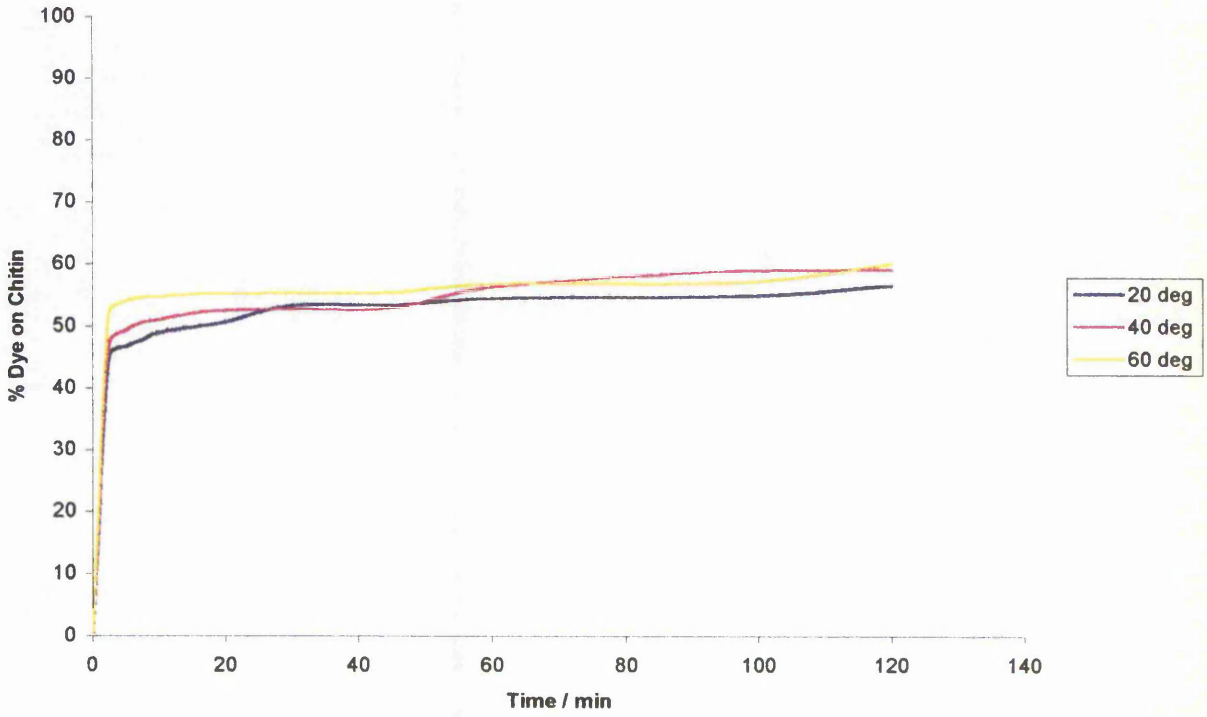


Fig. 3.5 Effect of temperature on the build up of C.I. Acid Blue 158 on chitin

The results show that the initial strike rate is not really affected by increasing the temperature of dyeing. It can be seen however that there is an increase in the adsorption of dye from 20^0C to 60^0C. Once the surface of the substrate becomes saturated however the increase in total dye absorbed is not really effected by temperature as all three dyeings give a final dye uptake of around 55%. This is unusual in that for acid dyes at acid pH there is a definite decrease in the dye adsorption at equilibrium with increase in temperature. This effect may be due to temperature not being as significant in metal complex formation as in normal acid dye adsorption.

3.1.4 Equilibrium Studies With Acid Dyes on Chitin

3.1.4.1 Introduction

The study of the distribution between dye adsorbed onto a substrate and dye remaining in the dyebath when the system is in a state of equilibrium can give important information as to the dyeing mechanism of the system under study. The state of equilibrium is reached when the rate at which dye is being adsorbed onto the polymer substrate from the dyebath is equal to the rate at which dye is desorbing off the polymer substrate into the dyebath.

3.1.4.2 Equilibrium Uptake of C.I. Acid Blue 158 under Neutral Conditions on Chitin Powder

Dyeings were carried out on chitin powder using C.I. Acid Blue 158 at several different concentrations. The dyeings were allowed to proceed until a state of equilibrium had been reached. The partition between the amount of dye on the chitin compared to the amount of dye remaining in the dyebath was calculated for each concentration and the equilibrium adsorption isotherm plotted (Figure 3.6). The information from this plot was then used to calculate and plot both the Freundlich and Langmuir equilibrium adsorption isotherms. The Freundlich type of isotherm can be expressed in the terms:

$$[D]_f = k[D]_s^{\ x}$$

where $[D]_f$ and $[D]_s$ are the concentrations of the dye in fibre (substrate) and the concentration of dye remaining in solution respectively, k is a constant and x is a fractional power. The characteristic of this relationship is such that if the logarithms of both sides are taken then a linear relationship is obtained.

$$\log [D]_f = \log k + x \log [D]_s$$

Therefore when a plot of the logarithm of the concentration of dye in fibre against the logarithm of the concentration of dye in solution is made a straight line should be obtained with a slope equal to x.

The Langmuir adsorption isotherm is of much more fundamental origin being derived by Langmuir on kinetic grounds for the adsorption of gases onto surfaces. The basis for this is that the dye is adsorbed onto a specific site in the fibre, and once that site is occupied no further adsorption can take place there. If the fibre contains $[D]_f$ moles of dye per kilogram in equilibrium with a dyebath concentration of $[D]_s$ moles per litre, and if we suppose that all the available sites are occupied by dye, the concentration within the fibre would be $[S]_f$. At equilibrium dye is constantly leaving and being adsorbed onto the fibre surface, and the rate of desorption $-d[D]_f/dt$ is proportional to the concentration of the dye adsorbed on the fibre surface, therefore:

$$-d[D]_f /dt = k_1 [D]_f$$

At the same time dye is also arriving at the fibre surface from the solution and some of this will be adsorbed. The rate at which the dye is adsorbed, $d[D]_f/dt$,

is dependent on the concentration of dye in solution as well as the number of unoccupied sites. This is proportional to $[S]_f$-$[D]_f$. Thus :

$$d[D]_f/dt=k_2[D]_s([S]_f-[D]_f)$$

At equilibrium the rates of adsorption and desorption are equal and so we can state:

$$[D]_f= k[D]_s([S]_f-[D]_f)$$

Where $k=k_2/k_1$

$$[D]_f = k[S]_f . [D]_s / 1+k[D]_s$$

If we invert the above we get:

$$1/[D]_f = 1/k[S]_f . [D]_s + 1/[S]_f$$

This shows that a plot of the reciprocal of the concentration of dye in the fibre against the reciprocal of the concentration of the dye in solution will be linear (Fig. 3.7).

As can be seen from the plots, C.I. Acid blue 158 appears to conform to the Langmuir type, indicating that adsorption is via a site specific mechanism. As there is no protonation of the amine groups within the chitin chain at neutral pH the adsorption mechanism is unlikely to be ionic, therefore it is suggested that the dye is being adsorbed by chelation with the central metal ion of the dye complex. These results support the conclusions reached on the basis of the dye uptake values at acid and neutral pH (Section 3.1.1.2)

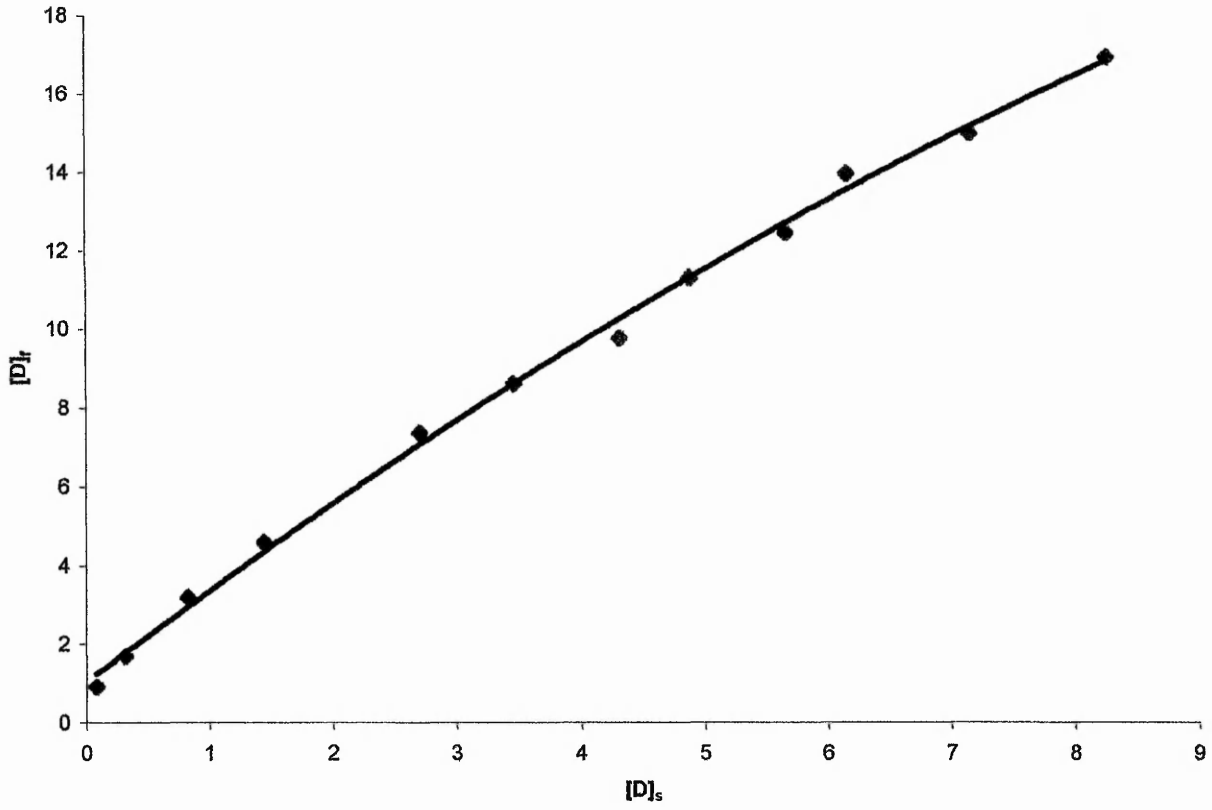


Fig. 3.6 Equilibrium Adsorption isotherm for C.I. Acid Blue 158 on chitin at 80^0C.

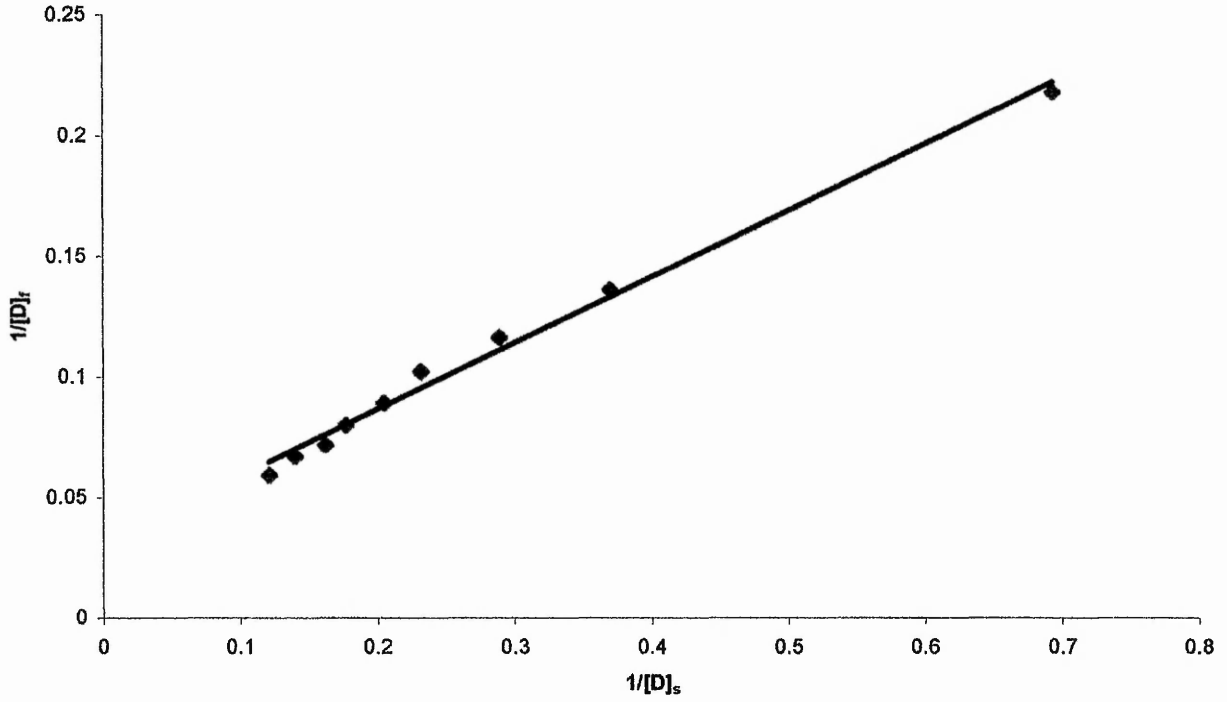


Fig. 3.7 Langmuir equilibrium adsorption isotherm for C.I. Acid Blue 158 at 80^0C

3.1.5 Colour Fastness

3.1.5.1 Introduction

Colour fastness is of great importance to textile processors as well as to end-users. Dyestuff manufacturers have been constantly developing dyes and dyeing processes to improve the colourfastness properties of dye/fibre systems. Colour fastness is also important to textile retailers and as a consequence the larger retail outlets have spent a lot of time developing tests to assess the level of colour fastness. Most colour fastness tests are based around the use of multifibre strip. This multifibre strip is a piece of woven fabric that incorporates six different textile substrates. These are, in the case of the multifibre obtained from the Society of Dyers and Colourists, secondary cellulose acetate (Dicel), bleached unmercerised cotton, nylon 6.6, polyester (Terylene), acrylic (Courtelle) and wool worsted. Pieces of this strip are generally "washed" with dyed samples and the level of transfer from sample to strip assessed with grey scales.

An important aspect of colour fastness is that colour fastness decreases as the depth of shade increases, i.e. the deeper the depth of shade produced the lower the colour fastness result will be.

3.1.5.2 Colourfastness of Acid Dyes on Chitin

Samples of chitin dyed with a range of acid dyes under both neutral and acid conditions were assessed for colour fastness. The dyes used were C.I. Acid Orange 7 (non-metallised acid dye), C.I. Acid Violet 90 (1:2 pre-

metallised acid dye), C.I. Acid Orange 74 and C.I. Acid Blue 158 (1:1 pre-metallised acid dyes). The dyes were applied at three depths, these being 0.5%, 1.0% and 2.0% owf. Acid pH was achieved by using 4.0% (owf) sulphuric acid.

The results of the colour fastness tests showed that in the case of C.I. Acid Orange 7 only the dyeing carried out with 4.0% sulphuric acid was dyed, the neutral-dyed sample being only slightly stained, and therefore no sensible comparison of fastness properties was possible. In the case of C.I. Acid Violet 90 the sample dyed with 4.0% (owf) sulphuric acid was fuller than the neutrally dyed sample and the colourfastness test reflected this, the fuller sample giving a worse result. In the case of C.I. Acid Orange 74 and C.I. Acid Blue 158 there was no noticeable difference in the results of the colour fastness tests. Both samples gave good results, the staining becoming more as the concentration of dyestuff on the substrate increased. As has already been noticed the dyeings done under neutral pH actually show a higher exhaustion than those carried out under acid conditions, and since wash fastness decreases with increase in depth of shade it could be argued that the dyeings carried out at neutral pH actually showed a higher degree of wash fastness. The wash-fastness results for C.I. Acid Violet 90, C.I. Acid Orange 74 and C.I. Acid Blue 158 are give in Tables 3.1, 3.2 and 3.3.

It should be noted that even though there appears to be only a small difference in colour fastness, a half grade improvement in colour fastness rating is very significant.

C.I. Acid Violet 90	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
0.5% Dye 4.0% H_2SO_4	5	4	5	4/5	5	4
0.5% Dye	5	4/5	5	4/5	5	4/5
1.0% Dye 4.0% H_2SO_4	5	3/4	4	4	5	3/4
1.0% Dye	5	4	4/5	4/5	5	4
2.0% Dye 4.0% H_2SO_4	5	3	4	4	5	3
2.0% Dye	5	3/4	4/5	4/5	5	3/4

Table 3.1. Fastness results for C.I. Acid Violet 90 on chitin powder

C.I. Acid Orange 74	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
0.5% Dye 4.0% H_2SO_4	5	4/5	5	4/5	5	4/5
0.5% Dye	5	4/5	5	4/5	5	4/5
1.0% Dye 4.0% H_2SO_4	5	4	5	4/5	5	4
1.0% Dye	5	4	5	4/5	5	4
2.0% Dye 4.0% H_2SO_4	5	3/4	5	4/5	5	3/4
2.0% Dye	5	3/4	5	4/5	5	3/4

Table 3.2. Fastness results for C.I. Acid Orange 74 on chitin powder

C.I. Acid Blue 158	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
0.5% Dye 4.0% H_2SO_4	5	4/5	5	4/5	5	4/5
0.5% Dye	5	4/5	5	4/5	5	4/5
1.0% Dye 4.0% H_2SO_4	5	4	5	4/5	5	4
1.0% Dye	5	4	5	4/5	5	4
2.0% Dye 4.0% H_2SO_4	5	3/4	5	4/5	5	3/4
2.0% Dye	5	3/4	5	4/5	5	3/4

Table 3.3. Fastness results for C.I. Acid Blue 158 on chitin powder

3.2 Dyeing Studies using Direct Dyes on Chitin

3.2.1 Introduction

As has been discussed in Section 2.13 chitin, chitosan and cellulose are all based around a β-(1.→4)-**D**-pyranose backbone, which gives them a flat linear polymer chain structure This indicates that dyes for cellulose may be applicable for dyeing chitin and chitosan. Direct dyes for cellulose are of particular interest because they can be divided into two distinct groups, those that contain copper ions and those that do not.

The polymer chains of cellulose are large planar ribbon like molecules and the long flat direct dye molecules are adsorbed onto the cellulose chains and held by the substrate using hydrogen bonding and Van der Waals forces as the

dye molecule lies flat along the polymer chain. There is no site specific adsorption involved with direct dyes and cellulose.

Copper-containing direct dyes were developed to enable their fastness characteristics to be enhanced by after-treating the dyed cellulose with a suitable fixing agent. In the case of the Indosol range of coppered directs marketed by Clariant, Indosol E-50 is used as the after treatment. The patent literature reveals that Indosol E-50 is a polybasic condensate of diethylenetriamine and an amide, such as cyanamide, dicyandiamide, guanidine or biguanide.

Coppered direct dyes can contain more than one copper ion per dye molecule. The copper ions within these dyes are thought to have one free co-ordination site available, which forms a complex between the dye and the fixing agent. This co-ordination site may also be available to form a complex between the dye and the amine group of chitin or chitosan.

3.2.2 Dying Studies Using Indosol SF Direct Dyes

3.2.2.1 Initial Studies

Dyeings were carried out on chitin using a range of coppered direct dyes from the Indosol SF range supplied as industrial samples from Clariant. As explained in Appendix 1 the precise chemical structures of these dyes has not been disclosed. The dyes selected from the range were:

Indosol Yellow SF-2RL

Indosol Rubinole SF-R

Indosol Blue SF-GL

Indosol Green SF-GLN

Indosol Brown SF-BR

Indosol Violet SF-B

Comparisons were made between these dyes with several uncoppered direct dyes. The uncoppered direct dyes used were:

C.I. Direct blue 29

C.I. Direct Red 79

C.I. Direct Green 59

All the dyeings showed excellent exhaustion, there being no noticeable difference in final exhaustion between the coppered and uncoppered dyes.

To try and assess if there was any difference in the fixation of the coppered and un-coppered dyes on the chitin, extractions were carried out on the dyed samples using water in a Soxhlet apparatus. Extraction was carried out for several hours and the results are shown in Table 3.4.

Dye	Comments
Indosol Yellow SF-2RL	No observable change
Indosol Rubinole SF-R	No observable change
Indosol Blue SF-GL	No observable change
Indosol Green SF-GLN	No observable change
Indosol Brown SF-BR	No observable change
Indosol Violet SF-B	No observable change
C.I. Direct Blue 29	Severe loss of colour
C.I. Direct Red 79	Significant loss of colour. Not as severe as C.I. Direct Blue 29
C.I. Direct Green 59	Significant loss of colour. Not as severe as C.I. Direct Blue 29

Table 3.4 Results of Soxhlet extractions for metallised and non-metallised direct dyes on chitin

From these results it can be seen that even though the uncoppered dyes exhaust to a similar level as those that are coppered, the coppered dyes appear to be fixed much more firmly to the substate. This suggests that the coppered dyes may be being adsorbed onto the substrate via a different mechanism. This also points towards the idea that the coppered direct dyes are forming a chelated metal complex with the chitin.

It is possible that there are two mechanisms of adsorption operating in the case of the Indosol dyes simultaneously, the first involving the complexing of the dye with chitin through the amine groups acting as ligands for the central copper ion. The second mechanism is the same as the normal adsorption of

direct dyes as in the case of cellulose, however it would appear that the amount of dye being adsorbed by the second mechanism is very small as there is no noticeable loss of dye during extraction. It must be noted that at low depths of shade it is very difficult to remove direct dyes from cellulose and it would be reasonable to assume that this is also the case for chitin and chitosan.

3.2.2.2 Effect of Electrolyte on the Adsorption of Direct Dyes on Chitin

The level of electrolyte is of vital importance in the dyeing of cellulose with direct dyes. It is considered to have a much greater influence on equilibrium in practice than variations in the dye content of the dyebath. As an example it has been observed that Chlorazol Sky Blue FF (C.I. Direct Blue 1) did not dye cuprammonium rayon in the absence of electrolyte[80]. Taking the view that dye adsorption is due to hydrogen bonding and Van der Waals forces between the dye and substrate and is opposed by the electrical surface charge of the substrate, it is unlikely that the attractive forces are removed by merely removing the electrolyte from the system. This being said the surface charge on the substrate is substantially increased by the removal of the electrolyte and under these conditions the electrical repulsion may outweigh the forces of attraction at all distances from the substrate surface.

In practical terms the addition of electrolyte to the dyeing system increases the adsorption of direct dye onto cellulose and so in the case of chitin a similar increase in adsorption may be expected.

Dyeings were carried out on powdered chitin with varying levels of electrolyte at several different depths of shade using a coppered and an uncoppered direct dye. The dyes used were:

C.I. Direct Blue 15 (uncoppered)

C.I. Direct Blue 218 (coppered)

The basic structure of these two dyes is the same except that C.I. Direct Blue 218 has two Cu(II) ions co-ordinated to it.

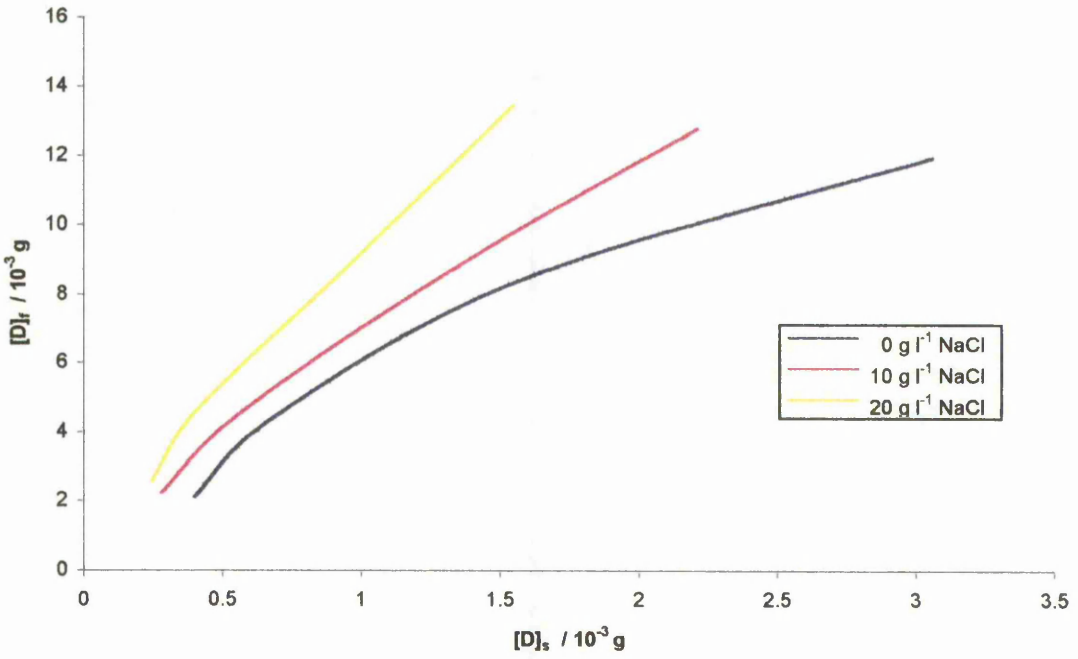


Fig. 3.8 Effect of electrolyte on the adsorption of C.I. Direct blue 15 on chitin at 80^0C

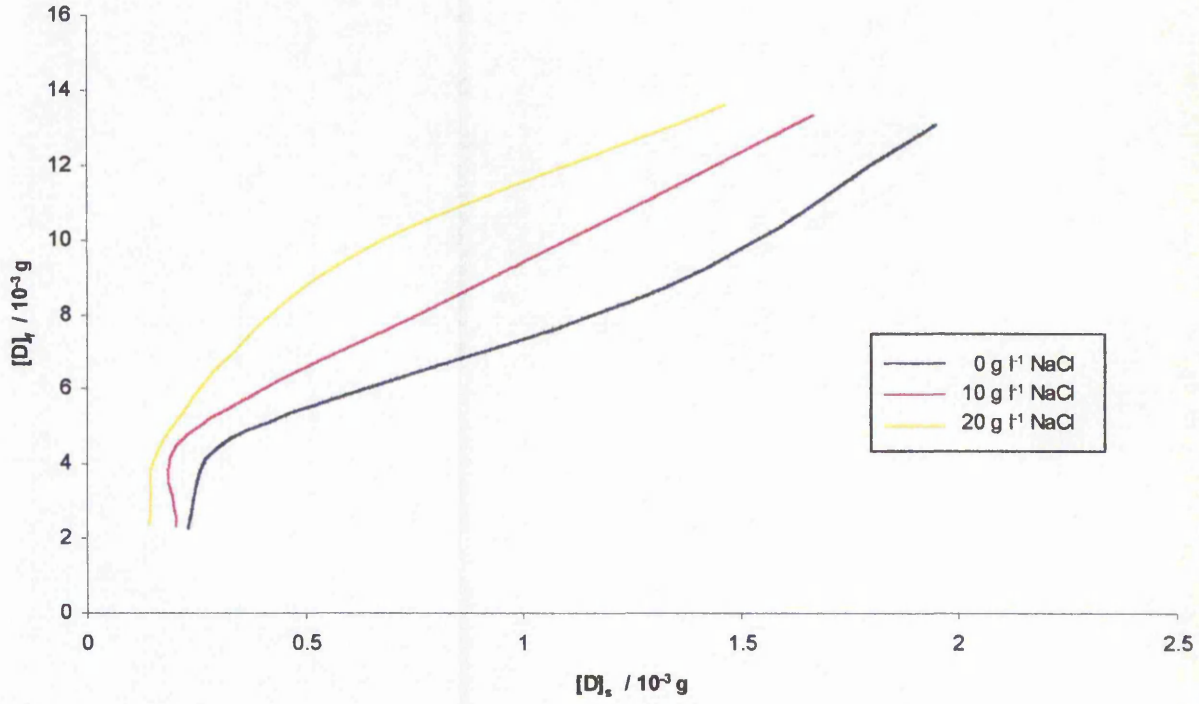


Fig 3.9 Effect of electrolyte on the adsorption of C.I. Direct Blue 218 by chitin at 80^0C

It can be seen from the plot that C.I. Direct Blue 15 behaves as expected, showing an increase of adsorption of the dye as the electrolyte concentration increases. This is also the case for C.I. Direct Blue 218

3..2.3 Comparison of Dye Uptake Between Cellulose and Chitosan

Samples of woven cotton were coated with chitosan. This was achieved by padding a solution of chitosan on to the cotton fabric, the fabric was then dried and neutralised. Comparisons of the treated fabric and untreated fabric were made using:

Indosol Blue SF-GL

C.I. Direct Blue 29

C.I. Direct Red 79

C.I. Direct Green 59

A sample of chitosan treated cotton fabric and a sample of the untreated cotton were dyed together in the same bath to assess the affinity of the dyes for the different substrates. After the dyeing had been completed the K/S values were recorded at λ_{max} for each dye. K/S values give a measure of the colour strength of dyes on textile substrates. The results are given below.

Dyestuff	K/S Value at λmax	
	Woven Cotton	Chitosan Treated Woven Cotton
Indosol Blue SF-GL	1.051	6.185
C.I. Direct Blue 29	2.536	3.63
C.I. Direct Red 79	2.322	4.79
C.I. Direct Green 59	1.986	3.59

Table 3.5 Comparison of K/S values for coppered and uncoppered direct dyes on cotton and chitosan treated cotton

The results show that all of the dyes have a higher affinity for the chitosan treated sample, but that the increase is much greater for the Indosol dye than for any of the uncoppered direct dyes.

Extractions were carried out on samples of the dyed substrates using water. The K/S values of the extracted samples were recorded and the decrease in colour strength calculated in terms of percentage.

Dyestuff	% Drop in K/S at λmax			
	Woven Cotton		Chitosan Treated Woven Cotton	
	20 min	240 min	20 min	240 min
Indosol Blue SF-GL	43%	65.8%	18.3%	19.0%
C.I. Direct Blue 29	57.5%	73.4%	57.9%	71.4%
C.I. Direct Red 79	56.9%	77.3%	62.6%	81.2%
C.I. Direct Green 59	27.6%	49.6%	23.2%	45.0%

Table 3.6 % Reduction in K/S values of coppered and uncoppered direct dyes after extraction with water.

It can be seen that the copper-containing Indosol dye shows much greater affinity for the chitosan treated samples than for the untreated cotton. The uncoppered dyes, although they also show a higher affinity for the treated sample, do not appear to be bound very firmly to either substrate, indeed the extraction results show a close correlation in percentage loss in colour strength for the uncoppered dyes from both the chitosan-treated and the untreated samples. It can be seen that there appears to be a more significant loss of colour from the chitosan treated cotton than from chitin powder dyed with

Indosol dyes; this may be due to chitosan having significantly more amine groups than chitin allowing it to adsorb significantly more dye and as already explained colour fastness is dependent on the depth of shade.

3.2.4 Studies of Build Up of Direct Dyes on Chitin

Build up experiments were carried out on chitin powder using two coppered and two uncoppered direct dyes. The uncoppered dyes were:

C.I. Direct Yellow 12

C.I. Direct Blue 15

The coppered dyes used were:

Indosol Yellow SF-2RL

C.I. Direct Blue 218

C.I. Direct Blue 15 and C.I. Direct Blue 218 were again chosen because they are the uncoppered and coppered version of the same dye.

It can be seen from the graphs that all four of the dyes show very similar build up properties. All the dyes show a rapid strike rate with over 50% of the dye adsorbed within the first five minutes of dyeing. The rate then appears to rapidly decrease and then remain steadily increasing. No saturation point appears to have been reached.

In the direct comparison between C.I. Direct Blue 15 and C.I. Direct Blue 218 it can be seen that the presence of the copper ions does not appear to effect the rate of adsorbance or to have any effect on the total amount of dye adsorbed. It has however been shown previously that the presence of the

copper ions appears to have quite a major effect on the fixation of the dyes to the chitin substrate.

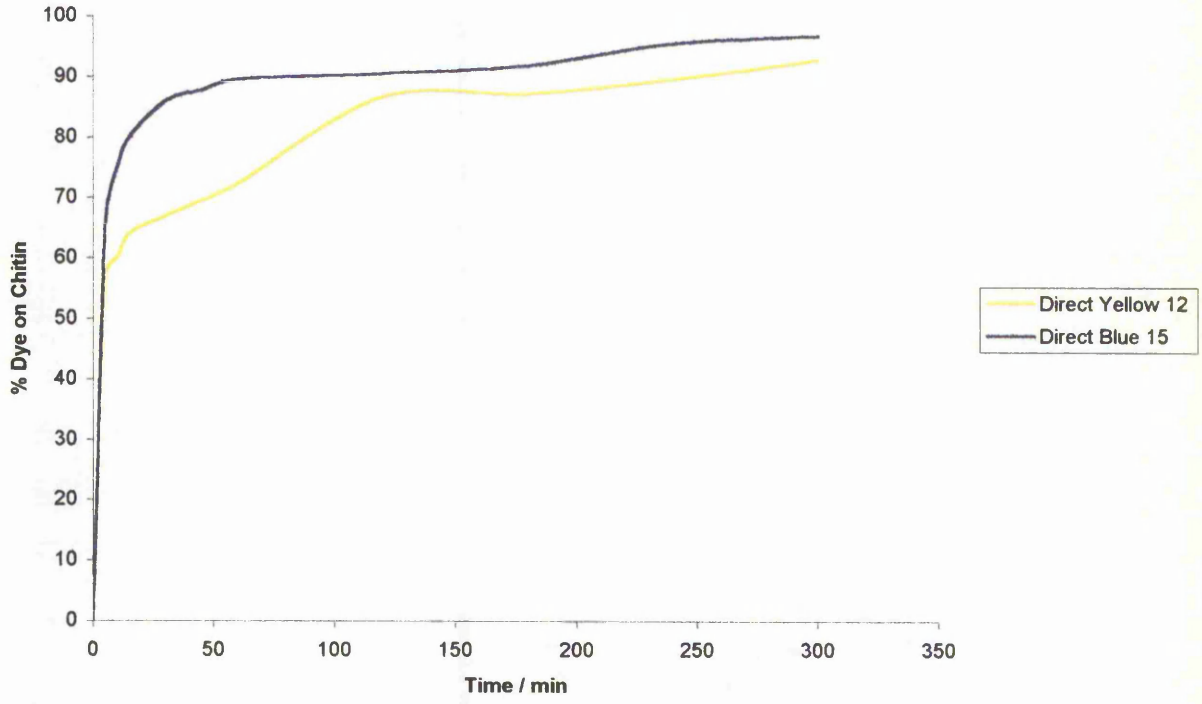


Fig 3.10 Plot of the build up of uncoppered direct dyes on chitin at 80^0C.

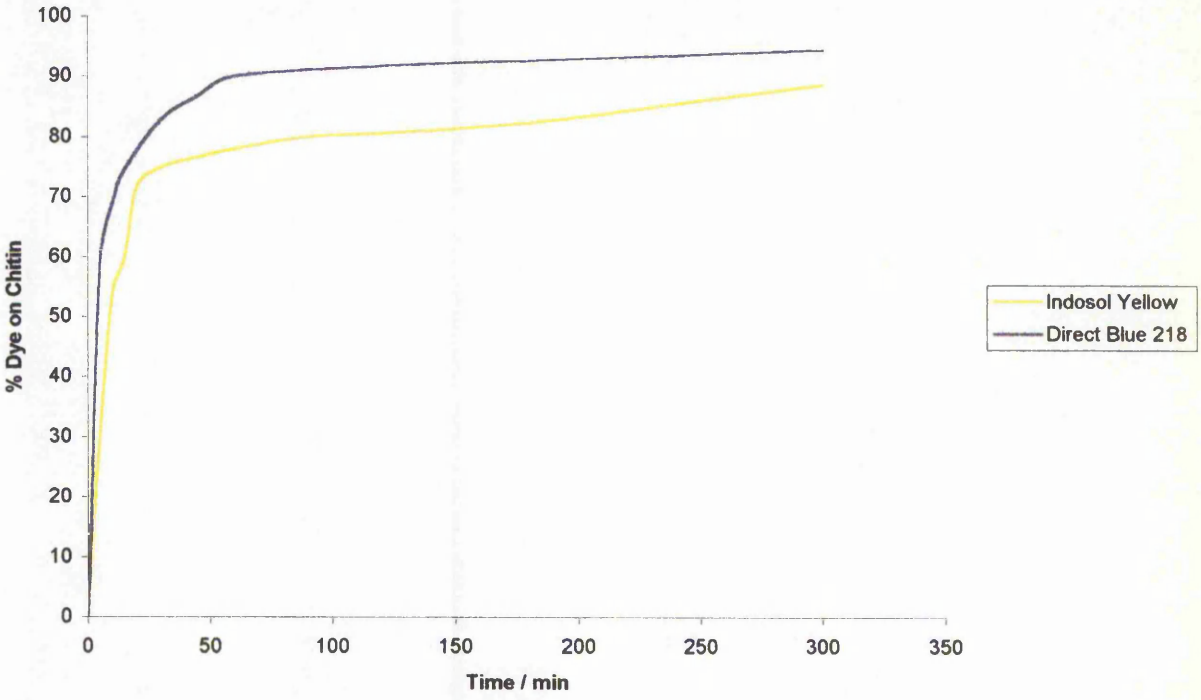


Fig 3.11 Plot of the build up of coppered direct dyes on chitin at 80^0C.

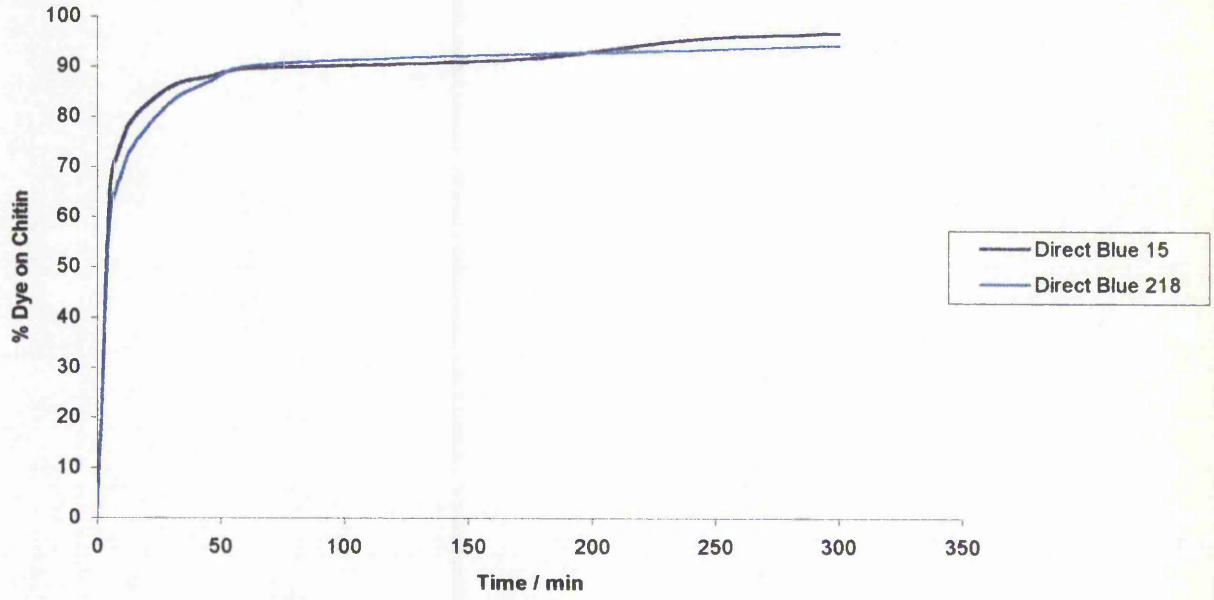


Fig. 3.12 Comparison of the build up of C.I. Direct Blue 15 and Direct Blue 218 on chitin at 80^0C.

3.2.5 Equilibrium Studies With Direct Dyes on Chitin

3.2.5.1 Introduction

The dyeing of cellulose with direct dyes has been shown to be completely reversible. Detailed studies have been carried out on this subject using C.I. Direct Yellow 12, and C.I. Direct Blue 1, on various cellulosic substrates such as cuprammonium rayon, cellophane film and viscose rayon as well as cotton. All of these studies found that the dyes used could be quite easily desorbed from the cellulose using water or a salt solution at 70°C.[81]

It has already been shown in the preceding sections that although the uncoppered direct dyes appear to behave in the same manner on both cellulose and chitin, both substrates showing this property of a reversible dyeing process, the dyes that contain copper ions do not show this property, indeed the dye appears to be very strongly bound to the substrate.

3.2.5.2 Comparison of the Equilibrium properties of Coppered and Uncoppered Direct Dyes

The equilibrium properties of an uncoppered and a coppered direct dye were studied. The dyes used were:

C.I. Direct Yellow 12

Indosol Yellow SF-2RL

Both Freundlich and Langmuir Equilibrium Adsorption Isotherms were derived for both dyes and the resulting plots are given below

It can be seen from the resulting plots that the uncoppered C.I. Direct Yellow 12 behaves in the same manner on chitin as it does with cellulose. But it can also be seen that the coppered Indosol Yellow SF-2RL shows quite a different behaviour, exhibiting the properties of a site specific dye by conforming to the Langmuir type equilibrium adsorption isotherm as opposed to the non site specific Freundlich equilibrium adsorption isotherm normally associated with direct dyes

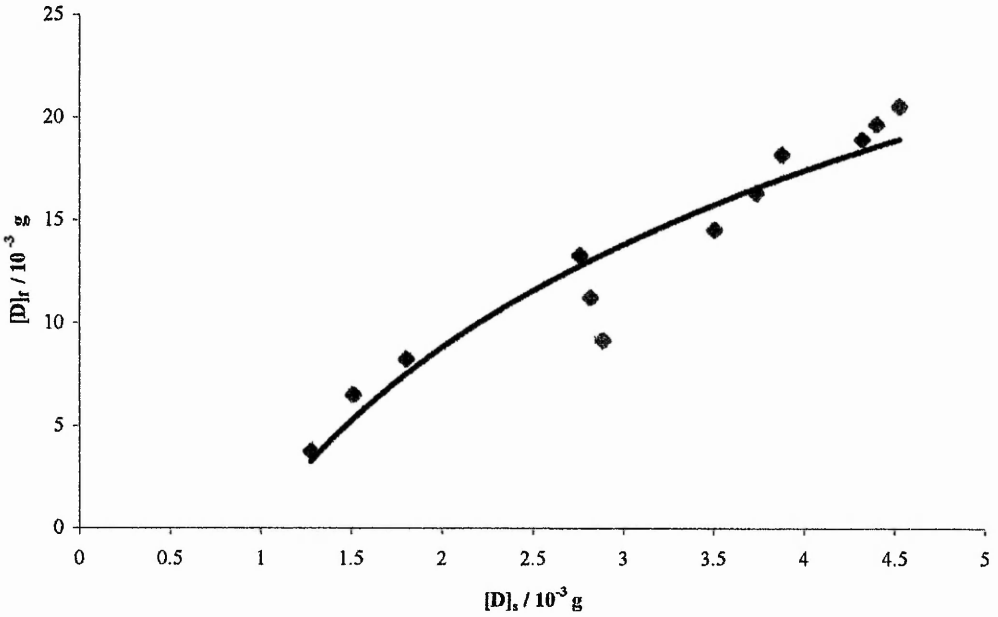


Fig. 3.13 Adsorption isotherm for C.I. Direct Yellow 12 on chitin at 80^0C

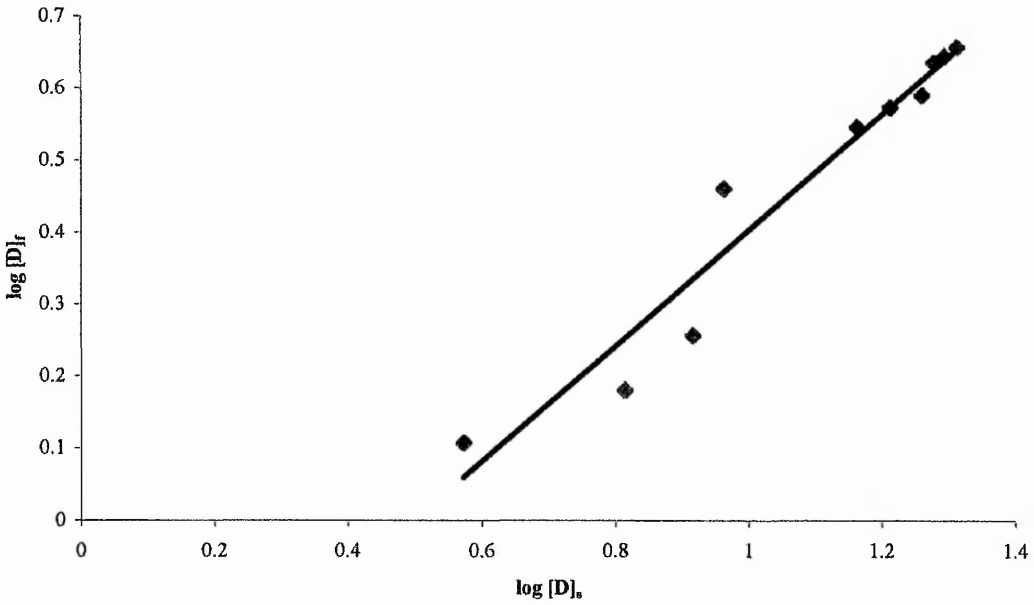


Fig. 3.14 Freundlich equilibrium adsorption isotherm for C.I. Direct Yellow 12 on chitin at 80^0C.

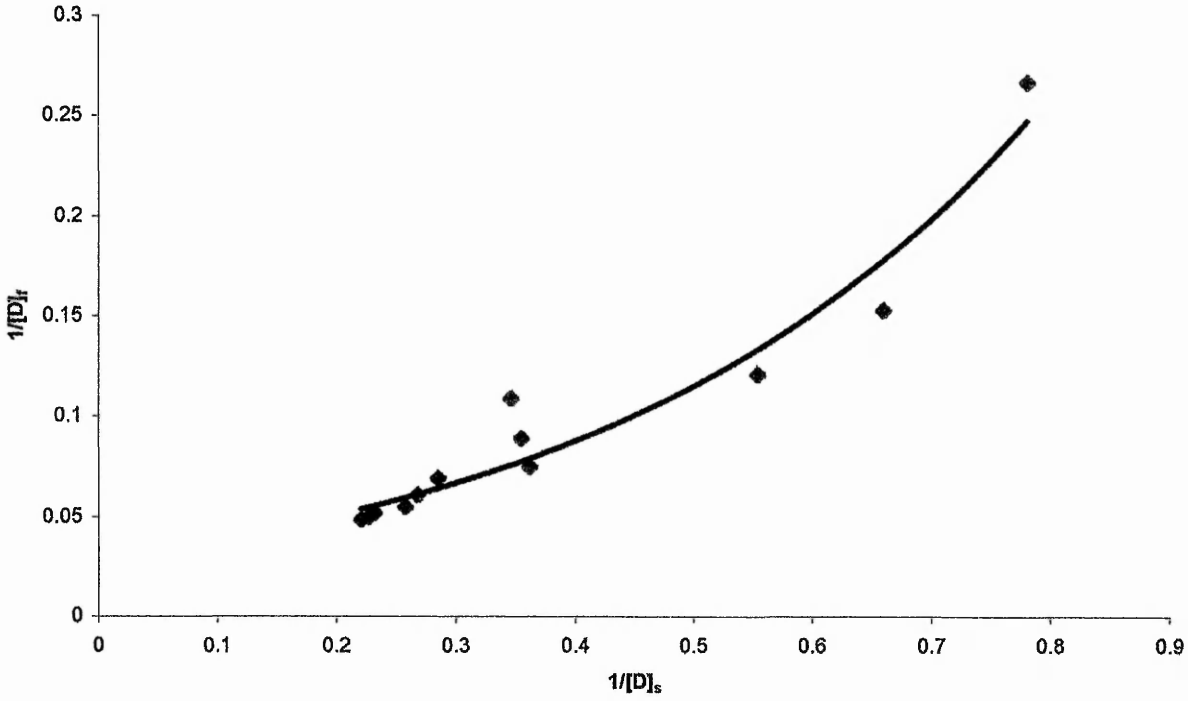


Fig. 3.15 Langmuir equilibrium adsorption isotherm for C.I. Direct Yellow 12 at 80^0C.

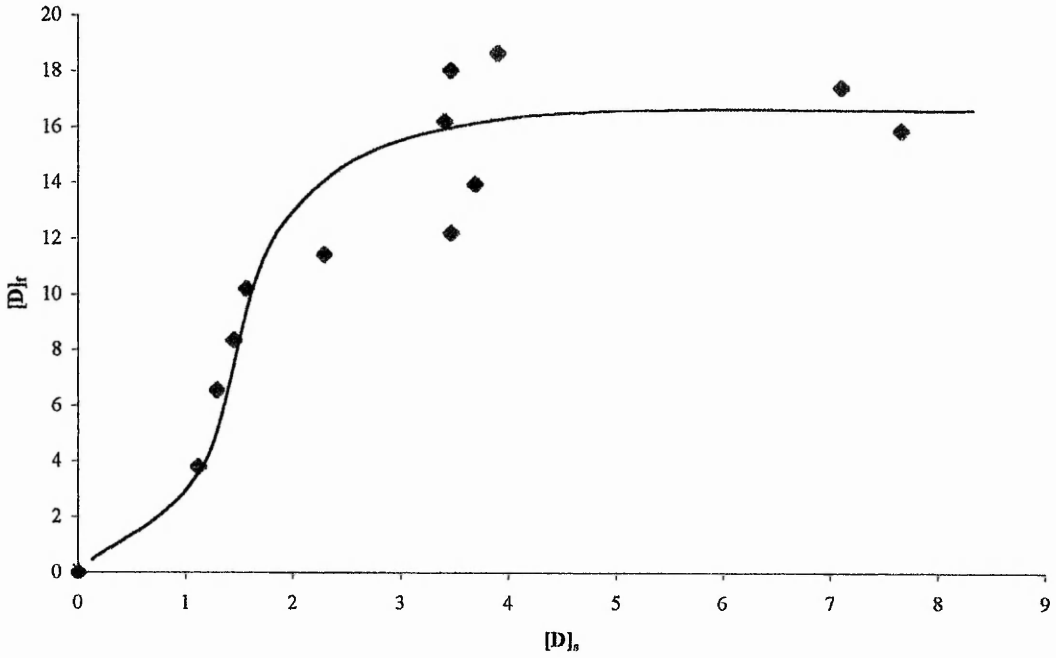


Fig. 3.16 Adsorption isotherm for Indosol Yellow SF-2RL on chitin at 80^0C

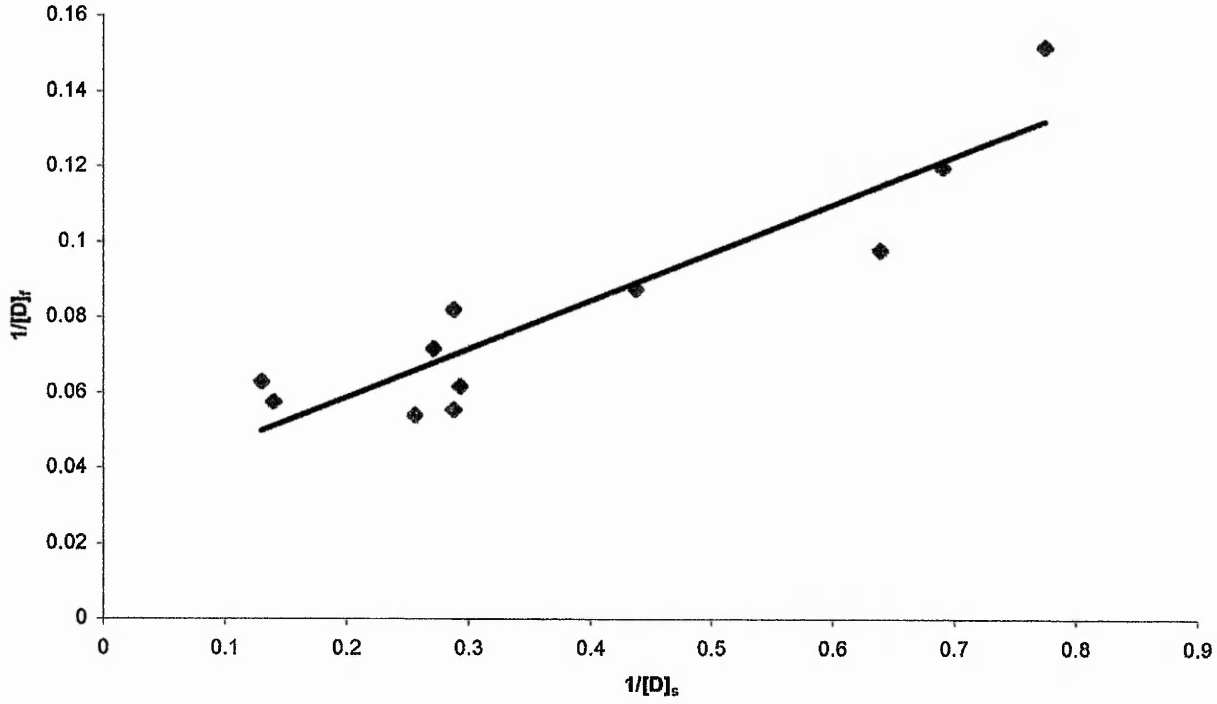


Fig. 3.17 Langmuir equilibrium adsorption isotherm for Indosol Yellow SF-2RL on chitin at 80^0C.

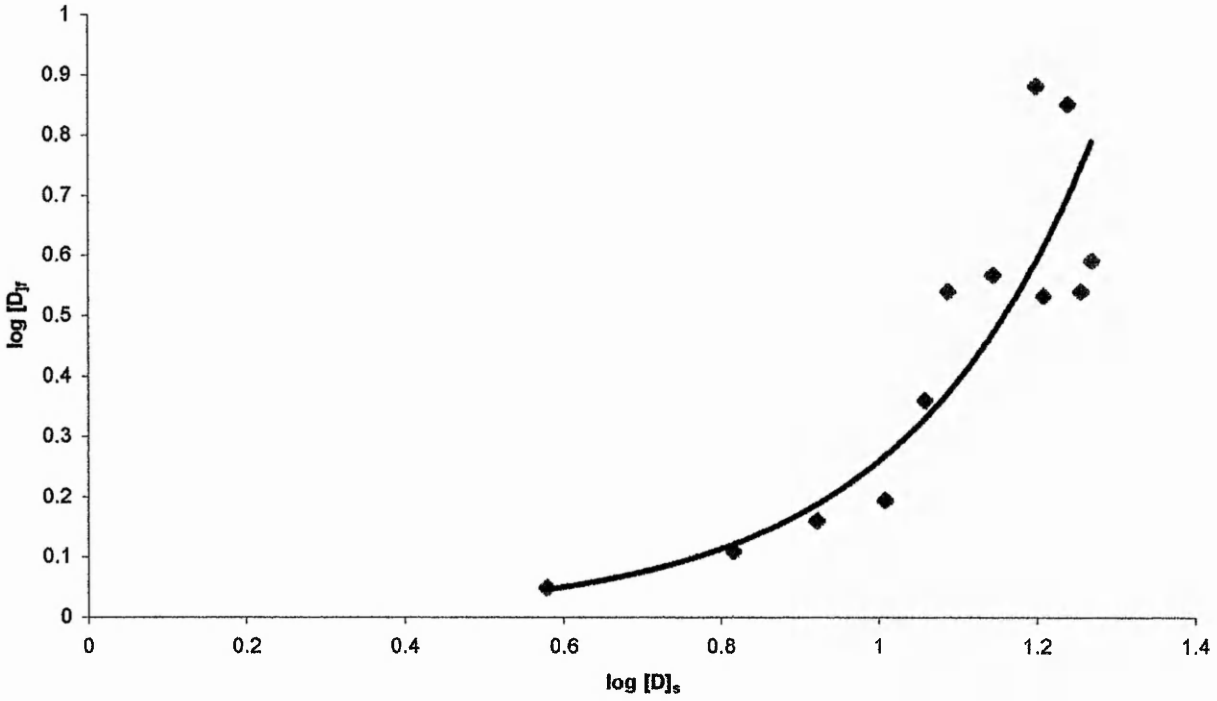


Fig. 3.18 Freundlich equilibrium adsorption isotherm for Indosol Yellow SF-2RL on chitin at 80^0C.

3.3 Diffusion Studies on Chitosan Film

3.3.1 Introduction

The mechanism of dyeing can be split into three phases:

1. Diffusion of the dye in the aqueous dyebath to the substrate surface
2. Adsorption of the dye onto the micellar surfaces at the outer surface of the substrate
3. Diffusion of the dye from the surface inside the substrate towards its centre.

It is generally assumed that the final step in the process is the slowest by a significant degree. The adsorption step is very rapid as all it involves is the impact of a dye molecule on the surface of the substrate. Diffusion of the dye molecules within the substrate is much slower than in the external solution due to the higher mechanical restraints to movement as well as any interaction between the dye and the substrate. From this it can be seen that dye molecules will arrive at the surface of the substrate much more rapidly than they can diffuse into the substrate itself, so that the dye will accumulate at the surface and an equilibrium will be established between the surface and the external solution. The equilibrium concentration at the surface will be determined by the concentration within the solution in accordance with the partition coefficient between the substrate and the aqueous phase. Once this state of equilibrium is achieved any further adsorption of the dye can only occur as dye diffuses from the surface to the centre of the substrate. If the dye

concentration in the external solution is kept constant the overall rate of dyeing must be controlled by the rate at which dye diffuses from the surface to the centre. This however is not the case if the concentration of the dye in the solution is limited and the degree of adsorption high as almost all of the dye can be accommodated at the substrate surface. Under these circumstances dyeing will appear to be very rapid as the rate is controlled by the first two stages, however this may lead to the substrate being "ring dyed"; this is when the surface of the substrate is dyed but the interior of the substrate is not.

A great deal of attention has been paid to the diffusion of dyes through textile substrates, particularly cellulose, where the use of cellophane has allowed detailed study of the process. Neale and Stringfellow[82] produced the first quantitative proof that a true state of equilibrium can ultimately be obtained, by looking at the adsorption of Chlorazol Sky Blue FF by cellophane.

In the measurement of the rate of diffusion within a substrate it is generally assumed that the rate of diffusion ds/dt of a dye across a unit area of substrate at a given point is proportional to the concentration gradient dc/dx of the dye at that point. This is given by Fick's equation[83].

$$ds/dt = -D dc/dx$$

The diffusion coefficient D is defined as being numerically equal to the amount of dye diffusing in unit time across unit area of the substrate under a unit concentration gradient. The value of D offers a convenient means of

comparing the behaviour of different dyes on the same substrate or the same dye on different substrates.

In this research diffusion experiments were carried out using the Sekaido roll technique (Section 5.). This technique involves rolling a film of polymer around a cylinder to simulate a solid piece of polymer substrate. The ends are sealed to prevent dye penetrating between the layers of polymer film. This means that the only way of dye reaching the inner layers of the film is by diffusing from the outer surface, through the first layer and into the next. This technique has been used for the calculation of the diffusion coefficients of dyes on cellulose and polyester, and the objective was to try to calculate the diffusion coefficients of some metallised and non-metallised dyes through chitosan film.

3.3.2 Diffusion of Direct Dyes Through Chitosan Film

3.3.2.1 Introduction

Several films were dyed with direct dyes in order to assess the best parameters for a more detailed study (Section 3.3.2.2). It was found that at 80^0C a time of 120 min gave diffusion through about eight layers of the film. Fig 3.20, 3.21 and 3.22 show examples of the dyed chitosan film. These are similar to the plots obtained for direct dyes on cellophane[84]. A calibration plot was also produced to allow the wet thickness of the film to be calculated (section 5.1.2.3). The diffusion characteristics of C.I. Direct Blue 15 on chitosan are similar to those of a direct dye on cellulose, as is the general relationship between the diffusion coefficient and the dye concentration.

Further diffusion experiments were carried out with C.I. Direct Yellow 12 to assess the effect of increasing electrolyte on the diffusion characteristics. Fig 3.21 shows the films produced with dyebaths containing 5 g l^{-1} NaCl and 15 g l^{-1} NaCl. Fig 3.19 shows the plot of optical density against distance for these films compared to that produced with 0g/l NaCl. It can be seen from the plot that increasing the electrolyte decreases the rate at which the dye diffuses through the chitosan film. This is similar behaviour to that shown by direct dyes on cellophane films[85].

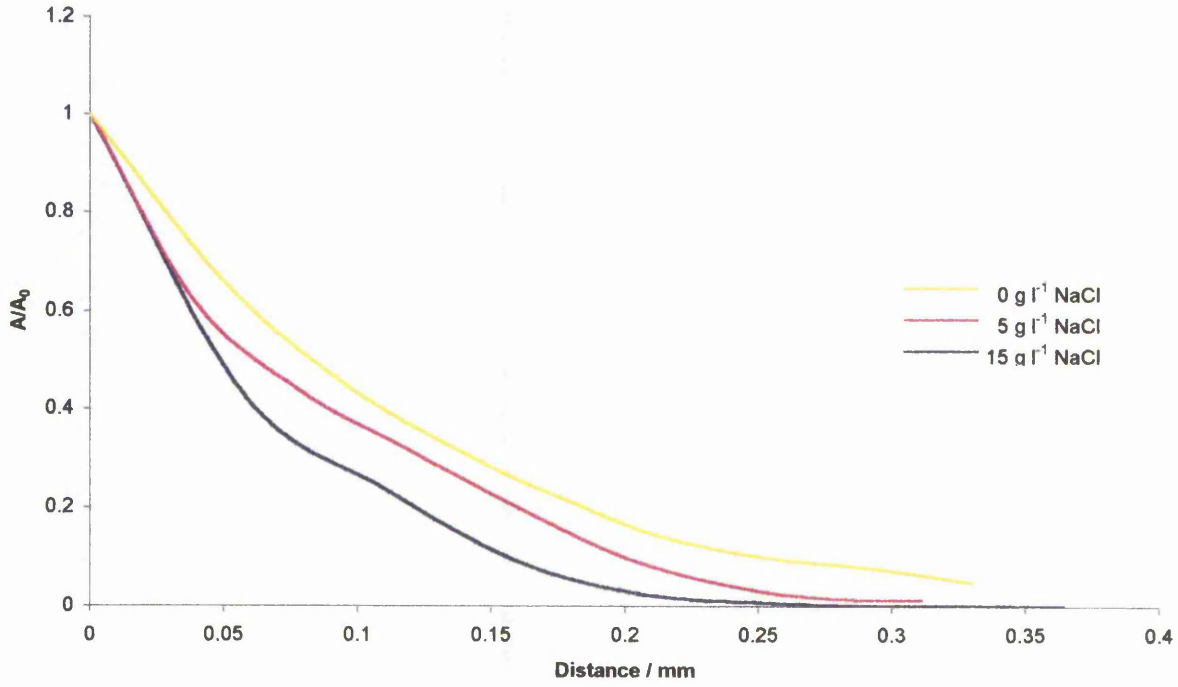


Fig 3.19 Effect of electrolyte on the diffusion of C.I. Direct Yellow 12 on chitosan film, 120 min at 80^0C

Fig. 3.20 Chitosan films dyed with (a.) C.I. Direct Yellow 12 and (b) C.I. Direct Red 24. 120 min at 80^0C

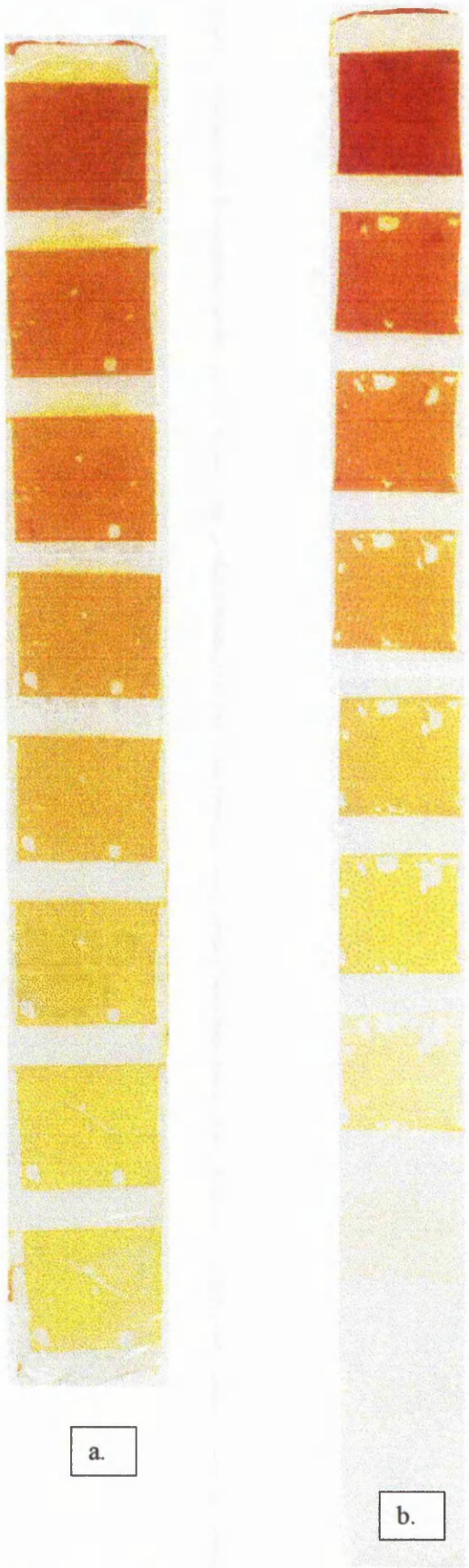


Fig 3.21 Effect of electrolyte on the diffusion of C.I. Direct Yellow 12. (a.) 5 g l^{-1} NaCl, (b.) 15 g l^{-1} NaCl, 120 min at 80°C

3.3.2.2 Comparison of the Diffusion of C.I. Direct Blue 15 and C.I. Direct Blue 218 through Chitosan Film

A comparison was made between the diffusion properties of C.I. Direct Blue 15 (uncoppered) and C.I. Direct Blue 218 (coppered) to assess if there was any difference in the mechanism by which these two dyes diffused through chitosan film. Both films were treated in the same way, but the dyed films show a dramatic difference. The uncoppered C.I. Direct Blue 15 had diffused into the roll as far as the eighth layer of the roll whereas, in the same time the coppered C.I. Direct Blue 218 had only dyed the outer layer. Fig. 3.22. shows the films produced; the pale blue staining on the film produced using C.I. Direct Blue 218 is due to a small amount of uncoppered dye being present in the commercial dye sample.

In order to get any meaningful results for calculating the diffusion coefficient it is necessary to have diffusion across several layers of the roll. Repeat experiments were made with C.I. Direct Blue 218 carried out over increasing time but it was found that even after about five days (104 hours) at 80^0C the dye had not diffused further than the outermost layer of the roll. A roll was processed at elevated temperature (130°C) to see if this increased the rate of diffusion and after eight hours at this temperature a film was obtained with three very dark layers and one very pale layer, the rest of the layers being completely undyed. Unfortunately the severe temperature conditions used caused the film to discolour and become very brittle making quantitative analysis difficult, but it is proposed that the plot of dye concentration against time would be similar to that obtained by ionic dyes on nylon under acid conditions, i.e. showing the characteristics of a site specific mechanism. Fig.

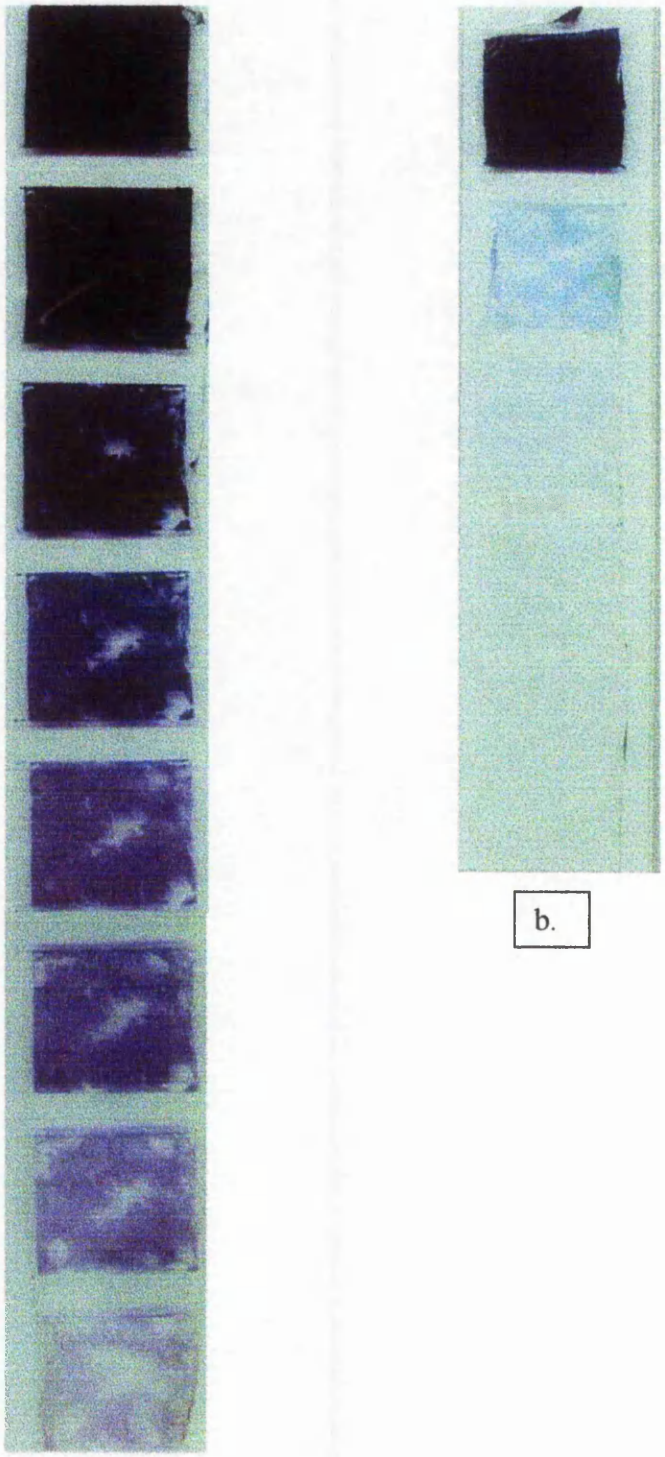


Fig. 3.22 Chitosan film dyed with (a) C.I. Direct Blue 15 and (b) C.I. Direct Blue 218.

3.23 shows the theoretical curve for C.I. Direct Blue 218. Analysis was possible for C.I. Direct Blue 15 however.

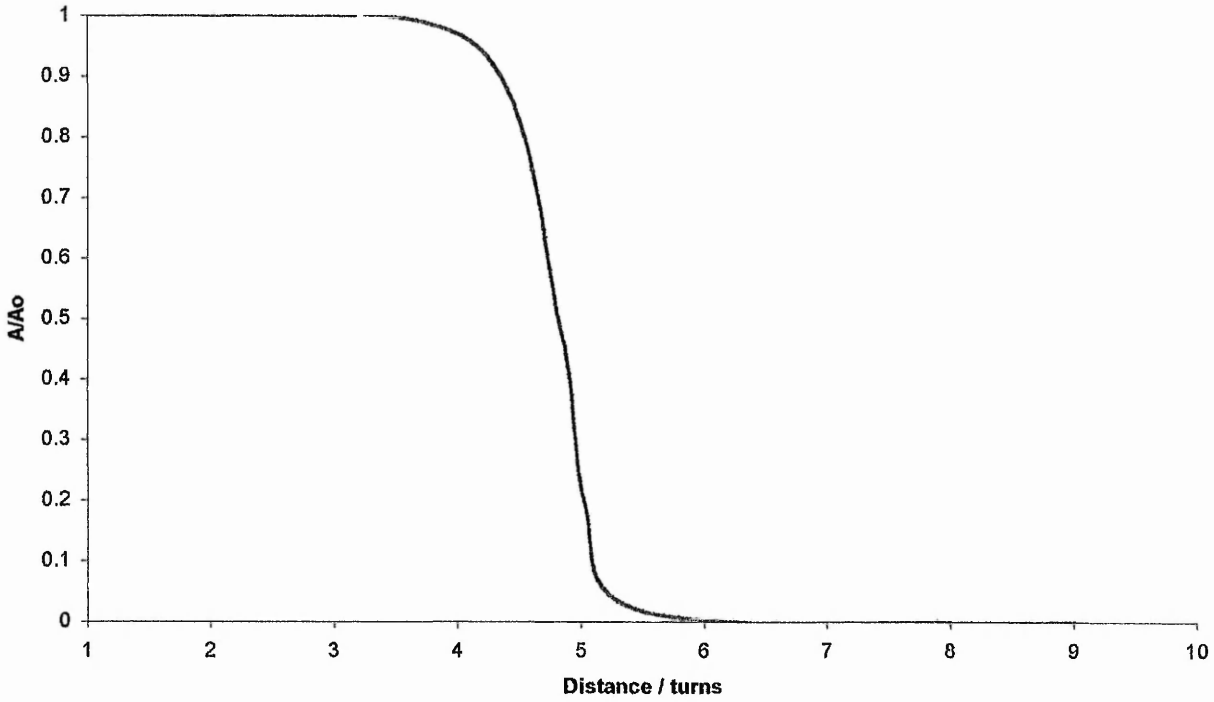


Fig. 3.23 Theoretical diffusion curve for C.I. Direct Blue 218

The film produced with C.I. Direct Blue 15 was analysed by dissolving up a portion of known area of each of the layers of the film and measuring the dye concentration spectrophometrically at 610 nm (λ_{max} for the dye). A plot was produced showing the concentration of dye against the distance from the surface.

The resulting plot bears a close resemblance to the plots derived for direct dyes on cellulose[83, 84]. The value of the diffusion coefficient was calculated at several values of dye concentration using the equation[86]:

$$Dc = \frac{1}{2t} * \frac{dx}{dc} * \int_0^c x.dc$$

Where:

Dc = Diffusion Coefficient

t = Time in seconds

$$\frac{dc}{dx} = \frac{1}{slope}$$

$$\int_0^c x.dc = \text{Area under the curve}$$

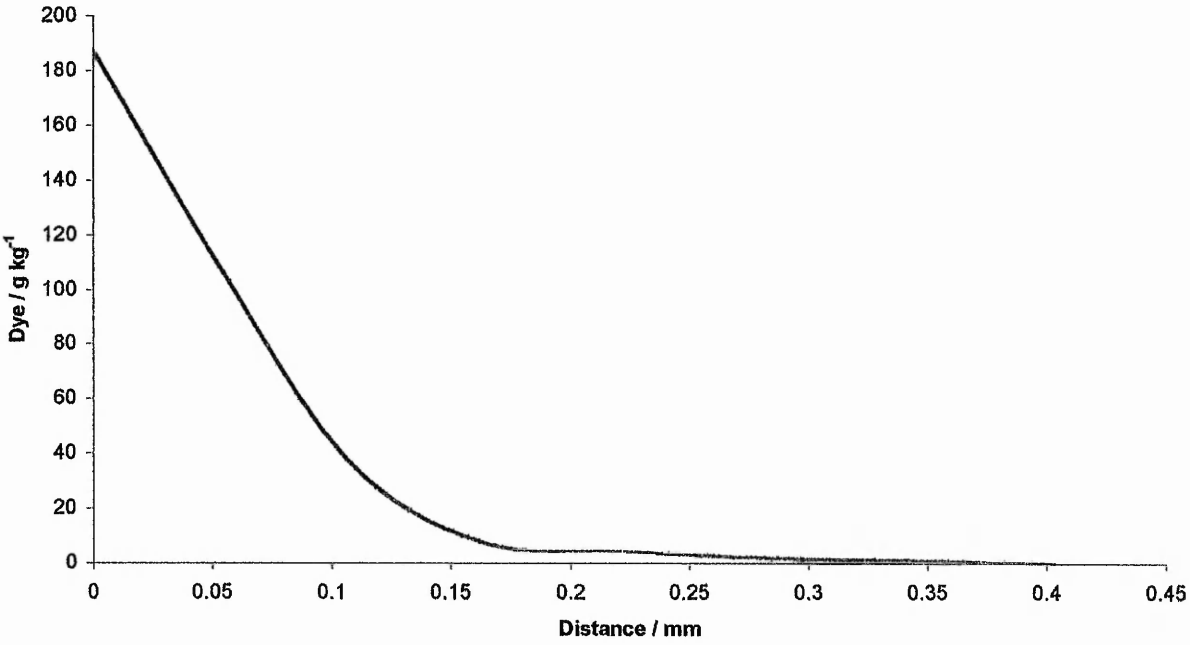


Fig 3.24 Diffusion of C.I. Direct Blue 15 through chitosan film at 80^0C

The diffusion coefficient was calculated at several different dye concentrations. Table 3.7 gives the value of Dc at the different concentrations.

Peters *et al*[84] examined the diffusion of Chlorozol Sky Blue FF in cellophane using a microdensitometer technique, he found that the relationship between the diffusion coefficient and concentration complied with the equation:

$$D_c = D_0(1+\beta c)$$

Where:

D_c = Diffusion coefficient

D_0 = Intercept on y-axis

β = Slope/D_0

c = Dye concentration g/100g of substrate

Dye / g kg^{-1}	Dc 10^7 / cm^2 s^{-1}
175	6.384
150	6.179
125	5.779
100	5.16
87.5	4.744
75	4.334
62.5	3.805
50	3.229
37.5	2.694

Table 3.7 Values of Dc at different dye concentrations for C.I. Direct Blue 15 on chitosan film, 120 min at 80^0C

Figure 3.25 is a plot of the values given in Table 3.7. It can be seen that the first part of the plot gives a straight line, and it is possible to apply the relationship derived by Peters to this portion of the plot and compare the values of D_0 and β for C.I. Direct Blue 15 in chitosan and Chlorozol Sky Blue FF in cellophane (Table 3.8).

	Chlorozol Sky Blue FF in cellophane	C.I. Direct Blue 218 in chitosan
D_0 (cm^2 s^{-1})	$1.5*10^{-9}$	$1.04*10^{-7}$
β	1.6	0.42

Table 3.8. Comparison of the values of D_0 and β for Chlorozol Sky Blue FF in Cellophane and C.I. Direct Blue 15 in Chitosan

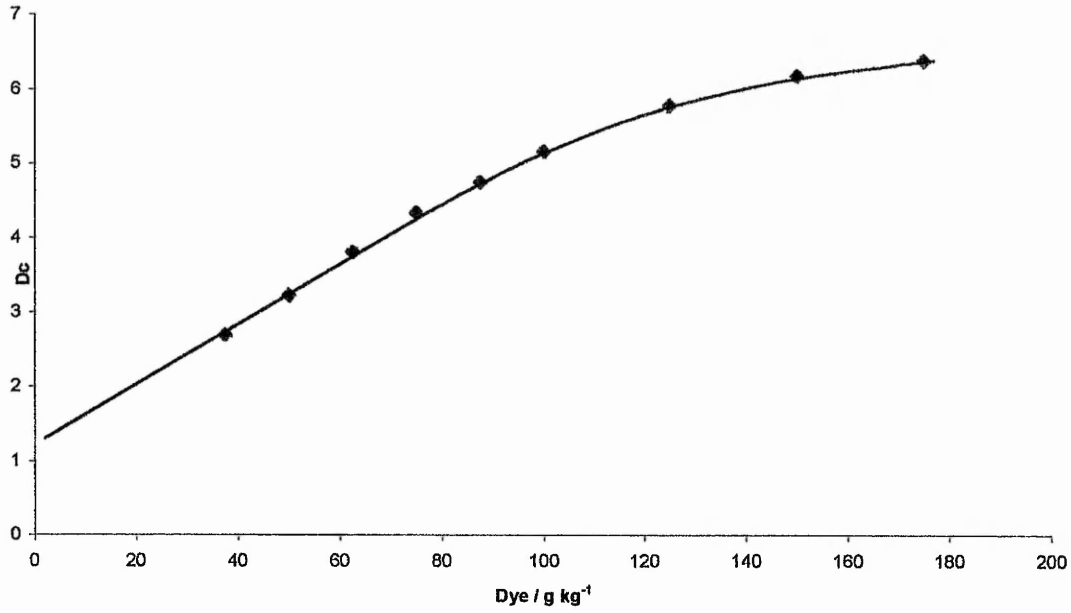


Fig. 3.25 Relationship between the diffusion coefficient (Dc) and dye concentration for C.I. Direct Blue 15 on chitosan

The diffusion characteristics of C.I. Direct Blue 15 on chitosan are similar to those of a direct dye on cellulose, as is the general relationship between the diffusion coefficient and the dye concentration. However the actual values of the diffusion coefficients are considerably larger for chitosan than for direct dyes on cellophane. This may be attributed to the highly amorphous nature of the chitosan film, which, unlike cellophane, was not stretched in any direction

during its preparation from solution. This would make diffusion through the film an easier process due to less mechanical restraint on the dye and hence give a higher value for the diffusion coefficient. Also the value of Dc increases with increase in dye concentration, and as chitosan adsorbs more dye than cellophane this will also lead to an increase in the diffusion coefficient. Also the experiments carried out by Peters *et. al.* were done using 5 g l^{-1} NaCl whereas no additional electrolyte was used in the above experiment, and as has been shown earlier the addition of electrolyte causes a reduction in the diffusion coefficient.

The behaviour of C.I. Direct Blue 218 in not diffusing further than the outer layer of the roll, even after over 100 hours at 80^0C is difficult to explain. One possible explanation is that due to the delicate nature of the chitosan film no tension could be applied to it during winding on to the Sekaido roll apparatus. It is therefore possible that there is not direct contact between the adjacent layers of the chitosan roll during dyeing; instead there may be a very thin water-filled gap between each layer. The uncoppered direct dye (C.I. Direct Blue 15) on reaching the inner surface of the outer layer desorbs from the chitosan film into this aqueous layer and is then adsorbed onto the outer surface of the next layer. Because of the high affinity of the coppered direct dye (C.I. Direct Blue 218) for chitosan, it does not desorb from the inner surface of the chitosan film into the aqueous layer and hence no transfer of dye between layers is possible.

The fact that when dyeing was carried out at elevated temperature (130^0C) for eight hours, three layers of film were dyed may be due to a combination of factors:

1. The film may be more swollen at 130^0C than 80^0C, making contact between adjacent layers more likely, thereby allowing transfer of the dye from one layer to the next.
2. The partition coefficient for the dye between chitosan and water is likely to move in favour of the aqueous phase as the temperature is increased, thus aiding desorption from the inner surface of the film into the aqueous phase, thus allowing transfer of the dye from one layer to the next.
3. The rate of dyeing is normally assumed to be doubled for every 10^0C rise in dyeing temperature, so that eight hours at 130^0C may be considered to be approximately equivalent to 250 hours at 80^0C.

However it is obvious from the shape of the concentration profiles that the mechanism of diffusion within the chitosan for the two dyes is different, C.I. Direct Blue 15 being similar to that of a direct dye in cellulose and C.I. Direct Blue 218 being similar to an acid dye in nylon, indicating that it involves specific site adsorption.

In an attempt to try to clarify if C.I. Direct Blue 218 will diffuse through chitosan film a further experiment was carried out. Two films were cast as before but one was cast at twice the thickness of the other. A sample was then taken from each film using the template to give two films with the same surface area but different thickness. These two films were then dyed separately at 80^0C for 8 hours at a liquor ratio of 400:1. The residual dye remaining in the dyebath was then measured and a comparison made between the dye uptake of the two films.

Weight of film (g)	Wet Thickness (mm)	Dye Concentration ($g\ l^{-1}$)	Absorbance at 600 nm	Dye Uptake[1] ("moles" kg^{-1})
0.05	0.14	$2*10^{-3}$	0.112	$25.8*10^{-6}$
0.0971	0.27	$2*10^{-3}$	0.358	$22.1*10^{-6}$
Blank			1.73	

[1] An arbitrary figure of 25000 was taken for the extinction coefficient for the dye

Table 3.9 Comparison of dye uptake between two chitosan films.

It can be seen from Table 3.9 that both films have very similar dye uptake in terms of moles per unit weight of chitosan. The ratio of the dye uptake is 1.17, and this shows that the dye is diffusing through the film. If both films were dyed uniformly to the same extent then the ratio should be 1.0; if dye was only being adsorbed onto the surface of the films then the ratio would be approximately 2.0.

Both of the dyed films were then placed in clean water, again at a liquor ratio of 400:1, and treated at 80^0C for a further 8 hours; the liquor was then measured for dye content. It was found that almost no dye had desorbed from the film into the water, the absorbance being 0.007 for the thin film and 0.014 for the thick film. These results suggest that metallised dyes will diffuse through chitosan, but due to the equilibrium partition being so heavily in favour of the chitosan the Sekaido roll technique does not give satisfactory results unless intimate contact between the layers can be made. A microdensitometer technique which uses a single piece of film would therefore be a more satisfactory technique to use.

3.3.3 Diffusion of 1:1 Pre-metallised Acid Dyes Through Chitosan Film

The diffusion of C.I. Acid Blue 158 was observed using chitosan film and the Sekaido roll technique. The resulting dyed film showed a heavily dyed outer layer, but no dye had penetrated further than this point. Further experiments were carried out with longer dyeing times, but even after several days, no dye appeared to penetrate further than the outer layer of the film. This would appear to indicate that although adsorption at the surface of the substrate is very rapid, the mechanism of adsorption is such that the dye molecule is bound very strongly to the substrate and therefore diffusion from the surface to the centre of the substrate is very slow. From these results it would appear that the mechanism of dye adsorption and diffusion of a 1:1 pre-metallised dye through chitosan is a site specific mechanism as with the metallised direct dyes, giving a diffusion curve similar to that shown in Fig. 3.20.

3.4 Dyeing Studies With Acid Dyes at Neutral pH on Wool and Nylon

3.4.1 Introduction

As has already been discussed, both chitin and chitosan contain amine groups that it is proposed are involved in metal complex formation with metallised dyes in which not all the co-ordination sites of the metal ion are occupied by ligands from the dye molecules. Wool and nylon also contain amine groups in their polymer chains and it is proposed that these groups are also capable of forming metal complexes.

The normal method of application for 1:1 pre-metallised acid dyes is from a bath containing 4-8% sulphuric acid. Under these conditions protonation of the amine groups occurs and dye adsorption is due to ionic bonding between the dye and the substrate. It is suggested that if the dyes were applied at neutral pH then any adsorption of the dye would be via a different mechanism as no protonation of amine groups would occur. Studies were carried out on samples of wool and nylon to investigate the adsorption of acid dyes at acid and neutral pH.

3.4.2 The Dyeing of Wool With Acid Dyes at Neutral pH

Dyeings were carried out on samples of scoured, bleached wool using a range of acid dyes under both acid pH and neutral pH. The dyes used were:

Non-metallised

C.I. Acid Blue 1

C.I. Acid Orange 7

1:1 Pre-metallised Acid Dyes

C.I. Acid Yellow 99

C.I. Acid Orange 74

C.I. Acid Red 183

C.I. Acid Blue 158

C.I. Acid Green 12

C.I. Acid Black 52

1:2 Pre-metallised acid dyes

C.I. Acid Violet 90

C.I. Acid Red 308

In the case of the non-metallised dyes the dyeings carried out under acid conditions showed good levels of exhaustion but the dyeings carried out at neutral pH showed no real uptake of the dye at all. The neutral dyeings were very unlevel and the dye was easily washed off showing that any colour on the substrate was more likely to be staining of the substrate rather than physical dyeing.

In the case of the 1:1 pre-metallised dyes there was a distinct increase in the level of exhaustion of the dyebath in the neutral dyeing compared to the dyeings carried out in the presence of acid. Of particular interest were the dyeings carried out using C.I. Acid Green 12; the dyeing at acid pH gave a very flat shade compared to the neutral dyeing which produced a much brighter shade. In all cases the dyes remained on the substrate after washing with hot water.

The 1:2 pre-metallised dyes showed similar behaviour under both sets of conditions. Both the level of exhaustion and the shade were very similar in each case. Again it was observed that the dye remained on the substrate after washing off in hot water.

It was observed that in the case of non-metallised and 1:1 pre-metallised acid dyes the behaviour of wool was consistent with that of chitin and chitosan, but in the case of the 1:2 pre-metallised dyes wool appears to be able to adsorb them quite satisfactorily whereas chitin and chitosan do not.

3.4.3 Colour Fastness of Acid Dyes on Wool

Colour fastness tests were carried out on the samples produced with acid dyes. In the case of the 1:1 pre-metallised acid dyes the results achieved under neutral conditions were comparable with those carried out at acid pH. In the case of the non-metallised acid dyes as no real dyeing had taken place no direct comparison of the colour fastness properties was possible. In the case of C.I. Acid Violet 90 (1:2 pre-metallised acid dye) the dyeing carried out at neutral pH showed a significant improvement over the sample dyed at acid pH.

C.I, Acid Yellow 99	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
1.0% Dye 4.0% H_2SO_4	5	4	4	5	4/5	4/5
1.0% Dye	5	45	4/5	5	4/5	4
C.I. Acid Blue 158	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
1.0% Dye 4.0% H_2SO_4	5	5	4/5	4/5	5	4/5
1.0% Dye	5	5	4/5	4/5	5	4/5
C.I. Acid Green 12	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
1.0% Dye 4.0% H_2SO_4	5	5	4/5	4/5	4/5	4/5
1.0% Dye	5	5	4/5	4/5	4	4/5

C.I. Acid Orange 74	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
1.0% Dye 4.0% H_2SO_4	4/5	4	4/5	4/5	5	4
1.0% Dye	4/5	4/5	4/5	4/5	5	4
C.I/ Acid Violet 90	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
1.0% Dye 4.0% H_2SO_4	5	3/4	4	5	4/5	4/5
1.0% Dye	5	5	4/5	5	5	5

Table 3.10 Colour Fastness of Acid Dyes on Wool at Acid and Neutral pH

3.5 The Dyeing of Wool Direct Dyes

3.5.1 Introduction

Direct dyes are not generally applied to wool although the fact that they contain sulphonic acid groups means that it is possible for them to act as milling acid dyes at acid pH. However as has previously been discussed, wool contains amine groups possibly capable of forming metal complexes and therefore a brief investigation of the possibility of applying pre-metallised direct dyes such as the Indosol SF range was carried out.

3.5.2 The Dyeing of Wool With Indosol SF Dyes

Dyeings were carried out at neutral pH, using samples of bleached wool. The dyes chosen for investigation were:

Indosol Yellow SF-2Rl

Indosol Rubinole SF-R

Indosol Blue SF-GL

Indosol Green SF-GLN

Indosol Brown SF-BR

Indosol Violet SF-B

These dyeings produced a good depth of shade and the dyebaths showed good exhaustion. The dyed wool showed no observable loss of colour after being rinsed in hot water.

3.5.3 Colour Fastness of Indosol Dyes Applied to Wool

Samples of wool dyed with Indosol SF dyes were tested for colourfastness using a standard test method for wool. The results obtained that these dyes show excellent fastness properties when applied to wool.

	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
Indosol Yellow SF-2RLl	5	4/5	5	5	5	4/5
Indosol Rubimole SF-R	5	4/5	4/5	5	5	4/5
Indosol Blue SF-GL	5	4/5	4/5	5/	5	4/5
Indosol Green SF-GLN	5	4/5	5	5	5	4/5
Indosol Brown SF-BR	5	4/5	4/5	5	5	4/5
Indosol Violet SF-B	5	4/5	4/5	5	5	4/5

Table 3.11 Colour Fastness of Indosol Direct Dyes on Wool at Neutral pH

3.6 The Dyeing of Nylon at Neutral pH

The above experiments using both acid dyes and direct dyes were repeated using nylon in place of wool. Although the results obtained were similar to those observed on wool, the depth of shade achieved on nylon was significantly less than that achieved on wool. This is expected as the amine group content of nylon is much less than that of wool. Both classes of dye again showed excellent wash fastness properties.

C.I, Acid Yellow 99	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
1.0% Dye 4.0% H_2SO_4	5	4	4	5	4/5	4/5
1.0% Dye	5	45	4/5	5	4/5	4
C.I. Acid Blue 158	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
1.0% Dye 4.0% H_2SO_4	5	5	4/5	4/5	5	4/5
1.0% Dye	5	5	4/5	4/5	5	4/5
C.I. Acid Green 12	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
1.0% Dye 4.0% H_2SO_4	5	5	4/5	4/5	4/5	4/5
1.0% Dye	5	5	4/5	4/5	4	4/5
C.I. Acid Orange 74	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
1.0% Dye 4.0% H_2SO_4	4/5	4	4/5	4/5	5	4
1.0% Dye	4/5	4/5	4/5	4/5	5	4
C.I/ Acid Violet 90	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
1.0% Dye 4.0% H_2SO_4	5	3/4	4	5	4/5	4/5
1.0% Dye	5	5	4/5	5	5	5

Table 3.12 Colour Fastness of Acid Dyes on Nylon at Acid and Neutral pH

	Secondary Cellulose Acetate (Dicel)	Bleached Unmercerised Cotton	Nylon 6.6	Polyester (Terylene)	Acrylic (Courtelle)	Wool Worsted
Indosol Yellow SF-2RLl	5	4/5	5	5	5	4/5
Indosol Rubimole SF-R	5	4/5	4/5	5	5	4/5
Indosol Blue SF-GL	5	4/5	4/5	5/	5	4/5
Indosol Green SF-GLN	5	4/5	5	5	5	4/5
Indosol Brown SF-BR	5	4/5	4/5	5	5	4/5
Indosol Violet SF-B	5	4/5	4/5	5	5	4/5

Table 3.13 Colour Fastness of Indosol Direct Dyes on Nylon at Neutral pH

4 Conclusions

A summary of the conclusions reached in this study is as follows:

1. Initial dyeing studies showed there to be a difference in the adsorption properties of 1:1 metal complex dyes on chitin powder at acid and neutral pH. This difference is caused because at acid pH dye adsorption involves ionic bonding between suitable groups on the dye molecule, such as sulphonic acid groups, and protonated amine groups along the chitin chain. In contrast to this at neutral pH a complex is formed between the central metal ion in the dye molecule and the amine groups in the chitin chain acting as ligands.
2. The addition of electrolyte to the dyebath reduces the negative surface charge on the chitin polymer allowing dye molecules to approach the polymer more readily, but there is a point at which the surface charge reaches a minimum after which further additions of electrolyte have little or no effect.
3. Increasing temperature has only a small effect on the final uptake of 1:1 pre-metallised acid dyes at neutral pH by chitin, which is not the case at acid pH.
4. The equilibrium adsorption of 1:1 pre-metallised acid dyes by chitin at neutral pH is by a site specific mechanism, but as there are no available sites for ionic interactions between the dye and the chitin it is most likely that a dye-metal-fibre complex is being formed.
5. The colour fastness of 1:1 pre-metallised acid dyes on chitin is higher than the same dyes applied at acid pH indicating that the mechanism of

adsorption results in a very stable dye-metal-fibre complex; metal complexes are very stable.

6. Although coppered and uncoppered direct dyes build up to the same level on chitin there is a significant improvement of the colour fastness of the coppered direct dyes over the uncoppered direct dyes. This again points to a different mechanism of adsorption for the different types of dye under the same conditions
7. Direct dyes of either type have a higher affinity for chitosan than for cellulose.
8. Equilibrium studies show that for uncoppered direct dyes at neutral pH the adsorption mechanism is similar to that associated with direct dyes on cellulose, whereas the coppered direct dyes showed a site specific adsorption mechanism similar to acid dyes on nylon at acid pH. This would strongly suggest that a complex was formed between the Cu(II) ion within the dye molecule and the amine groups of the chitin chain.
9. Examination of the diffusion characteristics of both coppered and uncoppered directs again show that the dyes diffuse through the chitosan via completely different mechanisms, the uncoppered direct dyes behaving as they would on cellulose, except that the value of the diffusion coefficient is greater on chitosan than on cellulose, whereas the coppered direct dyes again show a site specific mechanism of diffusion, although it was not possible to calculate absolute values for the diffusion coefficient. The 1:1 pre-metallised dyes when applied at neutral pH also show a site specific mechanism of diffusion, but again it was not possible to determine an overall value of the diffusion

coefficient. A microdensitometer technique may be a more suitable method for determining the diffusion coefficients of metallised dyes.

10. 1:1 pre-metallised dyes and coppered direct dyes may be applied to wool and nylon at neutral pH. Both types of dye give dyeings of a reasonable depth of shade and good-to-excellent wash fastness, showing that it is possible to form a metal complex between suitable dyes and the amine groups within the polymer chains.

5 Experimental

5.1 Materials

5.1.1 Chitin

The chitin used throughout this project was a commercial sample of crab chitin from India.

The chitin was hammer milled to a powder. The powder was separated by particle size and the fraction with particle size of between 200 and 500 μm used.

5.1.2 Chitosan

The chitosan used throughout this project was prepared from Indian crab chitin by Professor G. A. F. Roberts of the Nottingham Trent University

5.1.2.1 Powdered Chitosan

Powdered chitosan was prepared by dissolving 25 g of chitosan in 2.0 l of 0.1 M acetic acid. The solution was then filtered through polyester monofilament mesh to remove any insoluble material. The chitosan was then precipitated out using ammonia. The resulting white precipitate was filtered off and washed with distilled water until neutral to litmus. The solid was then washed with 80% methanol and then chopped in a Waring blender to give a powder. The solid was dried under vacuum.

5.1.2.2 Film Form

Chitosan films were produced by dissolving up 8 g of chitosan powder in 200 ml of 0.2 M acetic acid. The solution was stirred continuously until dissolution was complete. The resulting viscous solution was then filtered through polyester monofilament mesh. Any trapped air bubbles were removed from the solution by vacuum. The bubble-free solution was then poured onto a clean glass plate set on a level surface and skimmed to a depth of 0.9 mm using a doctor blade. The films were then allowed to dry in a clean dust free environment. Once the films were dry they were immersed in a tank containing a solution of 2% sodium carbonate and steeped for 30 minutes to neutralise. The neutralised films were then rinsed in distilled water to remove any residual sodium carbonate. After rinsing, the films were trimmed to the required size using a scalpel.

5.1.2.3 Film Thickness

For some of the experiments carried out it was important to know the thickness of the film being studied. This was achieved by producing a calibration plot for thickness against weight.

A film was produced by the method described in section 5.1.2.2. This film was then divided into ten segments along its length. Each of the segments was cut using a template of 30 mm x 30 mm to ensure that each segment had the same surface area. All ten segments were weighed individually and the total weight of all ten calculated. The ten segments were placed on top of each other and the thickness of all ten segments was measured using a digital micrometer.

Sample	Weight (g)
1	0.0352
2	0.0275
3	0.0279
4	0.031
5	0.028
6	0.0293
7	0.0186
8	0.0307
9	0.0282
10	0.0314
Total	0.2866

Table 5.1 Weight (g) of film segments

Total thickness of all ten segments = 0.3 mm

The thickness of each individual segment was then calculated using the formula:

$T_D = 0.3*w/0.2866$

$T_D = 1.047w$

Where

T_D = Dry thickness of segment (mm)

w = Weight of individual segment (g)

Sample	T_D (mm)
1	0.0369
2	0.0288
3	0.0291
4	0.0324
5	0.0293
6	0.0306
7	0.0195
8	0.0321
9	0.0294
10	0.0329

Table 5.2 Dry thickness of the film segments

Chitosan film does swell significantly in aqueous solutions such as dyebaths, and in certain circumstances the wet thickness is required. The original ten segments were steeped in distilled water for several hours and the total thickness again measured.

Total wet thickness = 0.81 mm

The wet thickness of the segments can then be related to the dry weight of the segment by the relationship

$T_w = 0.81*w/0.2866$

$T_w = 2.7913w$

Where:

T_w = Wet thickness of the sample

w = Dry weight of sample

5.2 Chemicals

The chemicals and solvents used were of general purpose reagent grade. The dyes used were taken from commercial samples, purified where necessary. All water used was distilled.

5.3 Spectroscopic Measurement

The absorbance of solutions were measured using a Unicam 8625 U. V. /Visible spectrophotometer. The measurement of reflectance for fabric samples was carried out using a Spectroflash 500 abridged spectrophotometer supplied by Datacolor International Ltd.

5.4 pH measurements

All pH measurements were carried out using a WPA CD 620 bench mounted digital pH meter. The meter was calibrated before use using commercially prepared buffer solutions. The term neutral pH used within this study refers to a pH range between 6.75 and 7.25.

5.5 Dyeing Machinery

Several different machines were used for dyeing depending on sample size and temperature requirements. For small samples of powdered chitin or chitosan, dyeing was carried out in a boiling tube placed in a Clifton water bath. Larger samples of powdered chitin, chitosan and fabric samples that

were dyed below 100°C, were dyed using an Ahiba Texomat laboratory dyeing machine. Any samples that required dyeing at elevated temperature were dyed using a Roaches fluid bed laboratory dyeing machine.

5.6 Characterisation of Chitin

5.6.1 Degree of *N*-acetylation

The degree of *N*-acetylation of the chitin was determined by the dye adsorption of C.I. Acid Orange 7 (see section 5.8), and was found to be 92% *N*-acetylated.

5.7 Characterisation of Chitosan

5.7.1 Degree of *N*-acetylation

The degree of *N*-acetylation of the chitosan was determined by the dye adsorption of C. I. Acid Orange 7 (see section 5.8), and was found to be 38% *N*-acetylated.

5.7.2 Molecular Weight

The molecular weight of the chitosan was determined by the limiting viscosity number. This was achieved by measuring the flow times of several concentrations of chitosan dissolved in 0.1 M acetic acid/0.2 M sodium chloride solvent system. The flow times were measured using a suspended level viscometer of modified Fitzsimmons type placed in a constant

temperature water bath set at 25°C. The flow times were measured until three consecutive results within 0.2 seconds of each other was achieved. The viscosity number was calculated using the formula[87]:

$$\text{Viscosity No.} = [(t_{sample}/t_{solvent})-1]/\text{Concentration}$$

Where the concentration is given in g l^{-1}

The Limiting Viscosity Number was determined by plotting Viscosity Number against Concentration in g l^{-1} and extrapolating back to a concentration of zero. This gave a value of 627.19 for the LVN.

The average molecular weight was then calculated using:

$$\log AMW = (\log LVN - \log(1.81*10^{-3}))/0.93$$

Therefore.

$$\log AMW = (\log 627.19 - \log(1.81*10^{-3}))/0.93$$

$$\log AMW = \log 5.957$$

This gives an average molecular weight for the chitosan of $9.05*10^{5}$

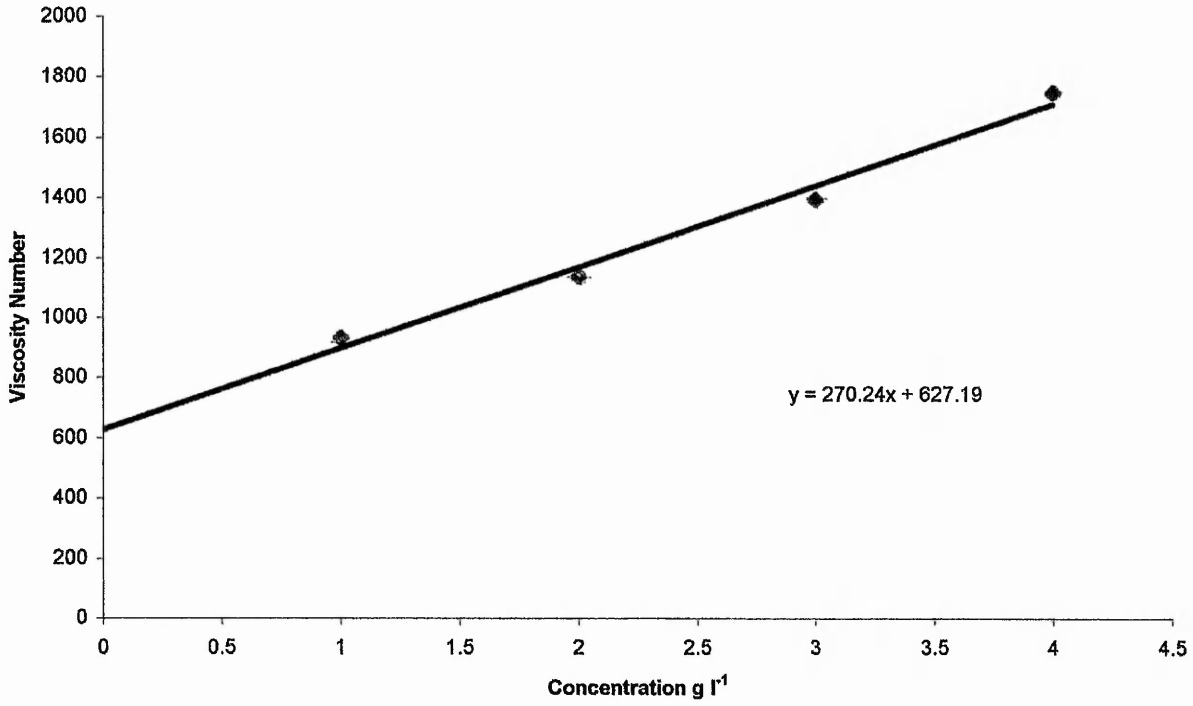


Fig 5.1 Limiting viscosity number for chitosan

5.8 Determination of the degree of N-acetylation by Dye Adsorption

When chitin and chitosan are treated with dilute acetic acid, protonation of the amine groups along the polymer chain occurs. These groups can then act as dye sites for anionic dyes. Although chitosan is normally soluble in dilute acetic acid it is not soluble if the acid solution contains an excess of anionic dye[29]. This effect can be used to determine the concentration of amine groups and hence the degree of *N*-acetylation.

5.8.1 Purification of C.I. Acid Orange 7

A sample of commercial grade C.I. Acid Orange 7 was extracted using boiling methanol in a Soxhlet apparatus. The refluxing was carried out until the methanol in the bottom flask became saturated. i.e. crystals of dye started to appear in the flask. Once this point had been reached the bottom flask was placed on a cork ring and allowed to cool slowly. Once the flask had cooled to room temperature it was placed in a refrigerator for 24 hours. The recrystallised dye was then filtered off, rinsed with methanol, and then dried.

5.8.2 Procedure[29]

Samples of chitin or chitosan were accurately weighed into a conical flask, 0.1 g in the case of chitosan and 0.2 g in the case of chitin. A stock dye solution was prepared from purified C.I. Acid Orange 7 and 0.1M acetic acid with a concentration of approximately $5*10^{-3}$ M of dye (1.75 g l^{-1}). Aliquots of this solution were added to the conical flask, 200 ml for chitosan and 100 ml for chitin. The flasks were stoppered and placed in a water bath set to 60°C. Once the flasks had reached temperature the pressure wass released and the flasks were then sealed and left at temperature for 16 hours. After this time the adsorption process should be complete. The solution was then be filtered through glass wool. This must be done quickly whilst the flask are at temperature to avoid any diffuse adsorption of the dye as the solution cools. The filtered solution must also be sealed to prevent any evaporation. The absorbance values of the solutions were then be recorded at 484 nm using 0.1

M acetic acid as the reference (a dilution factor of 100 is normally required). The degree of *N*-acetylation can then be calculated.

$$EW = (w*22500)/(\Delta A*f*v)$$

$$\text{Degree of } N\text{-acetylation } \% = [(EW-161)*100)/(EW+42)$$

Where:

EW= Equivalent weight of amine groups

w = oven dry weight of chitosan/chitin

ΔA = Difference of absorbance between reference and sample

f = dilution factor

v = volume of stock solution in litres

Therefore for the chitin used in this thesis the equiverlant weight of amine groups when:

w = 0.1028

ΔA = 0.044

is:

$EW = (0.105*22500)/(0.095*100*0.2)$

EW = 1243.42

And the degree of *N*-acetylation is:

Degree of *N*-acetylation % = [(2628.41-161)*100]/(2628.41+42)

Degree of *N*-acetylation % = 92.4%

5.9 Purification of Commercial Dyes

When necessary the samples of commercial dyestuffs were purified.

5.9.1 Purification of Acid Dyes

Acid dyes were purified using the same method as applied to C.I. Acid Orange 7 (Section 5.8.1).

5.9.2 Purification of Direct Dyes

The purification of commercial samples of direct dyes was carried out as follows. Commercial dye (5 g) was dissolved in 200 ml of boiling water, the resulting solution was then filtered through glass wool. A saturated solution of sodium acetate was prepared by dissolving 60g of sodium acetate in 50 ml of boiling water. The sodium acetate was then added to the filtered dye solution and allowed to cool slowly back to room temperature. Once the solution had reached room temperature, it was placed in a refrigerator for 24 hours. After the 24-hour period the recrystallised dye was filtered off under vacuum, rinsed with methanol and dried.

5.10 Initial Dyeing Studies on Chitin Powder

The initial dyeing studies carried out on chitin powder were performed using the following methods.

Dyeings were carried out on 0.5 g of chitin powder with 1% owf of dyestuff at a liquor ratio of 60:1. The dyebaths were prepared in boiling tubes by accurately pipetting 5 ml of a prepared dye solution with a concentration of 0.1% (1 g l^{-1}). The bath was then made up to 30 ml volume by pipetting 25 ml of distilled water. For the dyeings carried out under acid conditions 4% (owf) sulphuric acid was added to the bath by adding 2 ml of a prepared 1% solution and the quantity of water added adjusted accordingly to give the required final volume. Approximately 0.5 g of chitin powder was accurately weighed out and added to the prepared dyebaths. The boiling tubes were then sealed with Suba-seal caps and placed in a water bath set at 80^0C. Once the dyebaths had reached 80^0C the pressure was released using a hypodermic needle. Dyeing was carried out for 60 minutes with the tubes being regularly shaken to ensure even uptake by the chitin. After the 60 minutes had elapsed the tubes were opened and the chitin powder filtered off. The dyed chitin was rinsed in cold water, re-filtered and dried in an oven at 50^0C.

5.11 Extraction of Dyed Chitin

Extractions carried out on dyed chitin powder were done using 0.2 g of dyed chitin placed in an extraction thimble. The thimble was then placed in a Soxhlet apparatus and refluxed with water for 120 minutes.

5.12 Build Up of Dyes on Chitin Powder

Build up experiments were carried out on 6g of chitin powder in a total dyebath volume of 300 ml giving a liquor ratio of 50:1, at a dye concentration

of 2% in the case of acid dyes and 6% in the case of direct dyes. The dyebath was prepared in a conical flask. A 10 ml sample was removed from the dyebath for a reference, and the flask placed in a water bath set at 80^0C. Agitation of the dyebath was achieved using a magnetic stirrer, and the temperature was maintained using an immersion heater placed in the water bath and a relay contact thermometer placed in the dyebath. Once the dyebath temperature had been stabilised, the chitin powder was introduced to the dyebath and the flask sealed to prevent any evaporation from the dyebath. Uniform 10 ml fractions were removed from the dyebath at timed intervals. In the case of acid dyes these intervals were 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes and in the case of direct dyes the intervals were 5, 10, 15, 30, 45, 60, 120, 180, 240 and 300 minutes. The fractions were immediately filtered under vacuum to prevent any further uptake of dye by the chitin. The filtrate was then made up to 50 ml volumetrically. The reference sample was also made up to 50 ml and the absorbance values of the reference and samples recorded at λ_{max} for the particular dye under study. Accurate dilution of the reference and samples were made where necessary to give accurate absorbance measurements. The absorbance of the samples was compared to the absorbance of the reference and a plot of percentage dye on chitin against percentage dye remaining in solution produced.

5.13 Effect of Temperature on the Build Up of Dyes on Chitin

These experiments were carried out using the same method as in Section 5.12, except that a series of experiments were carried out with the same dye over a range of temperatures.

5.14 Effect of Electrolyte on the Adsorbance of Dye by Chitin

Three sets of four dyebaths were prepared in boiling tubes according to the table. The total dyebath volume was made up to 30 ml with water.

Electrolyte Concentration					
0 g l^{-1}		10 g l^{-1}		20 g l^{-1}	
Sample	Dye (10^{-3} g)	Sample	Dye (10^{-3} g)	Sample	Dye (10^{-3} g)
1	2.5	5	2.5	9	2.5
2	5.0	6	5.0	10	5.0
3	10.0	7	10.0	11	10.0
4	15.0	8	15.0	12	15.0

Table 5.3 Electrolyte concentration for individual dyebaths.

The electrolyte used depended on the class of dye being studied, for acid dyes it was sodium sulphate and for direct dyes it was sodium chloride.

Chitin powder (5 g) was added to each of the dyebaths and the tubes sealed with Suba-seal caps. The tubes were placed in a water bath set at 80^0C and once the dyebaths reached temperature the pressure was released using a hypodermic needle. Dyeing was carried out for 60 minutes and the tubes were agitated regularly to ensure even uptake of the dye by the chitin powder. After the dyeing had been completed the exhausted dye baths were filtered under vacuum to remove the chitin powder. The filtered dyebaths were made up to 50 ml volumetrically. The absorbance values of the exhausted dyebaths were

recorded at λ_{max} for the particular dye under study, dilutions being made when necessary. A plot of dye on chitin-$[D]_f*10^{-3}$ g kg^{-1}-against dye remaining in solution-$[D]_s*10^{-3}$ g l^{-1}-was prepared by from a calibration plot of absorbance against dye concentration $*10^3$ g previously prepared for each dye under study.

5.15 Equilibrium Studies

Equilibrium studies were carried out in boiling tubes. Twelve 25 ml dyebaths were made for each dye under study, each dyebath having a different but accurately known dye concentration. Table 5.4 shows the dye concentration recorded for C.I. Direct Yellow 12.

Sample	Dye (10^{-3} g)	Sample	Dye (10^{-3} g)	Sample	Dye (10^{-3} g)
1	5.02	5	14.056	9	22.088
2	8.032	6	16.064	10	23.092
3	10.04	7	18.072	11	24.096
4	12.048	8	20.08	12	25.1

Table 5.4 Dye concentration (10^{-3} g) of C.I. Direct Yellow 12

Chitin powder (0.5 g +/- 0.001 g) was added to each of the dyebaths and the tubes then sealed with Suba-seal caps. All twelve dyebaths were placed in a water bath set at 80^0C, and once the dyebaths had reached temperature the pressure was released using a hypodermic needle. Dyeing was carried out for 48 hours to ensure that equilibrium was reached, the dyebaths being agitated

regularly to ensure even uptake of the dye by the chitin. Once dyeing was complete the exhausted dyebaths were filtered under vacuum to remove the powdered chitin, and the filtered dyebaths made up to 50 ml volumetrically. The absorbances of the exhausted dyebaths were recorded at λ_{max} for the particular dye being studied, accurate dilutions being made where necessary. The quantity of dye remaining in the dyebath was calculated using the calibration data for the relevant dye and thus the amount of dye adsorbed by the chitin could also be calculated. From this data the equilibrium adsorption isotherm could be plotted. This is a plot of the amount of dye on chitin ($[D]_f$) against the amount of dye remaining in the exhausted dyebath ($[D]_s$) at equilibrium.

The equilibrium adsorption isotherm may be analysed in terms of either the Freundlich or the Langmuir equilibrium adsorption isotherms. The Freundlich is obtained by plotting log $[D]_f$ against log $[D]_s$. This will result in a linear plot if the mechanism of adsorption is non-site specific. The Langmuir equilibrium adsorption isotherm is obtained by plotting $1/[D]_f$ against $1/[D]_s$, in this case the plot will be linear if the mechanism of adsorption is site specific.

5.16 Colour Fastness of Dyed Chitin

1.0 g samples of powdered chitin were placed in pouches made from polyester monofilament mesh. The mesh pouches were sewn closed to prevent the chitin from spilling out. Dyeings were carried out with dye concentrations of 0.5%, 1.0% and 2.0% dye owf. For the dyeings carried out in acid conditions 4% owf sulphuric acid was added to the dyebath. The dyeing was

carried out in dyebaths of total volume of 300 ml using an Ahiba Texomat laboratory dyeing machine set to 80^0C for 60 minutes. Once dyeing was complete the polyester monofilament pouches were removed and rinsed in cold water, and then dried at room temperature.

The pouches containing the dyed chitin were then placed in a Roaches wash wheel, each wash pot containing one pouch of dyed chitin along with a 100 mm x 40 mm piece of multifibre strip (obtained from the Society of Dyers and Colourists). A solution of 5 g l^{-1} soap flakes was made and 100 ml of this solution was also added to each wash pot. The wash test was carried out at 50^0C for 30 minutes. Once the test had been completed the multifibre strip was removed from the wash liquor, rinsed in cold water and dried in a conditioned atmosphere. The conditioned atmosphere was 20^0C and 60% relative humidity. The dried pieces of multifibre were then mounted onto white card and assessed for staining visually using grey scales in a Verivide light cabinet under D65 illuminant.

5.17 Comparison between Chitosan-treated Cellulose and Untreated Cellulose

5.17.1 Preparation of Chitosan-treated Cellulose

A solution containing 1.0 g of chitosan powder dissolved in 200 ml of 0.1 M acetic acid was prepared. The solution was filtered through polyester monofilament to remove any insoluble material. A piece of scoured and bleached woven cotton was immersed in the solution until completely wetted out. The cotton sample was then run through a mangle to remove any excess chitosan solution. After being run through the mangle it was found that the

cotton had about a 100% pick up by weight of the chitosan solution. The treated fabric was then allowed to dry at room temperature. The dry fabric was neutralised by immersing in ammonia solution for fifteen minutes and was then allowed to dry at room temperature.

5.17.2 Dyeing of Chitosan-treated and Untreated Cellulose

A 1.0 g sample of chitosan-treated fabric and a 1.0 g sample of untreated cotton were dyed together in the same dyebath. The dyebath was made up with 2.0 % owf direct dye in a total volume of 300 ml and dyeing carried out at 80^0C for 60 minutes in a Ahiba Texomat laboratory dyeing machine.After the dyeing was complete the samples were removed from the dyebath and rinsed in cold water. The samples were allowed to dry at room temperature. The dried samples were then measured for reflectance using a Datacolor spectroflash spectrophotometer and the K/S value at λ_{max} recorded. Samples (0.2 g) were taken from both the untreated and chitosan-treated cellulose and extracted with water by refluxing in a Soxhlet apparatus for 20 minutes. The samples were then dried and the K/S values at λ_{max} recorded. The result for the extracted sample was then compared back to the original and the drop in K/S calculated in terms of percentage. The extraction was then repeated with a fresh 0.2 g sample of fabric but this time the extraction was carried out for 240 minutes. Again the result from the extracted sample was recorded and compared back to the original.

5.18 Diffusion Studies

5.18.1 Introduction

Diffusion studies were carried out on chitosan films (For the method of preparing these films see Section 5.1.2.2.) rolled onto a Sekaido roll apparatus.

5.18.2 Dyeing Procedure

The Sekaido roll consists of a metal cylinder around which the chitosan film is wound. A blanking plate is then placed along the film and the ends sealed with adhesive tape. The plate prevents dye from penetrating into the substrate thus allowing easier identification of the separate layers. The tape seals the edges of the roll preventing any dye from penetrating from the film edge.

The prepared roll was then placed in a dyebath containing 0.5 g of purified dye. Dyeing was carried out at 80^0C in an Ahiba Texomat laboratory dyeing machine. It was found that for non-metallised dyes 120 minutes was sufficient time for the dye to diffuse through several layers of the film, but for metallised dyes, even after several days at 80^0C, the dye had not penetrated further than the outer layer of the film. To overcome this lack of diffusion further dyeings were carried out at a temperature of 130^0C using a Roaches fluid bed laboratory dyeing machine. It was found that after 8 hours at this temperature diffusion had occurred across several layers of the film. Unfortunately the harsh conditions caused the film to become very brittle which made further handling and analysis very difficult.

After the dyeing was complete the Sekaido roll apparatus was removed from the dyeing machine and rinsed in cold water for 10 minutes to remove any excess dye from the substrate surface. The blanking plate and the tape was then removed and the film unrolled onto a glass plate. The film was dried at 50^0C in an oven.

5.18.2 Analysis of the Dyed Film

The dried film was cut into individual layers using the standard template used to determine the film thickness (Section 5.1.2.3). Each separate layer was accurately weighed and its wet thickness calculated. The individual layers were then placed in a sample bottle containing 20 ml of 5% sodium formaldehyde bisulphite addition complex and allowed to stand with occasional agitation, until the film had completely dissolved. In the case of the more heavily dyed outer layers it was found necessary to first treat the film with dilute ammonia and to gently apply heat to evaporate off the ammonia before adding the sodium formaldehyde bisulphite addition complex. Once all the film had dissolved the solutions were transferred to 50 ml volumetric flasks, and made up to volume with water. The dye concentration was then measured spectrophometricaly, dilutions being made when necessary. A plot of dye concentration against distance penetrated was then prepared.

From this plot it was possible to calculate the values of the diffusion coefficients at different dye concentrations (Table 3.7). A plot of the diffusion coefficient against dye concentration was then prepared.

5.19 The Effect of Electrolyte on The Diffusion of C.I. Direct Yellow 12 through Chitosan Film

Diffusion experiments were carried out as above using C.I. Direct Yellow 12. Three films were dyed, one film being dyed without any NaCl, one film being dyed with 5 g l^{-1} NaCl and one film being dyed with 15 g l^{-1} NaCl.

The films were dissolved in the manner already described and the absorbance measured spectrophometrically at 389 nm λ_{max} for this dye.

A plot of relative optical density against distance was produced.

5.19 Dyeing of Wool and Nylon

The dyeing of wool and nylon was carried out on 2.5 g samples of scoured fabric. The samples were scoured at 60^0C with soda ash for 20 minutes, then rinsed well in cold water for 10 minutes. Dyeing was carried out in an Ahiba Texomat Laboratory dyeing machine at 95^0C for 60 minutes at a liquor ratio of 150:1 and a dye concentration of 1% owf. For the dyeings carried out at acid pH 4% owf sulphuric acid was used. After dyeing the samples were rinsed in cold water for 10 minutes and dried in an oven at 50^0C. Spectroscopic analysis was carried out using a Datacolor smartmatch system. Measurement of the dyebath pH was done at the start and end of the dyeing cycle.

5.20 Colour Fastness on Wool and Nylon

Samples of dyed wool and nylon were tested for colour fastness as follows.

A 100 x 40 mm sample of the dyed fabric and a piece of multifibre strip also 100 x 40 mm were sewn together along one narrow edge. Each sample was then placed in a wash pot from a Roaches wash wheel. A solution of 5 g l^{-1} soap flakes was made freshly and 100 ml of this solution was added to each wash pot. The pots were sealed and placed in the Roaches wash wheel and washed at 50^0C for 30 min. At the end of the wash cycle the samples were rinsed in cold running water for 10 min. After rinsing the samples were spread out on a mesh drying tray ensuring that the wool or nylon was not in direct contact with the multifibre strip to avoid any contact staining. Once the samples were dry the multifibre strip was mounted onto plain white card and the level of staining assessed with grey scales in a Vervide light cabinet under D^{65} illuminant.

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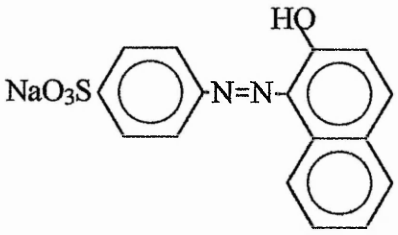
Appendix 1

Structures of Dyes Used

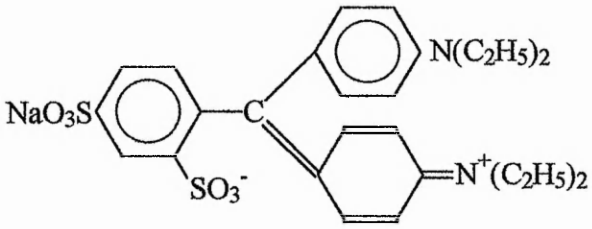
Acid Dyes

Non-metallised Acid Dyes:

C.I. Acid Orange 7

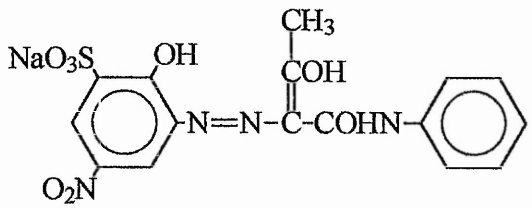


C.I. Acid Blue

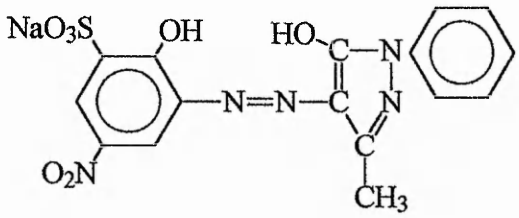


1:1 Metal Complex Dyes

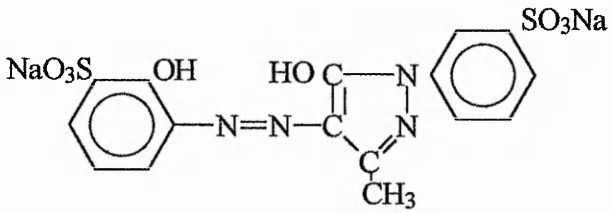
C.I. Acid Yellow 99



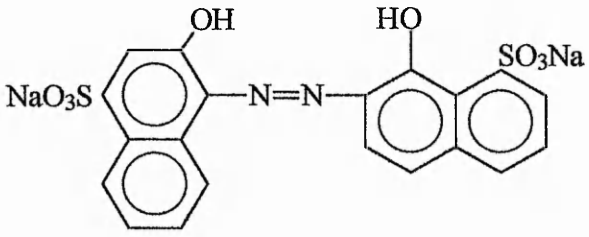
C.I. Acid Orange 74



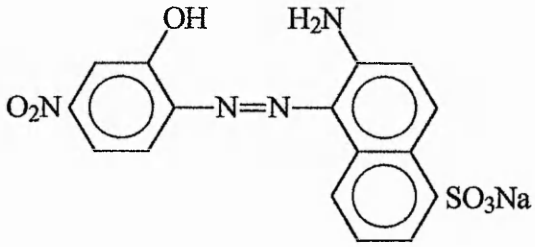
C.I. Acid Red 183



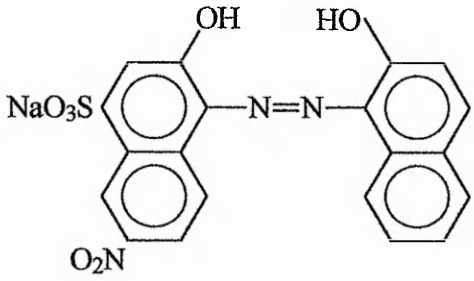
C.I. Acid Blue 158



C.I. Acid Green 12



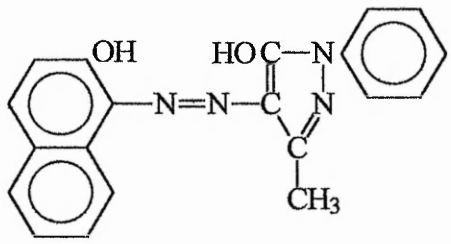
C.I. Acid Black 52



All the 1:1 pre-metallised acid dyes used are complexed with Cr(III)

1:2 Metal Complex Acid Dyes

C.I. Acid Violet 90



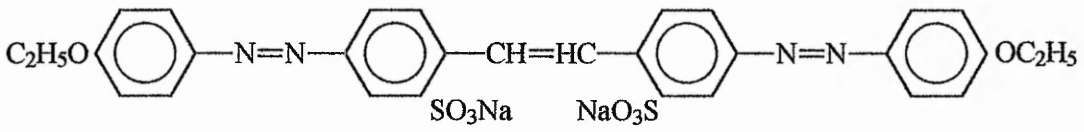
C.I. Acid Red 308

Structure not disclosed.

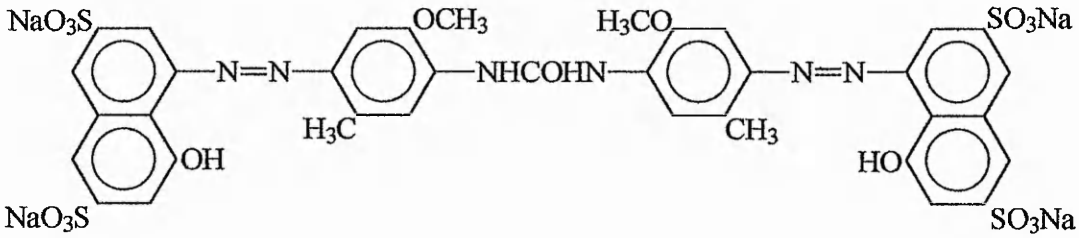
<u>Direct Dyes</u>

Non-metallised direct dyes

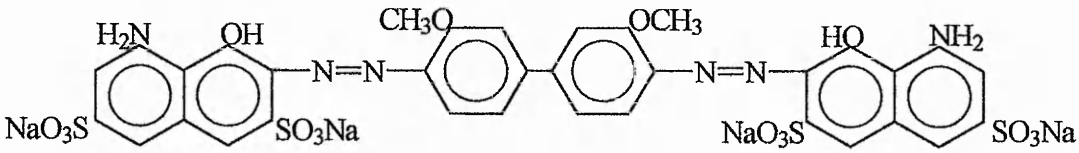
C.I. Direct Yellow 12



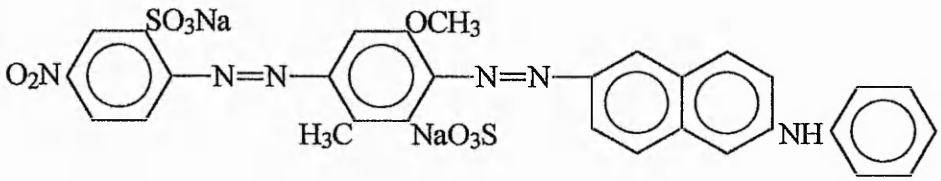
C.I. Direct Red 79



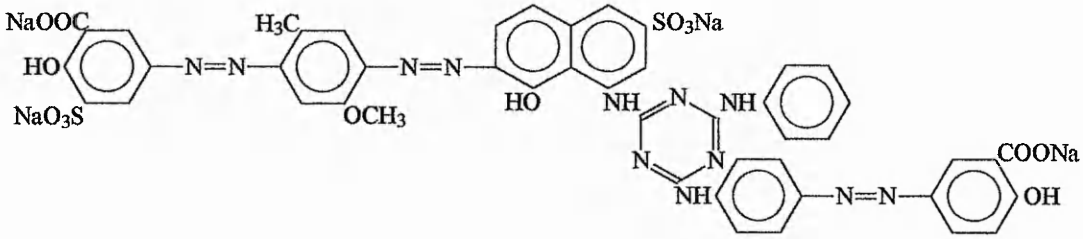
C.I. Direct Blue 15



C.I. Direct Blue 29



C.I. Direct Green 59



Metallised direct dyes

C.I. Direct Blue 218

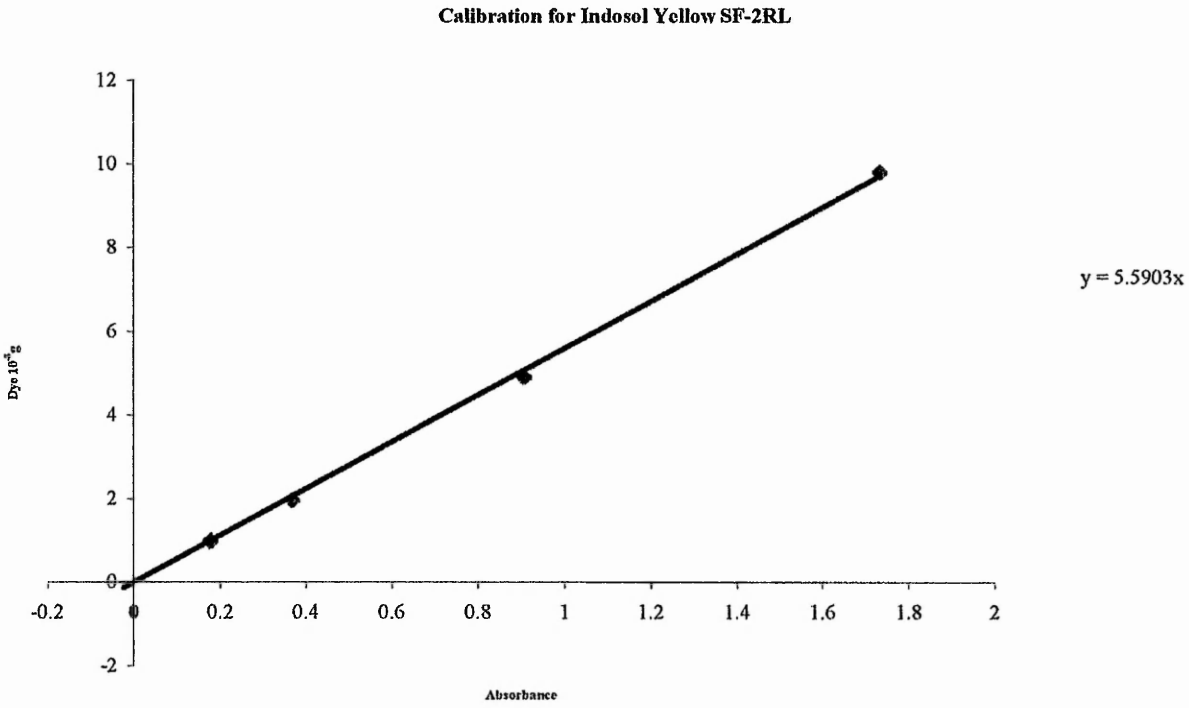
This Dye has the same structure as C.I. Direct Blue 15 but has two copper ions complexed to it.

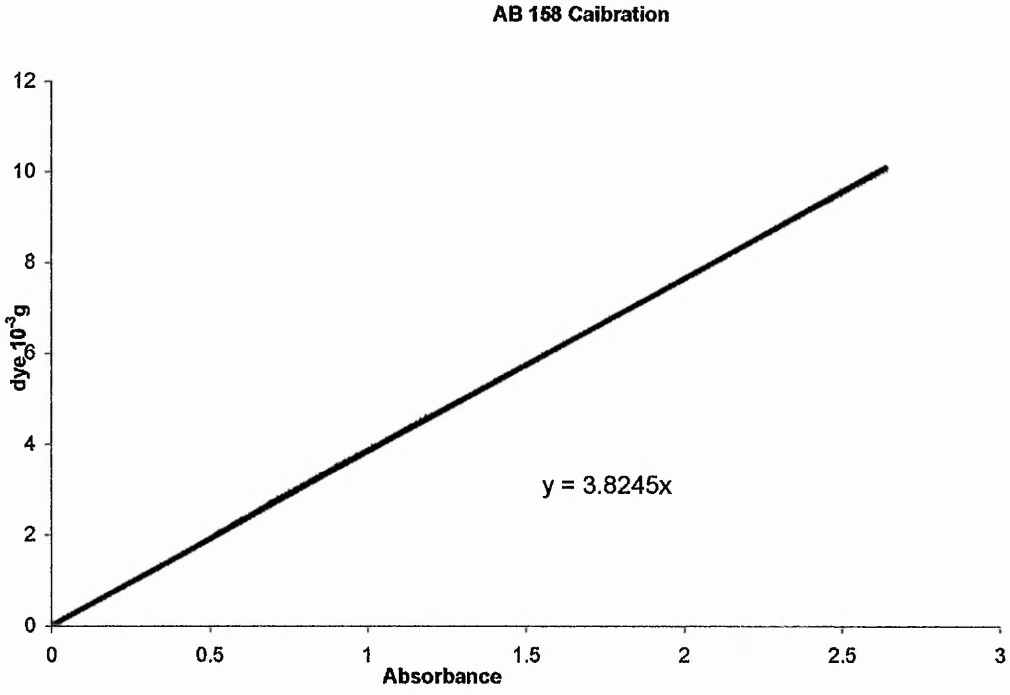
Indosol dyes

The structure of these dyes has been withheld by the manufacturer and the only structural information available is that all the members of this range are copper complexes.

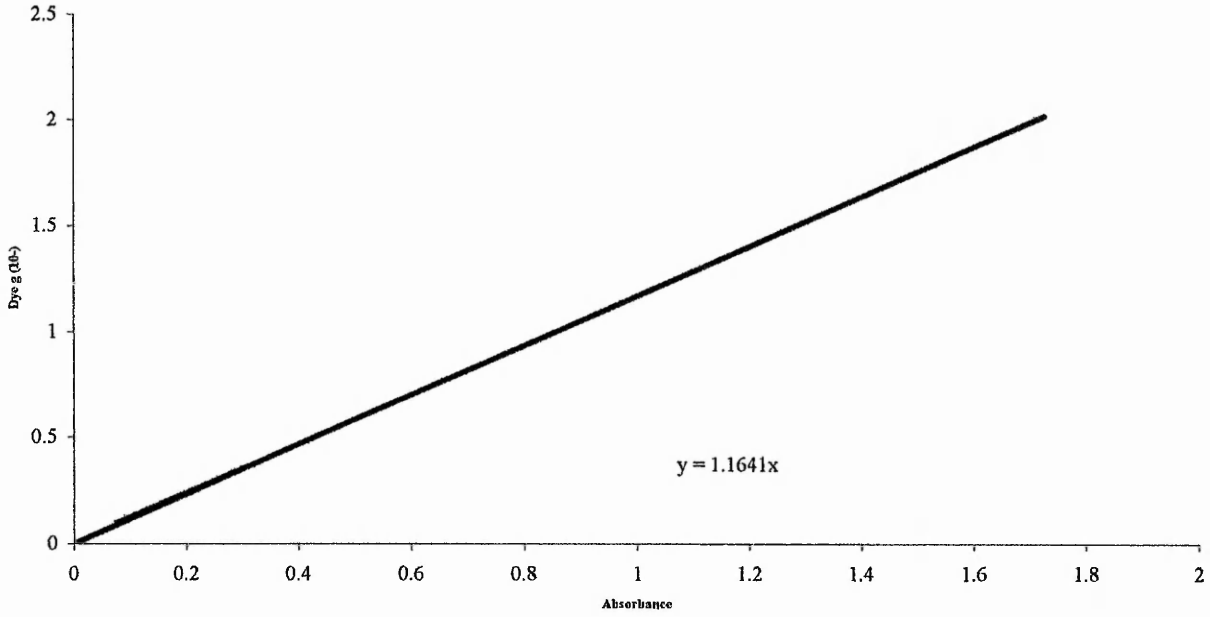
Appendix 2

Calibration Charts

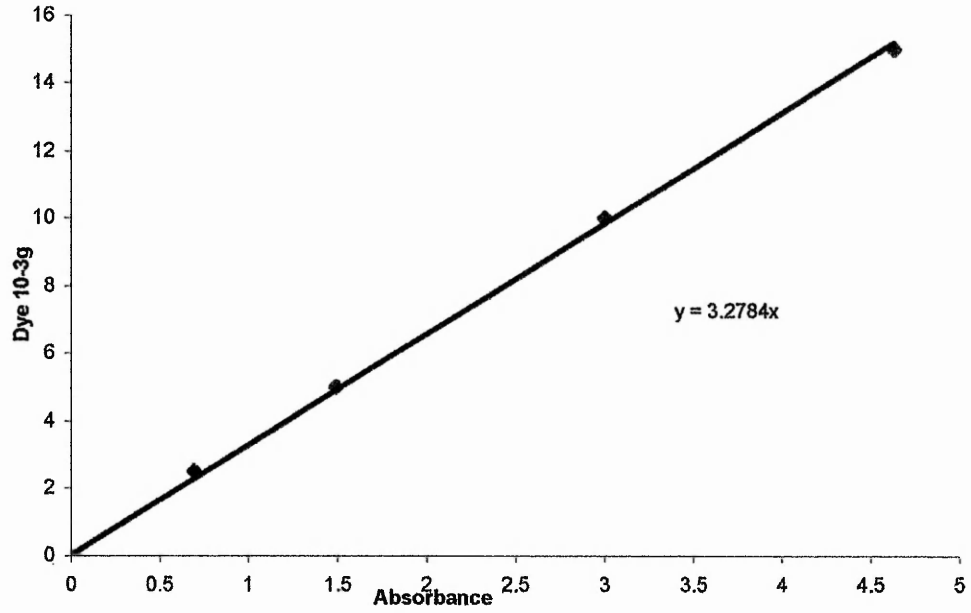


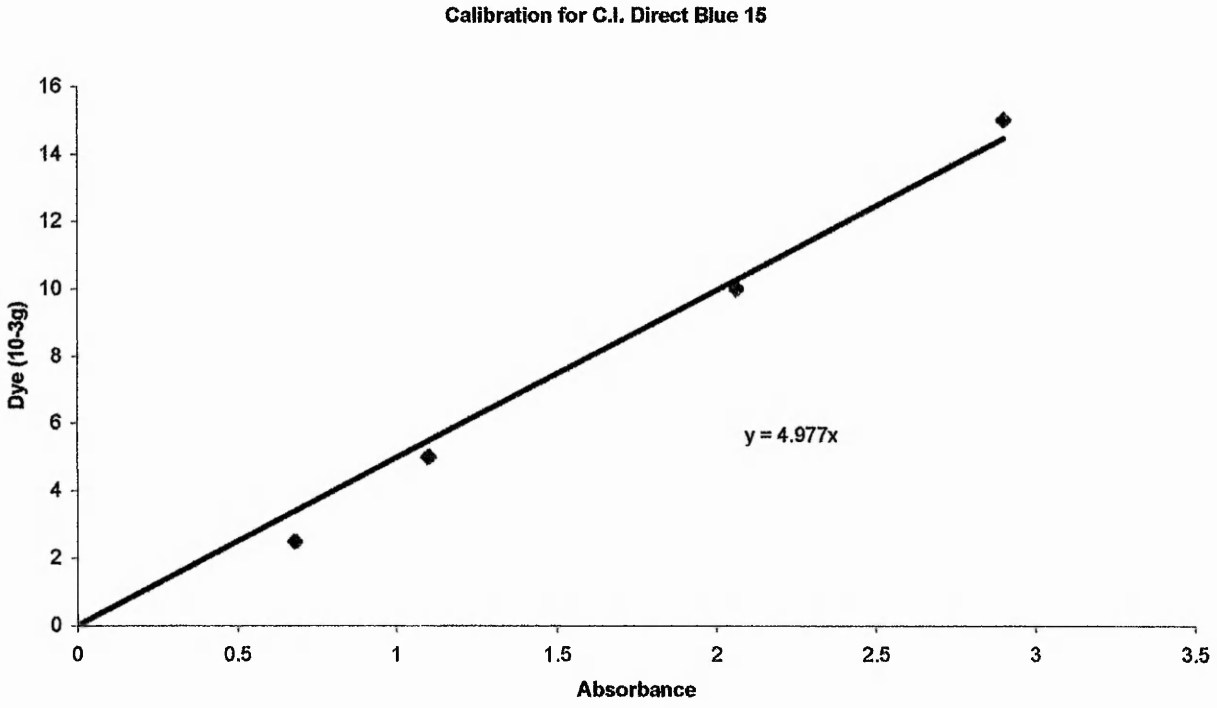


Calibration For Direct Yellow 12



Calibration for C.I. Direct Blue 218




Calibration for C.I. Direct Blue 15
Dye (10-3g)
Absorbance
y = 4.977x
0
2
4
6
8
10
12
14
16
0
0.5
1
1.5
2
2.5
3
3.5