STUDIES ON THE FLUORESCENCE & PHOTOYELLOWING PROPERTIES OF WOOL

by

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TEXT BOUND CLOSE TO THE SPINE IN THE ORIGINAL THESIS

CONTENTS

Page Number

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STATEMENT

The experimental work in this thesis has been carried out by the author in the Department of Chemistry at The City University, London and in the laboratories of the International Wool Secretariat, Technical Centre, Ilkley, between October 1983 and September 1986. The work has not been presented and is not being presented for any other degree.

G. ISMAIL
August, 1987

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ABSTRACT

Basic concepts of photochemistry are outlined.

Detailed descriptions of the morphology of wool, its chemical composition and the probable mechanism towards its photodiscolouration (yellowing) process are discussed. The use of fluorescent whitening agents (FWAs) to brighten photoyellowed wool and some processes for the prevention of photoyellowing are briefly outlined.

Phthalimides, as FWAs for wool, have been synthesised. They have been shown to behave in a similar fashion to other conventional FWAs, when applied to wool, despite the high fluorescence quantum yields observed with some of them in solution. Some drawbacks from their use as whiteners for wool have also been encountered.

Potential FWAs containing halogen atoms which intramolecularly quench fluorescence have been synthesised and applied to wool in a number of ways. Application by cold pad-batch methods revealed that the complete displacement of all halogen atoms by nucleophilic groups in wool did not take place. Treatment of these materials with morpholine, sodium carbonate or water was necessary to develop the full potential fluorescence yield of the FWA on the fabric. That the fluorescence of FWAs can be quenched by the heavy atom effect was shown by the fact that no fluorescence was observed when commercial FWAs were applied to brominated wool. If the FWA treated brominated fabrics were subjected to a reduction treatment then the bromine atoms were removed and the fluorescence of the FWA was restored. Application of the potential FWAs by exhaustion at high temperatures always led to strong fluorescent fabrics implying that the halogen atoms were completely displaced using this application method but the question remains as to how much of the displacement leads to covalent bonds between the fabric and the FWA.

Several stilbenes have been shown to sensitise the formation of singlet oxygen which is capable of oxidising indole residues, e.g. tryptophan. Some stilbenes were found to quench singlet oxygen, e.g. disodium 4,4′-diaminostilbene-2,2′-disulphonate. Such materials when applied to wool sensitised the photoyellowing of wool and proved to be no more stable than conventional stilbene fluorescent whitening agents.

Different chemically treated wools have been exposed to UV radiation in the presence of different aqueous reductive bleaching agent solutions. It was observed that thiourea dioxide gave the best performance, and in the case of wool serge, led to a fabric having a better light fastness. An interesting beneficial synergistic

effect was uncovered when the reducing agents were used in conjunction with formaldehyde. Biphenols (models for dityrosine) have also been padded onto wool and exposed to light in the wet state to mimic the photoyellowing of dityrosine. o,o′-Biphenol treated wool was exposed to light in the presence of the reducing agents and these were found to decrease the amount of discolouration produced by degradation of o,o′-biphenol.

Both intramolecular and intermolecular fluorescence quenching by disulphide bond containing compounds have been studied in order to further establish the view that the disulphide bond containing cystine residue of wool quenches the fluorescence of the excited states of some closely located amino acid residues, e.g. tryptophan. Intermolecular quenching of fluorescence, in solution, has been found to be dependent on the lifetime of the excited singlet state of the fluorescer. Strong quenching of the intramolecular type, in solution, has been clearly demonstrated. Further quenching studies of the intermolecular type were also carried out on wool. Neither the fluorescence of a conventional FWA or of kynurenine, a photo-oxidation product of tryptophan, have been observed to be quenched by cystine residues, many of which lie on the surface of the wool. Application, under basic condition in air, of poly-ethylene glycol dimercaptoacetate onto the surface of wool pre-treated with the above conventional FWA led to strong fluorescence quenching of the applied FWA. Some future work remains to be performed in order to make some firm conclusions on this particular study.

FUTURE PUBLICATIONS

Some of the work contained in this thesis is going to be published in J.S.D.C. as outlined below:-

1. The photosensitising properties and photostability of stilbene fluorescence whitening agents.

   R.S. Davidson, G.M. Ismail and D.M. Lewis.

2. The efficiency of reaction between reactive fluorescent whitening agents and wool.

   R.S. Davidson, G.M. Ismail and D.M. Lewis.

3. Retardation of the photoyellowing of wool and wool treated with fluorescent whitening agents by the action of reducing agents.

   R.S. Davidson, G.M. Ismail and D.M. Lewis.

The work below has been presented at the following scientific meeting:-

The production of fluorescent brightening agents and their application to wool.

European Postgraduate Symposium on Photochemistry, The City University, London, April 11-13, 1984.

CHAPTER 1

INTRODUCTION TO PHOTOCHEMISTRY

1.1 Preface

Photochemistry is the branch of chemistry concerned with the action of light (mainly visible and ultraviolet) on chemical systems. It is essentially the chemistry of atoms and molecules in electronically excited states. Photochemical reactions have been studied for many decades but it is only with the development of sophisticated instrumentation that detailed mechanistic investigations of fast photochemical processes has become possible. As there are many textbooks [1 - 4] which provide excellent reviews of photochemistry this chapter merely provides a basic introduction to the creation, general properties and decay pathways open to excited states.

1.2 The Grotthus-Draper and Stark-Einstein Laws

There are two basic laws of photochemistry. The first, known as the Grotthus-Draper Law, states that only light which is absorbed is capable of initiating a photochemical process. The second, the Stark-Einstein Law, is derived from quantum theory and states that when an atom or molecule undergoes a photochemical change it is as a result of the absorption by the atom or molecule of a single photon. Because excited states normally have very limited lifetimes, it is seldom that an atom or molecule in an excited state will have time to absorb a further photon to give a still higher energy state. However, there is evidence that this may happen under conditions of intense irradiation. It is even possible

for a molecule to absorb two photons simultaneously (biphotonic absorption) under extreme conditions [5]. Despite these apparent deviations, the essential point of the law remains - molecules absorb radiation in discrete quanta, and the number of excited molecules produced is proportional to the number of photons absorbed. The energy of photons is related to the frequency (ν) of the radiation by Equation 1.1.

$$E = h\nu = hc/\lambda \qquad (1.1)$$

where h = Planck's constant,
c = velocity of light,
ν = frequency of radiation
and λ = wavelength of the radiation.

1.3 Primary and Secondary Processes

The absorption of light by molecules and the formation of excited states lies in the province of spectroscopy. Photochemistry is concerned primarily with the fate of the excited state once formed, i.e. with the nature of the process

$$A^* (+B) \longrightarrow C + D \ldots\ldots$$

where A* is a molecule in an electronically excited state, and C and D etc. are products of the primary photochemical reaction. The mechanism by which C and D etc. are formed may involve A* reacting with a ground state molecule B. The process shown above falls into the category 'excitation/chemical change'. It is likely

that there will be competitive photophysical processes by which A* returns to the ground state. In general the lifetimes of excited states are short, and the photon flux densities available from technologically important light sources are sufficiently low, to ensure that the instantaneous concentration of excited molecules is small, so that reactions between excited molecules are unlikely.

In many situations of technological importance, the products of the primary photochemical reaction act as intermediates which engage in secondary non-photochemical processes to produce final products. There may be a number of competing secondary reactions and the ratio of final products may depend on the concentration of products of the primary reaction.

1.4 Absorption of Light. The Beer-Lambert Law

The absorption of monochromatic radiation by a homogeneous solution is governed by the Beer-Lambert Law which states that when a beam of incident intensity I_o passes through a layer of thickness d, its intensity is reduced to I, where

$$I = I_o 10^{-\epsilon cd}$$

ϵ is the extinction coefficient, and c the concentration of the absorbing species. If d is in cm, and c is the molar concentration (moles/litre), ϵ is the molar extinction coefficient, and has the units litres/mole.cm.

The absorbance or optical density of a layer is defined as

$$A = \log_{10} I_o / I = \epsilon cd$$

The transmittance T is defined as I/I_o, and therefore

$$T = 10^{-A} \text{ and } \therefore A = -\log T$$

The Beer-Lambert Law may be derived theoretically if it is assumed that the rate of loss of photons is proportional to the rate of bimolecular collisions between photons and the absorbing species [6]. If there is no molecular interaction ϵ is independent of c, but varies with wavelength, temperature and solvent.

If there is more than one absorbing species, the total absorbance is the sum of the absorbances of the individual components

i.e. $A = \sum \epsilon cd$,

provided that the components do not interact.

If the material is heterogeneous, and there are discrete phases with different refractive indices, reflection and scattering will complicate matters.

1.5 Molecular Excitation and its Consequences

1.5.1 Molecular Orbitals

Molecules are formed by the joining of atoms by chemical bonds. The simplest description of bond formation and the electronic structure of molecules is given by molecular orbital theory. Molecular orbitals are derived from the linear combination of atomic orbitals (LCAO). It is assumed that only valence shell

electrons are involved, and that inner shell electrons remain in their atomic orbitals. When a single bond is formed between two atoms, the atomic orbitals of the individual atoms combine to form a molecular orbital which embraces both nuclei and accommodates both electrons. When two atomic orbitals A and B, represented by wavefunctions Ψ_A and Ψ_B combine, two different molecular orbitals are possible, represented by wavefunctions Ψ_1 and Ψ_2, depending on the method of combination:

$\Psi_1 = \Psi_A + \Psi_B$ the atomic orbitals are combined <u>in phase</u>

$\Psi_2 = \Psi_A - \Psi_B$ the atomic orbitals are combined <u>out of phase</u>

For efficient combination the individual atomic orbitals must have similar symmetry, comparable energies and the ability to overlap. The in phase combination Ψ_1 gives a molecular orbital which has an energy less than that of the atomic orbitals, and this molecular orbital is known as a <u>bonding</u> orbital. Conversely the out of phase combination Ψ_2 gives a molecular orbital which has an energy higher than that of the atomic orbitals, and this is known as an <u>anti-bonding</u> orbital. Normally when a bond is formed the two electrons from the atomic orbitals occupy the bonding molecular orbital, and the internuclear distance adjusts to minimise the energy. The total number of molecular orbitals formed is equal to the number of atomic orbitals participating, but normally only the bonding orbitals are occupied, each by a pair of electrons having opposite spins.

When carbon atoms are incorporated in molecules it is normal for all the valence electrons to be used in bond formation. In the case of elements in Groups V, VI and VII of the periodic table, there are 'lone pairs' of electrons which do not participate. These non-bonding electrons are known as 'n' electrons.

If the atomic orbitals which combine to form a molecular orbital are symmetrical about the internuclear axis, the molecular orbital is known as a σ type if bonding, and σ^* if anti-bonding. Atomic orbitals of the s type always combine in this way, and p orbitals can do so. In the case of carbon, the hybridisation of s and p orbitals to give sp, sp^2 and sp^3 hybrids yields atomic orbitals which again combine to give σ and σ^* types. σ type molecular orbitals provide the main framework of organic molecules. The other types of molecular orbital which are important in organic chemistry are those produced by the lateral overlap of two parallel p orbitals, and these are called π and π^* molecular orbitals. The ways in which σ, σ^*, π and π^* orbitals are formed are shown in Figure 1.1. Note that when a molecular orbital is anti-bonding, there is a node between the nuclei. The node represents a point where the wavefunction is zero, and therefore where the probability of finding an electron is also zero. A single bond, for instance between two carbon atoms, is formed by a σ orbital, while a double bond is a combination of a σ orbital plus one π orbital, and a triple bond is a combination of a σ orbital and two π orbitals. Electrons in σ orbitals can be considered to be localised to

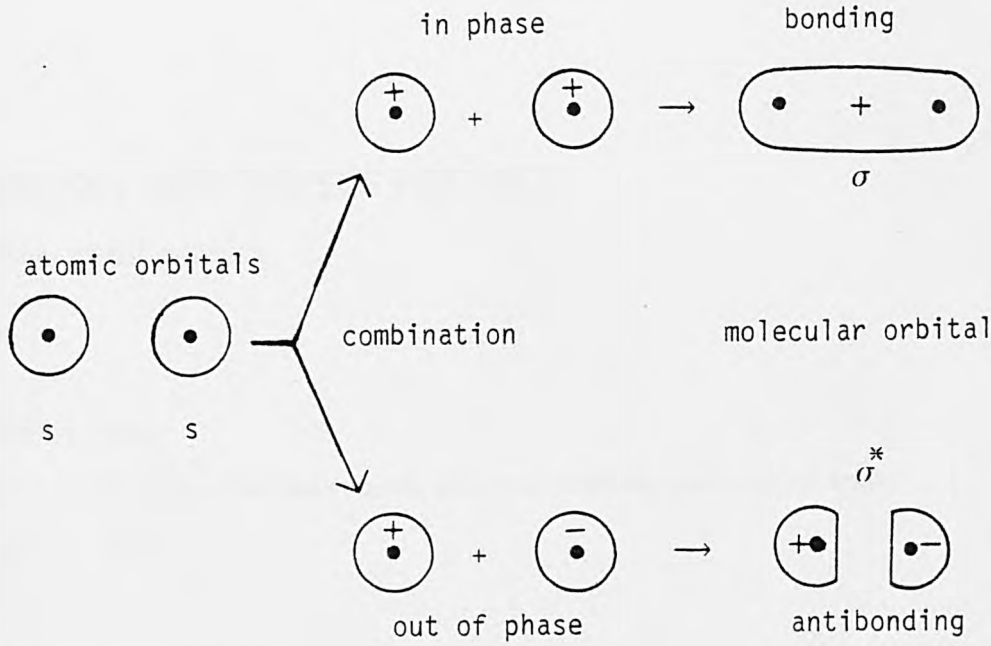


Figure 1.1a. Combination of s atomic orbitals to form σ (bonding) and σ^* (antibonding) molecular obitals

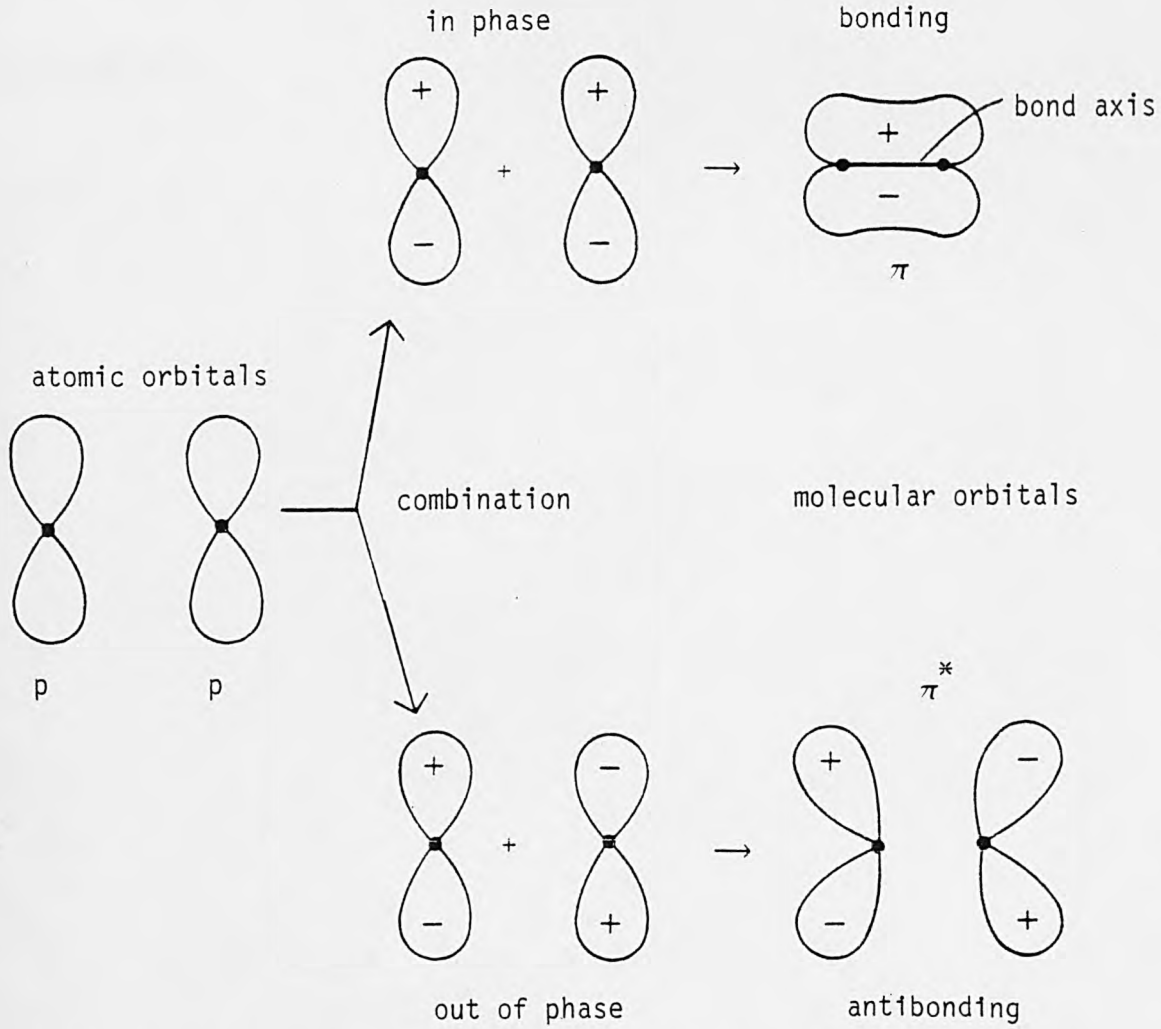


Figure 1.1b. Combination of p atomic orbitals to form π (bonding) and π^* (antibonding) molecular orbitals

the volume between and around the nuclei of the atoms forming the bond. When double bonds are conjugated, as in the benzene ring, the π orbitals merge, and therefore electrons in the π orbitals are delocalised and can move freely around the conjugated π system.

1.5.2 Electronic Excitation in Molecules

Electronic excitation takes place when an electron in a bonding molecular orbital, or an n atomic orbital, is raised into an anti-bonding orbital. In most organic molecules σ orbitals have the lowest energy, with the energies of π, π^* and σ^* orbitals being progressively higher. The energy of π orbitals is strongly influenced by the degree of conjugation. The energy of n orbitals may lie above or below that of the π orbitals. The most common transitions are $n \longrightarrow \pi^*$, $\pi \longrightarrow \pi^*$, $n \longrightarrow \sigma^*$ and $\sigma \longrightarrow \sigma^*$ and are depicted in Figure 1.2. Depending on which transition takes place, a variety of molecular excited states are possible. An excited state derived from an $n \longrightarrow \pi^*$ transition is known as an n,π^* state, from a $\pi \longrightarrow \pi^*$ transition as a π,π^* state, and so on. The excited molecule contains two unpaired electrons. If the spins are opposed the state has a multiplicity of one, and is a <u>singlet</u>, while if the spins are parallel the state has a multiplicity of three and is a <u>triplet</u>. In almost all organic molecules the ground state is a singlet, and therefore the various singlet states can be designated, in order of increasing energy, S_0, S_1, S_2 etc., while the equivalent triplet states are T_1, T_2 etc. Triplet states have lower energies

than the equivalent singlet states.

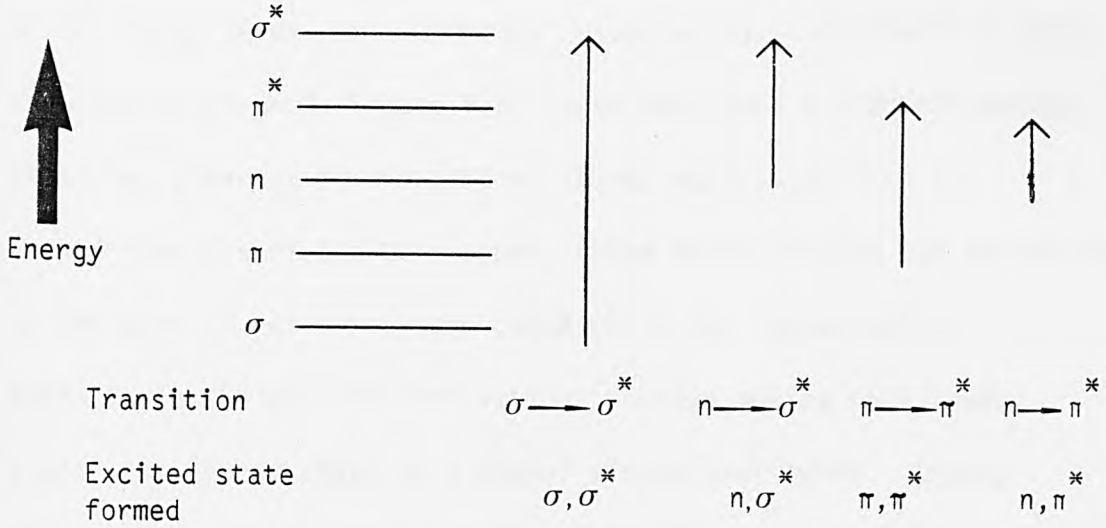


FIGURE 1.2

The electronic excitation processes in organic molecules (where n = non-bonding orbital; σ and π = bonding orbitals; σ^* and π^* = anti-bonding orbitals).

Electronic energy is not the only type of quantised energy possessed by a molecule. Unlike an atom, molecules can have vibrational and rotational energy, and it is possible that when a transition takes place there is a change not only in the electronic state but also in the vibrational and rotational states. This explains why the absorption spectra of molecules consist of bands rather than lines.

1.5.3. The Jablonski Diagram

Transitions between energy states in molecules may be shown on an energy level, or 'Jablonski' diagram [7], a schematic example of which is shown in Figure 1.3. Note that each electronic energy state has a series of vibrational states associated with it. Transitions are of two main types, those which involve the absorption or emission of radiation, and those which are radiationless. Radiationless electronic transitions involve moving to a lower electronic energy state at a higher vibrational level. Excess vibrational energy can be readily dissipated to other molecules. A radiationless transition between states of the same multiplicity is known as 'internal conversion' (IC), while when a change in multiplicity occurs the radiationless transition is known as 'intersystem crossing' (ISC). ISC is favoured if the singlet state is n, π^*, as the energy gap between singlet and triplet is then small [8].

As with atomic orbitals there are selection rules which govern the possible transitions between excited states. Perhaps the most significant selection rule is that transitions which involve the absorption or emission of radiation can only take place between states of the same multiplicity. This implies that for almost all organic molecules the immediate result of absorption is the formation of an excited state. If this state is S_2, S_3 etc., it will in all probability decay rapidly to either the lowest excited singlet state (S_1) or the lowest triplet state (T_1) by

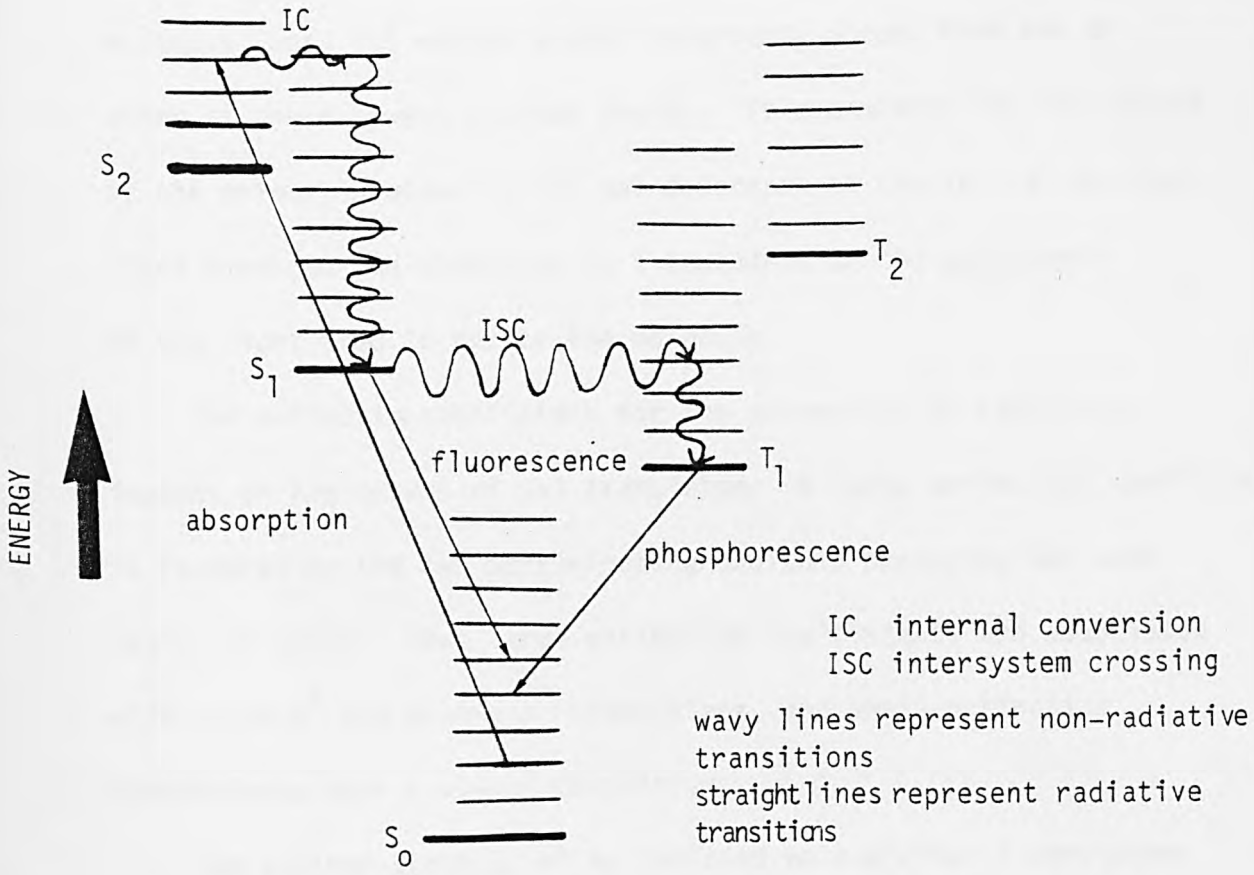


Figure 1.3. The Jablonski diagram. This shows the singlet and triplet levels, each of which has many vibrational levels associated with it, and the possible transitions.

Process	First Order Rate Constant (s^{-1})		
	General Range		Benzophenone (in benzene)
Absorption	$10^{15} - 10^{16}$	very fast	
$S_2 \overset{IC}{\rightsquigarrow} S_1$	$10^{11} - 10^{14}$	very fast	10^{12}
$S_1 \overset{IC}{\rightsquigarrow} S_0$	$10^{6} - 10^{12}$	in competition	
$S_1 \xrightarrow{\text{fluorescence}} S_0$	$10^{6} - 10^{9}$	in competition	10^{6}
$S_1 \overset{ISC}{\rightsquigarrow} T_1$	$10^{4} - 10^{12}$	in competition	10^{10}
$T_1 \overset{ISC}{\rightsquigarrow} S_0$	$10^{-1} - 10^{5}$	slow	10^{5}
$T_1 \xrightarrow{\text{phosphorescence}} S_0$	$10^{-2} - 10^{4}$	slow	10^{2}
vibrational relaxation	10^{12}	very fast	10^{12}

internal conversion and/or intersystem crossing [9]. Therefore, the primary photochemical or photophysical process by which the molecule loses its energy almost invariably occurs from one or other of these lowest excited states. This explains why the nature of the primary process(es) is not dependent on the initial excited state produced and therefore is independent of the wavelength of the light used to excite the molecule.

The extinction coefficient for the absorption of radiation depends on the nature of the transition. A large extinction coefficient is favoured by the two participating orbitals occupying the same region of space. Thus large extinction coefficients are associated with $\pi \longrightarrow \pi^*$ and $\sigma \longrightarrow \sigma^*$ transitions, and small extinction coefficients with $n \longrightarrow \pi^*$ transitions.

The excited state S_1 of an isolated molecule has a very short lifetime (only true for high absorption extinction coefficient transitions, i.e., $\pi \longrightarrow \pi^*$ and $\sigma \longrightarrow \sigma^*$ transitions) because the process $S_1 \longrightarrow S_0 + h\nu$ is facile. This process is fluorescence. A chemical reaction of S_1, or indeed intersystem crossing, needs to be very rapid to compete with this radiative decay mechanism. The process $T_1 \longrightarrow S_0 + h\nu$, on the other hand, is forbidden, or to be more precise has a low probability. The lowest triplet state therefore has a relatively long lifetime, because the radiative decay (phosphorescence) is so slow. Therefore intersystem crossing to a triplet state favours a non-radiative decay mechanism, and most technologically significant photochemical processes are derived from the chemical reactivity of the triplet state.

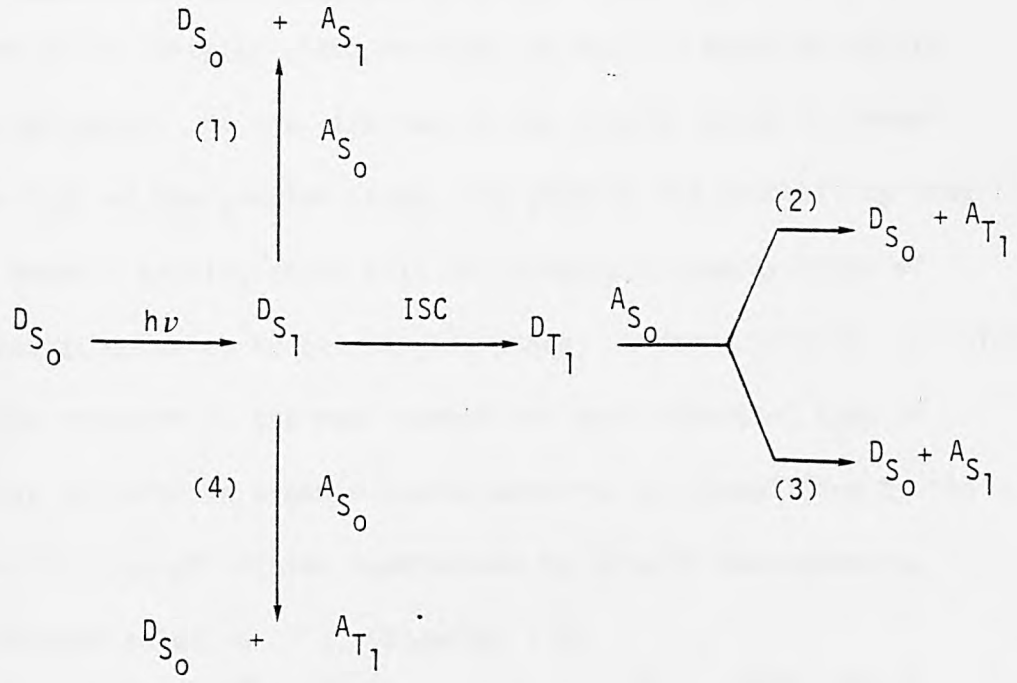
1.6 Sensitization and Quenching by Energy Transfer

1.6.1. Intermolecular Energy Transfer

An excited molecule in either the singlet or triplet state may be deactivated by transfer of energy to another molecule having lower lying singlet or triplet energy levels, the excited molecule returning to the ground state whilst the other molecule is elevated to a higher energy state. The excited molecule can be referred to as the donor (D), the other molecule being the acceptor (A) and the energy transfer process can be summarised by Equation 1.2.

$$D^* + A \longrightarrow D_{S_o} + A^* \qquad (1.2)$$

The overall process can be considered as either the donor molecule sensitizing the formation of an excited state of the acceptor, or as the acceptor molecule quenching the excited state of the donor. Thus, the donor molecule can be regarded as a photosensitizer and the acceptor molecule can be regarded as a quencher. The acceptor molecule can quench either the singlet or triplet state of the donor and result in the sensitization of the acceptor molecule to either its singlet or triplet state, as outlined in Scheme 1.1.



Scheme 1.1

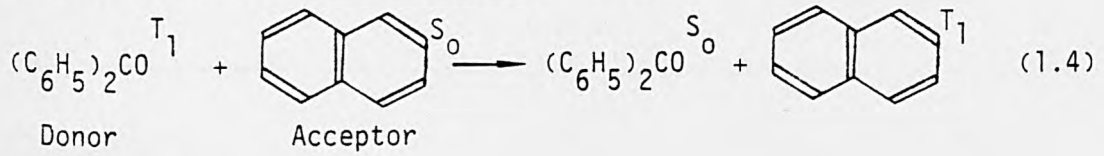
Scheme 1.1 shows four important types of energy transfer processes as follows:-

(1) Singlet - Singlet energy transfer : This takes place over relatively both short and long distances and gives rise to an excited singlet state of the acceptor as a result of energy transfer from the donor's excited singlet state. Thus, if there is sufficient overlap of the emission spectrum of the donor and the absorption spectrum of the acceptor, the donor fluorescence is quenched and acceptor fluorescence is sensitized. An example of singlet - singlet energy transfer is the sensitization of biacetyl fluorescence by energy transfer from singlet state acetone, in cyclohexane [10] solution, (Equation 1.3).

$$(CH_3)_2CO^{S_1} + CH_3COCOCH_3^{S_o} \longrightarrow (CH_3)_2CO^{S_o} + CH_3COCOCH_3^{S_1} \quad (1.3)$$

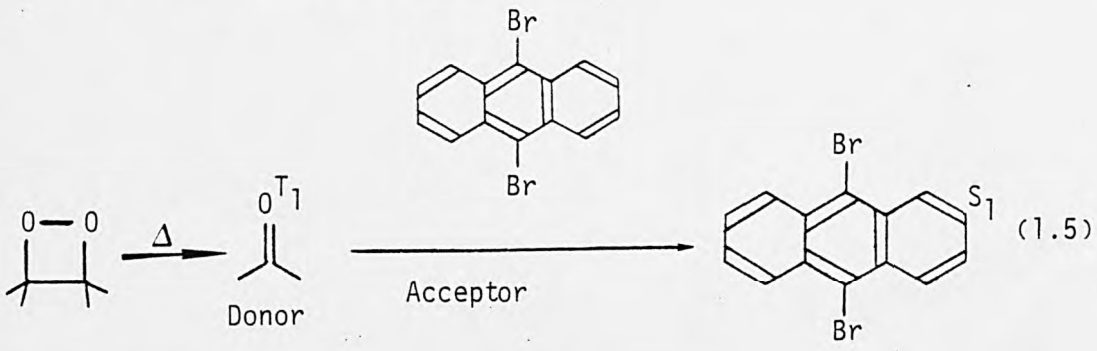
Donor Acceptor

(2) Triplet - Triplet energy transfer : In this case an excited donor in its triplet state produces an excited acceptor in its triplet state. As the lifetime of the triplet state is longer than that of the singlet state, the greater the probability that the donor's triplet state will participate in energy transfer processes compared to the singlet state. Indeed, triplet - triplet energy transfer is the most common and most important type of energy transfer in organic photochemistry, as exemplified by the sensitization of triplet naphthalene by triplet benzophenone, in benzene solution [11], (Equation 1.4).



(1.4)

(3) Triplet - Singlet energy transfer : In solution, energy transfer from a triplet donor to an acceptor to give the excited singlet state of the acceptor is relatively rare, however triplet - singlet energy transfer from thermally produced triplet acetone to appropriate acceptors, such as 9, 10 - dibromoanthracene, has been observed [12]. In this case, which utilises a chemiluminescence technique, the thermolysis of tetramethyl - 1, 2 - dioxetane yields triplet acetone which undergoes triplet - singlet energy transfer to 9. 10 - dibromoanthracene to give the singlet state of the anthracene derivative [12], (Equation 1.5).

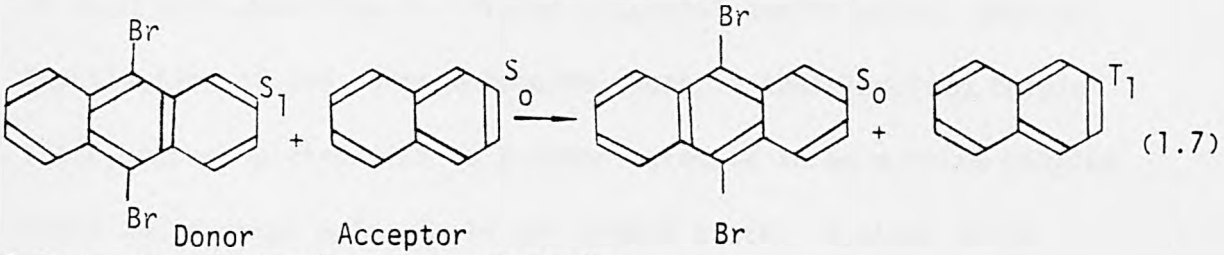


(1.5)

Oxygen is an efficient quencher of the triplet excited states of organic molecules and often gives rise to the formation of oxygen molecules in the excited singlet state, (Equation 1.6).

$$D^{*}_{T_1} + {}^{3}O_2 \longrightarrow D^{S_o} + {}^{1}O_2 \qquad (1.6)$$

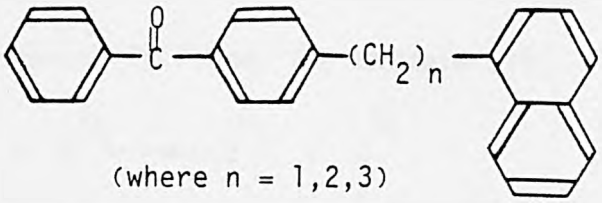
(4) Singlet - Triplet energy transfer : This is the rarest of the four energy transfer processes and involves an excited singlet donor and ground state singlet acceptor to produce an excited triplet acceptor. For example, the fluorescence of 9, 10 - dibromoanthracene is quenched by naphthalene, in toluene solution, to give triplet naphthalene [13], (Equation 1.7).



(1.7)

1.6.2. Intramolecular Energy Transfer

Energy transfer can also take place between different chromophores within the same molecule, and is termed intramolecular energy transfer. For example, both singlet and triplet energy transfer have been studied in the compound below [14, 15].



(where n = 1,2,3)

Using an excitation wavelength of 313 nm the naphthalene moiety is promoted to its singlet state. Singlet energy transfer to the benzophenone moiety then proceeds with high efficiency (n = 1, 98%; n = 2, 80%; n=3, 94%). Intersystem crossing ensues,

with unit efficiency, forming the benzophenone triplet which then undergoes triplet energy transfer to naphthalene (n = 1,2,3, 100%) to yield the naphthalene triplet which, if in a low temperature glass, undergoes phosphorescence. Using an excitation wavelength of 366 nm starts the above process at the benzophenone singlet state.

1.7 Triplet - Triplet Annihilation

If the concentration of the donor molecule is high, then a bimolecular self-quenching process can result. An example of such self-quenching is triplet - triplet annihilation, whereby deactivation of two donor molecules, both in their excited triplet state, occurs giving rise to a donor molecule in an excited singlet state and a donor molecule in the ground state. Systems which are subject to quenching <u>via</u> triplet - triplet annihilation often show delayed fluorescence which emanates from the donor molecule that is generated in its excited singlet state, as shown in Scheme 1.2. Triplet - triplet annihilation has been observed for anthracene and naphthalene in n - hexane solutions, biacetyl in benzene solutions and benzophenone in freon, for example [16].

$$D^{T_1} + D^{T_1} \longrightarrow D^{S_0} + D^{S_1}$$

$$D^{S_1} \longrightarrow D^{S_0} + h\nu_{DF}$$

(where $h\nu_{DF}$ = delayed fluorescence)

Scheme 1.2

1.8. Concentration Quenching

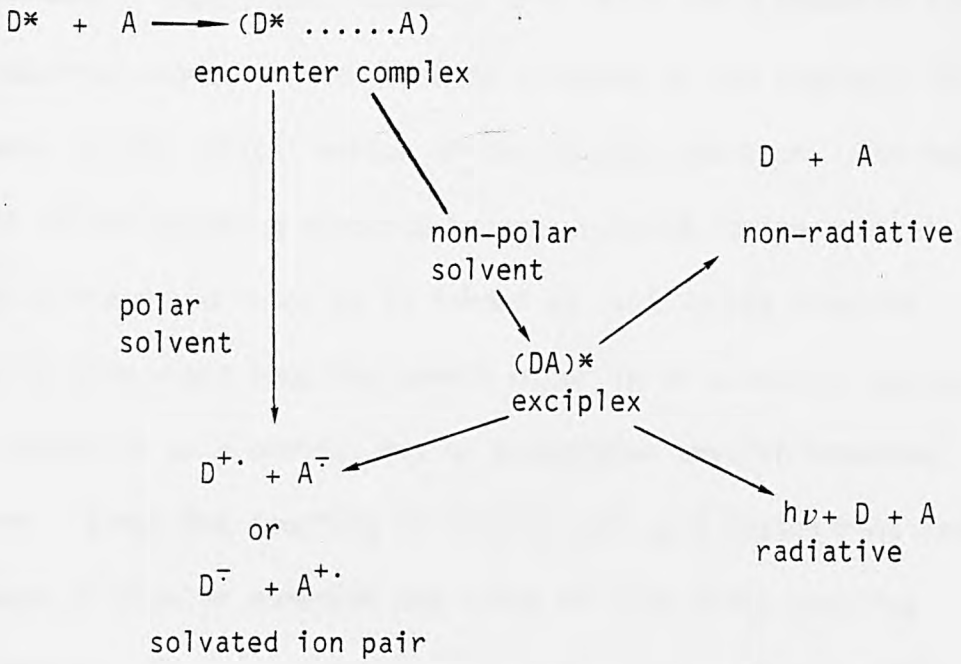
Concentration quenching can also lead to deactivation of the singlet state characterised by a decrease in fluorescence intensity with increasing amounts of the compound under investigation. The fluorescence quenching which is observed in such cases results from interaction of a molecule in its excited singlet state with a molecule of the same species in its ground state to produce an excited dimer or excimer, (Equation 1.8). For example, pyrene fluorescence is progressively quenched as the concentration of pyrene in the solution is increased and this is accompanied by the growth of a broad structureless emission band, corresponding to the pyrene excimer, at longer wavelength than the pyrene fluorescence [17].

$$D^{S_1} + D^{S_0} \longrightarrow (DD)^{*} \longrightarrow D^{S_0} + D^{S_0} + h\nu \qquad (1.8)$$

1.9. Quenching Via Electron Transfer

That quenching of excited states of organic molecules can occur <u>via</u> electron transfer processes is well documented [18], however doubt exists as to the exact nature of the intermediate species involved, which could be an exciplex or an encounter complex. Complete electron transfer occurs in polar solvents leading to the formation of solvent separated radical ion pairs. In non-polar solvents incomplete electron transfer may occur to form an intermolecular charge - transfer complex in an excited state (termed an exciplex), which may relax by either a radiative or

non-radiative process. The parameters for the above mechanism of electron transfer quenching are summarised in Scheme 1.3.



Scheme 1.3

The first example of exciplex emission was the finding of new structureless fluorescent bands in the spectrum of perylene quenched by aromatic amines in non-polar solvents [19]. Radical ion formation has been observed in the reactions of various singlet state aromatic hydrocarbons with amines in aprotic polar solvents which give rise to hydrocarbon radical anions and amine radical cations [20].

1.10. Spin-orbit Coupling and Heavy Atom Effect

For organic molecules, spin-orbit interactions usually provide the major mechanism for intersystem crossing. Since the electron is charged and 'spinning' it is expected to have spin angular

momentum as well as magnetic moment. The electron inverts its spin, i.e. changes the direction of its magnetic moment in a $S \longrightarrow T$ transition. Clearly this calls for a magnetic interaction. The required magnetic interaction is provided by the magnetic field produced by the orbital motion of the charged electron. The magnetic moment of the spinning electron becomes coupled to the orbital magnetic field and hence it is termed as 'spin-orbit coupling'.

The spin-orbit coupling cannot occur in an s orbital because an electron in an s orbital has an associated angular momentum of zero. Since the coupling of orbital and spin motion requires exchange of angular momentum and hence no spin-orbit coupling is expected. Figure 1.4 shows an over simplified planar model of an electron in a p orbital. The magnetic torque (force) is generated by an electron travelling in an orbital which has a shape of "figure 8" about the nucleus, and a spin-flip can occur with a simultaneous orbital momentum change.

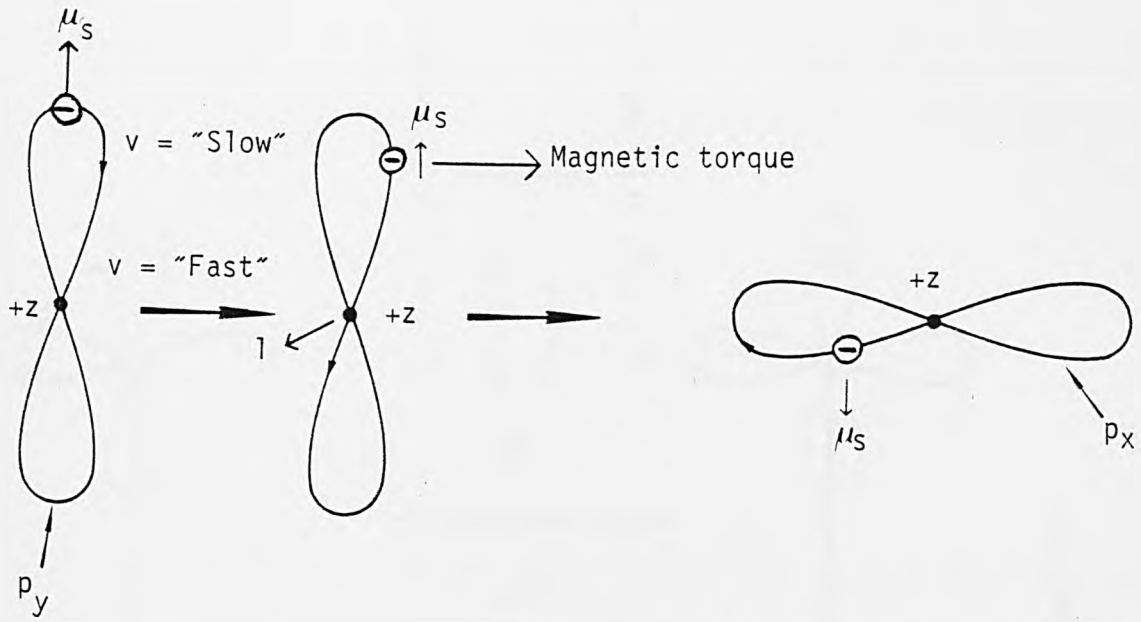
The spin-orbit coupling takes place only if (i) the orbital transition involved possesses the character of a $p_x \longrightarrow p_y$ orbital jump, for example $^1n\pi^* \longrightarrow {}^3n\pi^*$ (Fig. 1.5), and (ii) the orbital transition is localized on a single atom.

The triplet state produced under spin-orbit coupling can be written in the following form:

$$\Psi_T = \Psi_T^o + \sum_K \frac{\langle \Psi_{S_K}^o \mid H_{SO} \mid \Psi_T^o \rangle}{(E_T - E_{S_K})} \Psi_{S_K}^o \quad (m)$$

where S_K is the Kth singlet state and E_T and E_S are the energies

Spin-orbital description



Vector model description

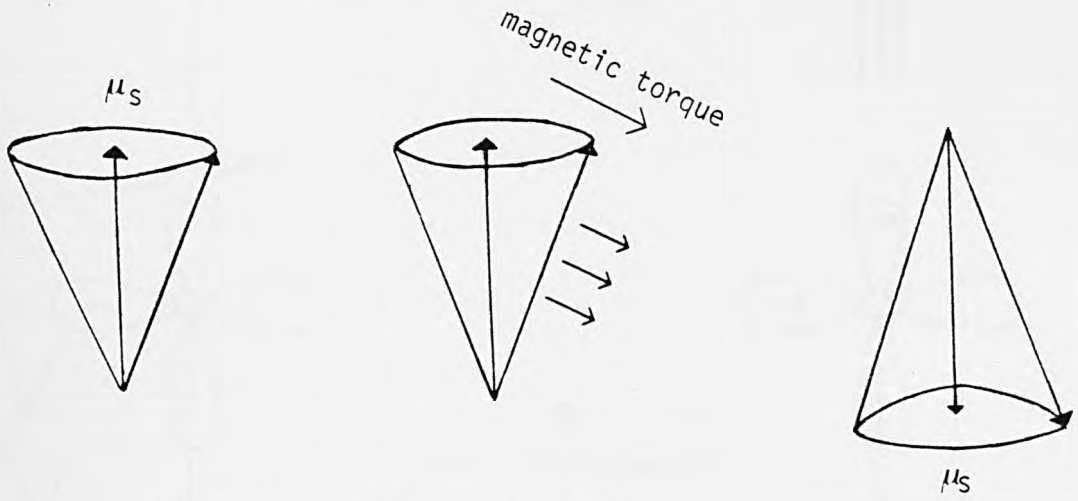


Figure 1.4. Spin-orbit coupling.
μ_s = electron-spin magnetic moment
l = orbital angular momentum

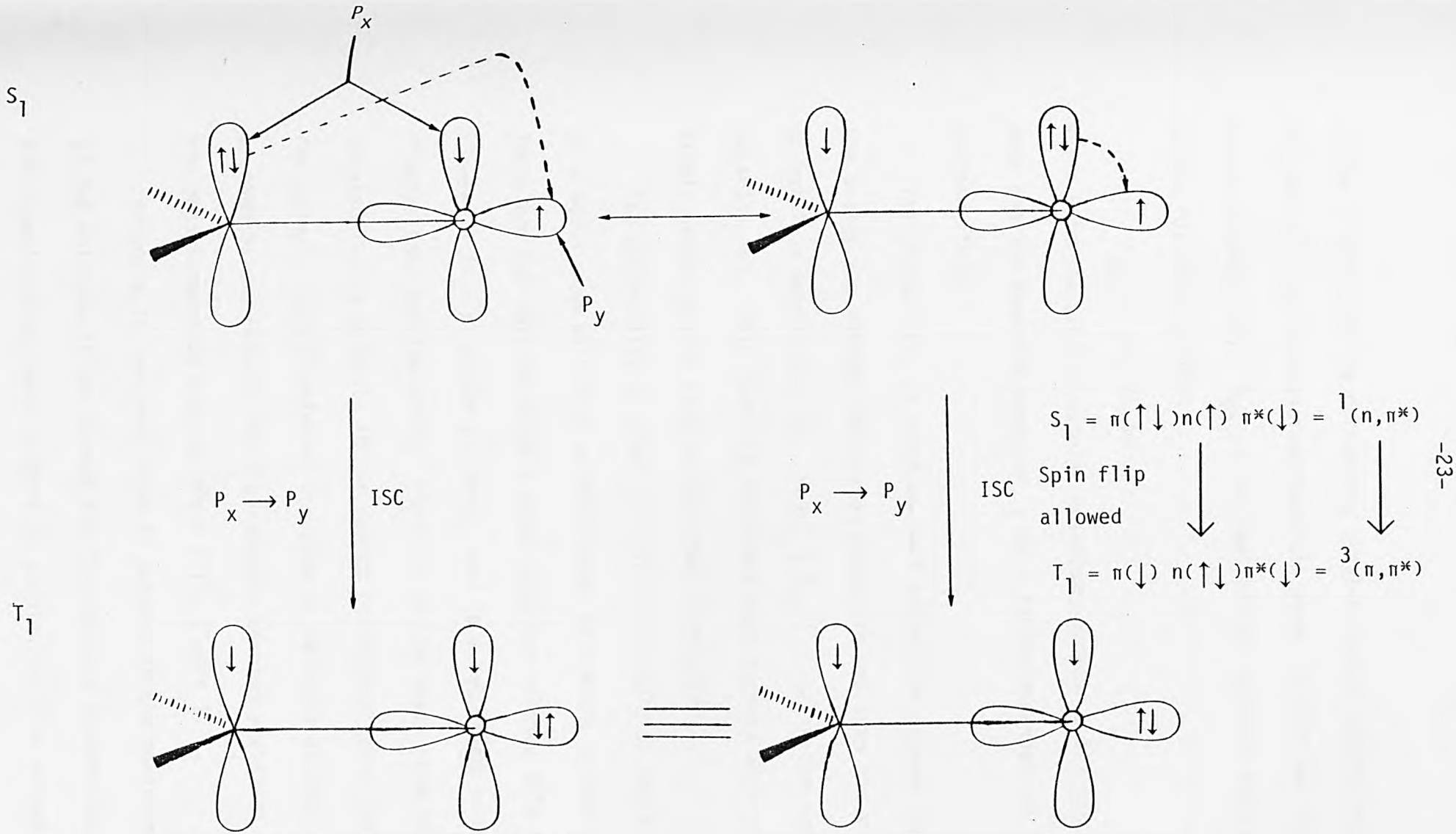


Figure 1.5. Quantitative orbital description for spin-orbit coupling: ${}^1(n,\pi^*) \longrightarrow {}^3(\pi,\pi^*)$ ISC in C=O group.

of the triplet and the perturbing singlet states respectively. Ψ_S^o and Ψ_T^o are the wavefunctions of 'pure' singlet and triplet states respectively. H_{SO} is the Hamiltonian operator and is equivalent to the following product:

$$H_{SO} = K\zeta \ (L.S)$$

where L is the orbital angular momentum operator and S is the spin angular momentum operator, ζ is a factor dependent on the nuclear field.

The probability of the S⟶T transition depends upon the energy gap between the states concerned and upon the size of matrix elements such as $<\Psi_{S_K}^o \mid H_{SO} \mid \Psi_T^o>$ in the above equation (m). This quantity increases very rapidly with increasing atomic number giving rise to the heavy atom effects.

The probability of electron spin flips would be increased if a heavy atom with high atomic number is present in the system. There are two ways by which a heavy atom can enhance this transition (S⟶T) in a molecule and hence lead to a reduction in the fluorescence quantum yield. Firstly, if the heavy atom is incorporated into a molecule it can quench the fluorescence intramolecularly. For example, substitution of hydrogen by halogens at the 1-position of naphthalene reduces the fluorescence quantum yield and increases the phosphorescence quantum yield [21], (Table 1.1).

Secondly, if the heavy atom is present in the environment of the molecule it can quench the fluorescence intermolecularly. For example xenon, when present in a solution of an aromatic

hydrocarbon, such as anthracene or naphthalene, has been shown to quench the fluorescence and enhance the rate of triplet formation of the molecule [22]. Similar intermolecular quenching effects are observed from the quantum yields of fluorescence and phosphorescence of aromatic hydrocarbons in different halogenated solvents as exemplified by the study of naphthalene in halogenated propanes [23], (Table 1.2).

TABLE 1.1 [21]

Compound	Quantum Yields[(a)]	
	Fluorescence	Phosphorescence
Naphthalene	0.55	0.055
1-Chloronaphthalene	0.058	0.3
1-Bromonaphthalene	0.0016	0.27
1-Iodonaphthalene	<0.0005	0.38

(a) Data for rigid solution at 77K.

TABLE 1.2 [23]

Solvent	Naphthalene Quantum Yields[(a)]	
	Fluorescence	Phosphorescence
Ethanol/Methanol	0.55	0.055
1-Chloropropane	0.44	0.08
1-Bromopropane	0.13	0.24
1-Iodopropane	0.026	0.35

(a) Data for rigid solution at 77K.

1.11. Quantum Yield

The efficiency of a primary photophysical or photochemical process is measured by the quantum yield for that process. If molecule A absorbs a photon to give an excited state A^*, which can then lose its energy by several different processes, the quantum yield for any one of these processes is defined as the number of molecules undergoing that process divided by the number of photons absorbed. This is the definition of primary quantum yield and as a consequence of the Stark-Einstein Law, it has a maximum value of unity. However, primary quantum yields are often difficult to measure. A product quantum yield Φ for a product P which is formed from a photoreaction of an initially excited molecule A may be expressed as

$$\Phi = \frac{\text{number of molecules of P formed}}{\text{number of quanta absorbed by A}}$$

$$= \frac{\text{rate of formation of P}}{\text{intensity of absorbed radiation}}$$

Product quantum yields are much easier to measure. The number of quanta absorbed can be determined by an instrument called an actinometer, which is actually a standard photochemical system whose quantum yield is known. An example of the information which can be learned from quantum yields is the following. If the quantum yield of a product is finite and invariant with changes in experimental conditions, it is likely that the product is formed in a primary rate-determining process. Another example : in some reactions, the product quantum yields are found to be

well over 1 (perhaps as high as 1000). Such a finding indicates a chain reaction.

1.12. Utility of Quenching Studies to Investigate Photochemical Reactions

Photosensitisation and quenching are important photochemical techniques which can be used to identify the multiplicity of the excited state, or states, from which a given photochemical reaction occurs. The kinetics of photochemical quenching are described by the Stern-Volmer [24] equation:-

$$\frac{\Phi_o}{\Phi_q} = 1 + Kq.\tau.[Q] \qquad (1.9)$$

where Φ_o = the quantum yield in the absence of quencher,

Φ_q = the quantum yield in the presence of quencher at a concentration [Q],

Kq = rate constant for quenching

and τ = lifetime of the excited state in the absence of quencher.

This equation can be applied to the quenching of phenomena such as fluorescence, phosphorescence and product formation. A plot of Φ_o/Φ_q versus [Q], under ideal conditions, will result in a straight line with an intercept at 1 and a gradient equal to $Kq.\tau$. If either Kq or τ is known the other can be calculated from a Stern-Volmer plot. Curvature of the plots will result if excited states of more than one multiplicity are being quenched. The greater the difference between the triplet energies of the

sensitiser and quencher molecules the more efficient will be the quenching process. By using quenchers with a wide range of triplet energies estimates for the value of the triplet energy of a sensitiser molecule, for which such data is not available, can be determined. The incident radiation should selectively excite the sensitiser molecule. If reaction rates are being studied, kinetic analysis should be confined to the first 10% of reaction in order to minimise any interference with the quenching process by the reaction products. If a substance quenches both the fluorescence and a photochemical reaction of a substrate it can be concluded that the reaction occurs, at least in part, via the excited singlet state. Some compounds, such as oxygen and naphthalene, are efficient quenchers of triplet states so if the presence of either oxygen or naphthalene retards the rate of a photochemical reaction the mechanism of that reaction must involve the excited triplet state.

Photosensitisation has proved useful for creating excited states which may be difficult to obtain by direct irradiation. For example, it is difficult to produce singlet oxygen by direct absorption of light (in the microwave region of the electromagnetic spectrum) but via energy transfer from suitable donors, e.g. dyes, it is facile. Photosensitisation is also a convenient technique for the creation of triplet excited states of highly fluorescent compounds which have low quantum yields of triplet production.

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CHAPTER 2

THE STRUCTURE, COMPOSITION AND YELLOWING OF WOOL

2.1. STRUCTURE OF WOOL

Wool is the animal fibre of outstanding importance. It has special features which distinguish it from other hairs, but which render it supremely valuable as a textile fibre. The differences are of a physical nature, and the basic tissues and chemical structure of all animal hairs, including wool, are similar. Wool fibres have a somewhat swollen root at the base and a tip towards which they taper, ultimately becoming a fine point.

The wool fibre is complex in structure and composed essentially of three tissues, the cuticle, the cortex and the medulla. The cortical cells make up the bulk of the fibre and the cuticle cells encase the cortex. The cuticle cells overlap each other like the tiles on a roof, the exposed scale edges pointing towards the fibre tip (see Figure 2.1). They are responsible for the difference in the coefficient of friction of the wool fibre when measured in the with-scale and against-scale directions.

The cuticle of fine wool fibres is normally only one cell thick (except where cells overlap), whereas the cuticle of human hair and other coarse fibres consists of a series of overlapping cells. Cuticle cells of Merino wool (this is most frequently used for chemical study) are generally rectangular, with dimensions of about 20 x 30 x 0.5μm [2,3]; they represent about 10% by mass of the whole fibre [4]. Microscopic examination of fibre cross-sections shows that each cuticle cell is comprised of an enzyme-resistant exocuticle, and an enzyme-digestible endocuticle [5,6],

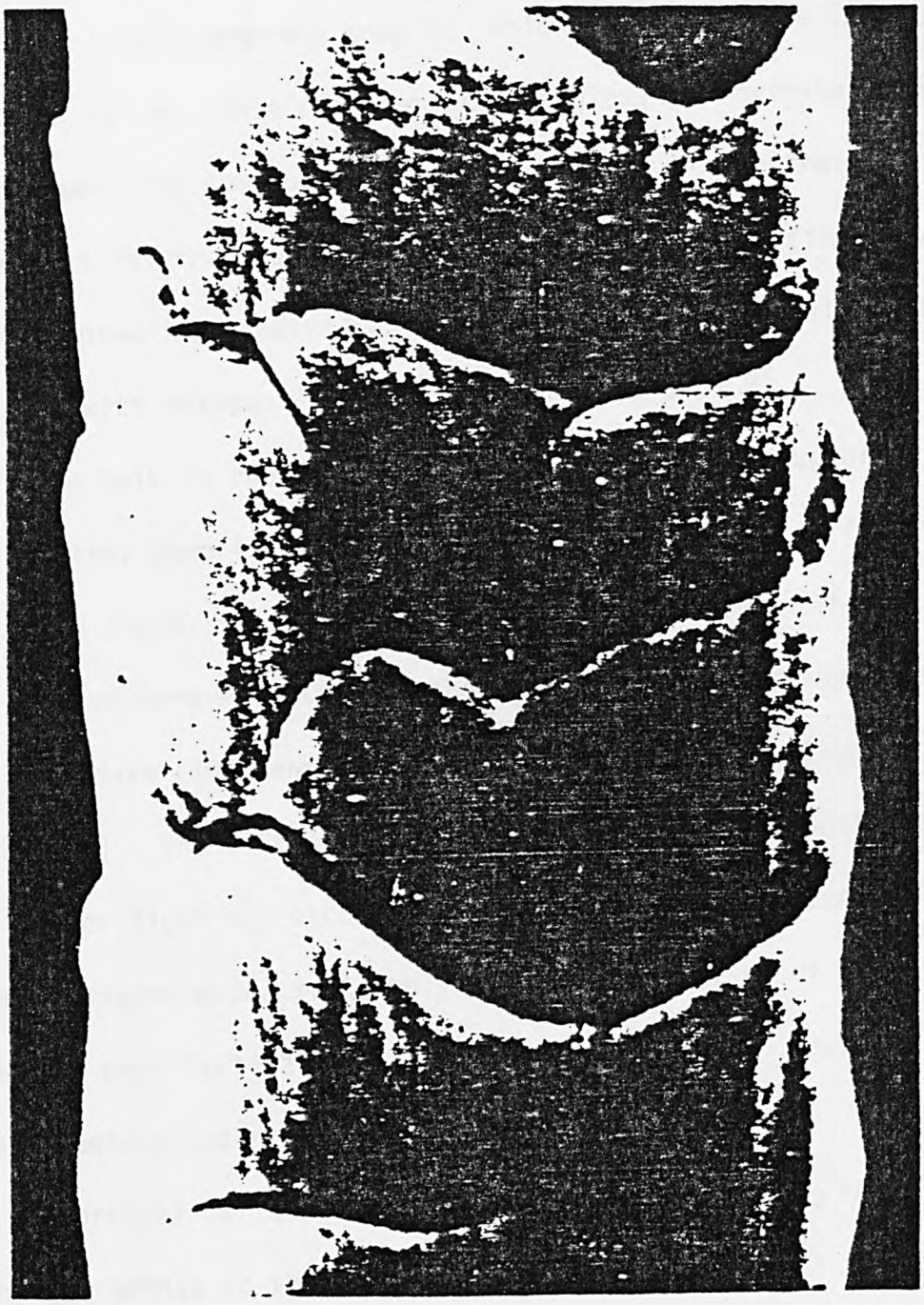


Figure 2.1. Scanning electron micrograph of a Merino wool fibre (from Anderson et al [1]).

surrounded by a thin hydrophobic membrane, the epicuticle [2].

Cuticle cells are separated from the underlying cortical cells by a cell membrane complex, which also surrounds individual cortical cells. This cell membrane complex, approximately 25nm thick, performs the function of cementing cells together and is sometimes referred to as intercellular cement. The fibre may be disrupted into individual cells by attacking the cell membrane complex with enzymes [7], or with formic acid [4,8].

The bulk of the wool fibre (about 90%) is made up of cortical cells; they comprise the cortex, which is generally differentiated into two parts, the orthocortex and the paracortex. The orthocortex is stained more heavily than the paracortex by basic dyes [9], by acid dyes [10], and by salts of lead, mercury, silver and gold [11 - 13]. The differential staining permits easy identification by either light microscopy (detects dyes only) or electron microscopy (detects metal salts only). The distribution of the ortho- and the para-cortical cells is bilateral, and each forms an approximate hemi-cylinder, as illustrated in Fig. 2.2.

Cortical cells are spindle-shaped, approximately 95μm long and 5μm across at the maximum width. They consist of highly organised rod-like microfibrils, sometimes called filaments (approximately 10μm x 7.2nm diameter), packed in an amorphous matrix. The microfibrils occur in bundles, called macrofibrils, which are more tightly packed in orthocortical than in paracortical cells. Consequently the latter contain a higher proportion of

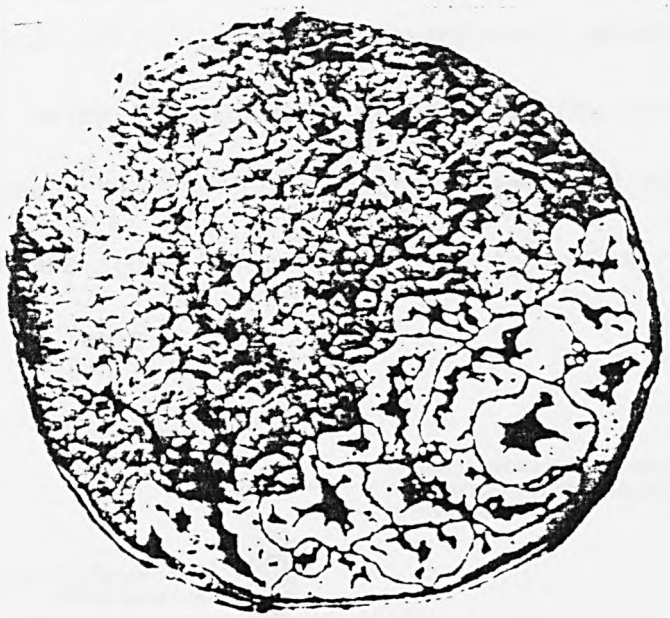


Figure 2.2. Electron micrograph of a cross-section of a Merino wool fibre showing the darkly stained orthocortex and the lightly stained paracortex (from Fraser et al.[14]).

matrix (see Figure 2.3).

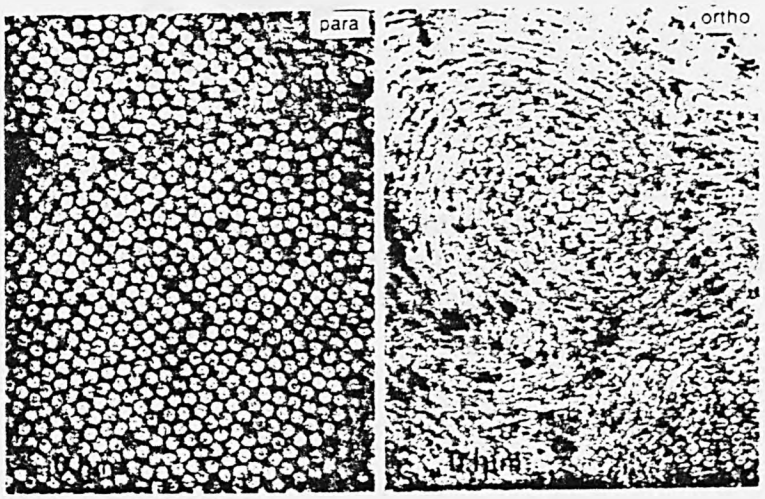


Figure 2.3. Selected areas from a cross-section of a wool fibre at high magnification. Note how the lightly stained microfibrils are more closely packed in the matrix in orthocortical cells than in paracortical cells (from Fraser et al.[14]).

Cortical cells also contain cytoplasmic debris (nuclear remnants), which are much more abundant in the paracortex than in the orthocortex. All of the above-mentioned features of the wool fibre are shown schematically in Figure 2.4. Their dimensions are shown in Table 2.1.

Figure 2.4. Sketch of a broken section of a fine wool fibre showing the major cellular components and the detailed structure within them.

Table 2.1. Approximate dimensions of the components of a Merino wool fibre (μm).

Component	length	width	diameter	thickness
fibre	100,000	-	20	-
cuticle cell	30	20	-	0.5
cell membrane complex	-	-	-	0.025
cortical cell	95	-	5	-
macrofibril	10	-	0.3	-
microfibril	1	-	0.007	-

2.1.1. Fine structure

It will be seen from Fig. 2.4 that the cortical cells are composed of macrofibrils, each of which contains a number of microfibrils lying parallel with each other. The microfibril is of the order of 7.5nm in diameter and electron microscopy has shown that there may be protofibrils with still smaller diameters. There is reason to believe that the microfibril consists of a sheath of nine protofibrils surrounding two situated in the centre.

The protofibrils themselves appear to be made up of three alpha helices which are the fundamental structure of the keratin molecule. Cortical fibrils, however, do not consist entirely of alpha helices arranged in an orderly manner parallel with the longitudinal axis of the fibre, but something like two-thirds is amorphous, with the keratin molecules orientated in a random manner, constituting a zone known as the matrix. In both regions

the molecules are bound together with disulphide linkages, and they are more numerous in the matrix. Such a structure is illustrated diagrammatically in Fig. 2.5.

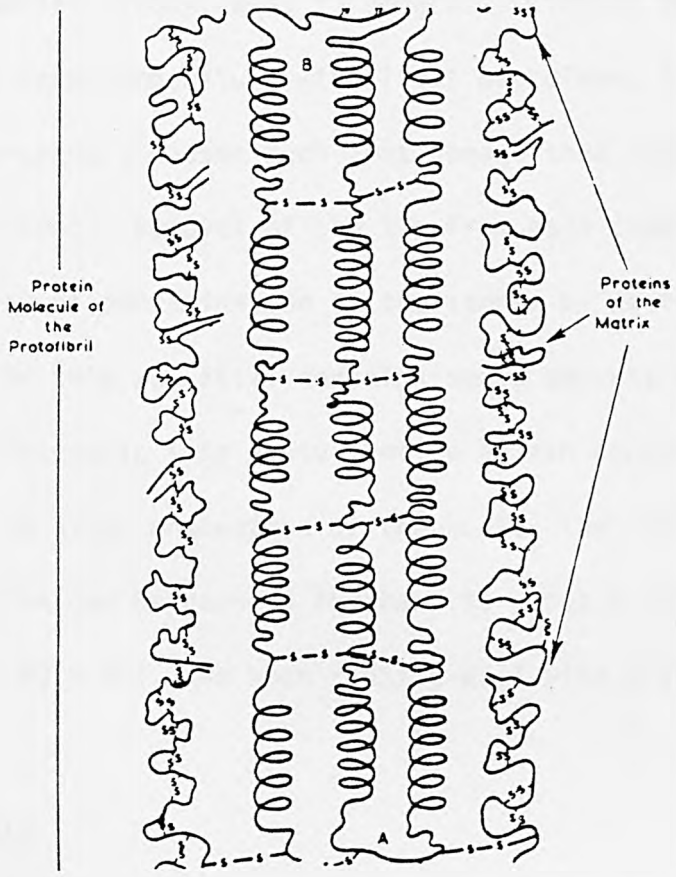


Figure 2.5. Protofibril matrix model for wool [15].

2.2 CHEMICAL COMPOSITION OF WOOL

Raw wool, when first shorn from the sheep's back, often contains less than 50% of clean fibre due to contamination by wool wax, suint, sand, dirt and vegetable matter. Wool was is the secretion of the sebaceous glands of the sheep and suint is the dried residue of the secretion of the subiferous glands. Together, wool wax and suint may comprise 20 - 50% of the weight

of the raw wool. They are removed from the wool along with sand and dirt by scouring, an industrial operation in which the wool is agitated with hot aqueous soap or detergent solutions.

For scientific study, wool is generally cleaned by successive extraction at room temperature with light petroleum, ethanol and water. The procedure causes much less damage than industrial scouring processes. Removal of the tip from each staple excludes the possibility of contamination of the sample by weathered wool. Wool cleaned in this way still contains small amounts of lipid material and inorganic ions equivalent to an ash content of approximately 0.5 - 1% after combustion of the wool. The residue left after combustion can be reduced further (to about 0.1%) by soaking the wool in 0.001M HCl, and then rinsing well with distilled water.

2.2.1. Keratin

When all impurities have been removed, keratin remains which belongs to the group of compounds classed as proteins which are the ultimate stage of complexity of organic matter before it becomes living tissue. A brief description of the chemistry of proteins is desirable in order to understand the properties of wool fibre.

Proteins are giant molecules built up by the condensation of a number of comparatively simple alpha amino acids, in which the amino nitrogen is attached to the $-CH_2-$ group adjacent to the carboxyl radical, e.g. $CH_3 \cdot CH\ (NH_2) \cdot COOH$ (alanine). The simplest of the α- amino acids is glycine, $CH_2(NH_2) \cdot COOH$, two

molecules of which condense as follows:

$$HOOC \cdot CH_2 \cdot NH_2 + HOOC \cdot CH_2 \cdot NH_2 \longrightarrow HOOC \cdot CH_2 \cdot NH \cdot OC \cdot CH_2 \cdot NH_2 + H_2O$$

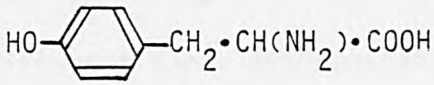
(1)

The compound (1) still contains a primary amino group which can be condensed with another molecule of glycine to give the product represented in formula (2)

$$HOOC \cdot CH_2 \cdot NH \cdot OC \cdot CH_2 \cdot NH \cdot CO \cdot CH_2 \cdot NH_2 \qquad (2)$$

By continuing this condensation a very large molecule, known as peptide, can be built up in which the group $-NH \cdot CO \cdot CH_2-$ is repeated very many times. Peptides are degradation products of proteins and therefore of a similar but somewhat less complex structure. Glycine is the simplest $\alpha-$ amino acid, but as many as thirty others have been isolated from proteins, of which four examples are:

Alanine $CH_3 \cdot CH(NH_2) \cdot COOH$

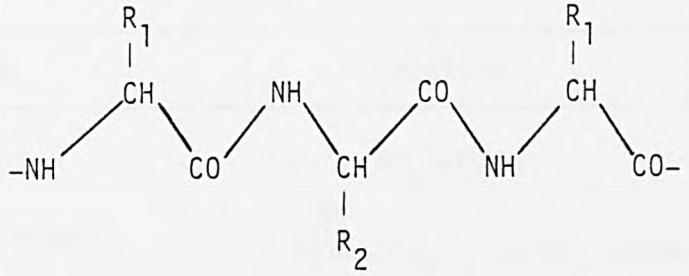
Tyrosine 

Leucine $(CH_3)_2 \cdot CH \cdot CH_2 \cdot CH(NH_2) \cdot COOH$

Serine $HO \cdot CH_2 \cdot CH(NH_2) \cdot COOH$

Because the amino group which condenses with the carboxyl radical of the adjacent molecule is in the $\alpha-$ position, the whole of the remainder of condensation polymers in the molecules in the more complex amino acids appear as side chains substituting one of the hydrogen atoms in the CH_2 component of the main skeleton.

Thus an extremely simple peptide, built up from tyrosine and leucine residues, would have the structural formula:



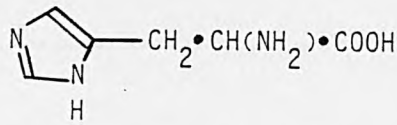
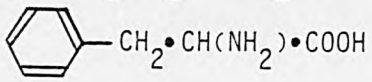
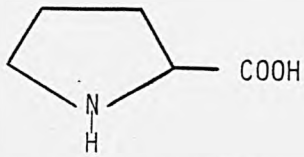
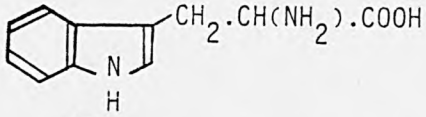
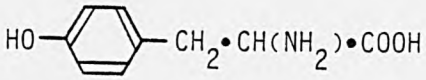
in which R_1 is the tyrosine residue (HO-C$_6$H$_4$-CH_2-) and R_2 the leucine residue [$(CH_3)_2CH \cdot CH_2$-].

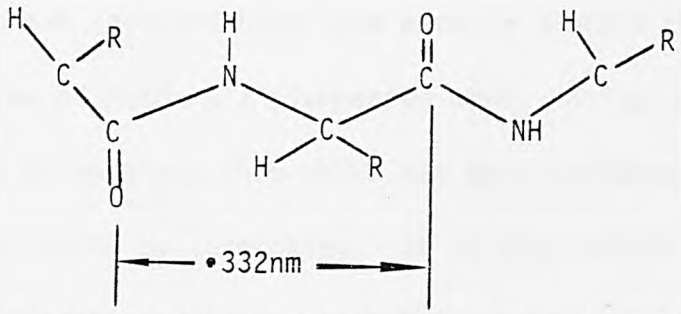
By means of careful hydrolysis and chromatographic methods it is possible to isolate and estimate all the amino acids of keratin. The composition is shown in Table 2.2[16].

Out of the thousands of proteins which are polymerides of amino acids only keratin and one or two others are fibre-forming. It was not until Astbury applied methods of X-ray analysis that it was established that the long-chain keratin molecules are organized in orientated and amorphous regions. Unstretched and stretched wool fibres show different X-ray diffraction diagrams, corresponding with two forms known as α - and ß- keratin respectively. The pattern for ß- keratin is shown in Fig. 2.6, indicating a repeat period in the longitudinal axis of 0.334nm. This corresponds with 0.332nm spacing of the zigzag structure of the fully extended peptide chain.

Table 2.2

Amino acids in keratin

Name	Structure	Per cent
Alanine	$CH_3 \cdot CH(NH_2) \cdot COOH$	5.5
Arginine	$H_2NC(=NH)NH \cdot (CH_2)_3 \cdot CH(NH_2) \cdot COOH$	7.1
Aspartic acid	$HOOC \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	6.2
Cystine	$HOOC \cdot CH(NH_2)CH_2 \cdot S \cdot S \cdot CH_2CH(NH_2)COOH$	11.0
Glutamic acid	$HOOC \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	12.2
Glycine	$CH_2(NH_2) \cdot COOH$	5.8
Histidine		0.8
Leucine	$(CH_3)_2CH \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	7.6
Lysine	$H_2N(CH_2)_4 \cdot CH(NH_2) \cdot COOH$	2.6
Methionine	$CH_3 \cdot S \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	0.5
Phenylalanine		2.8
Proline		7.5
Serine	$HO \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	11.5
Threonine	$CH_3 \cdot CH(OH) \cdot CH(NH_2) \cdot COOH$	7.0
Tryptophan		0.9
Tyrosine		4.0
Valine	$(CH_3)_2CH \cdot CH(NH_2).COOH$	5.6
Isoleucine	$CH_3CH_2CH(CH_3) \cdot CH(NH_2) \cdot COOH$	3.1



In α- keratin the longitudinal spacing is 0.514nm, as shown in Fig. 2.7. Astbury and Bell [17] explained the elasticity of wool, together with the alterations in the X-ray diagrams, by the existence of a fold in the peptide chain in the unstretched state.

Figure 2.6 X-ray diffraction diagram of ß-keratin.

Figure 2.7. X-ray diffraction diagram of α - keratin.

Great advances have been made in protein chemistry since the time of Astbury's pioneering work. Polyalanine, like keratin, exists in an alpha form which may be transformed into the beta configuration by stretching. It is much easier to interpret X-ray diffraction diagrams of substances of known constitution, such as polyalanine, than those obtained from the infinitely more complex keratin molecule.

The pattern yielded by the alpha form of polyalanine corresponds with the theoretical requirements of a helical structure as shown in Fig. 2.8 and as represented diagrammatically in Fig. 2.10. A cable of three helical strands, however, as in Fig. 2.11, conforms better with the X-ray diffraction diagram.

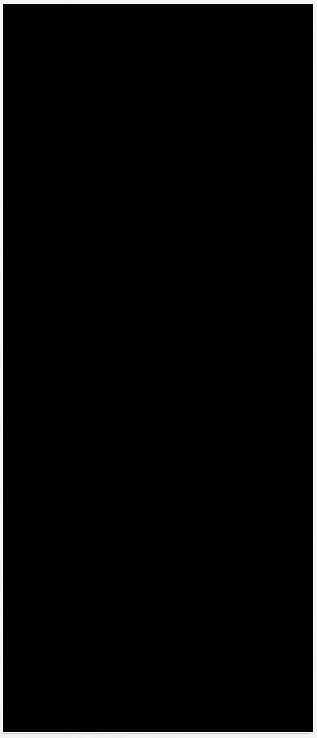


Figure 2.8. α- Keratin

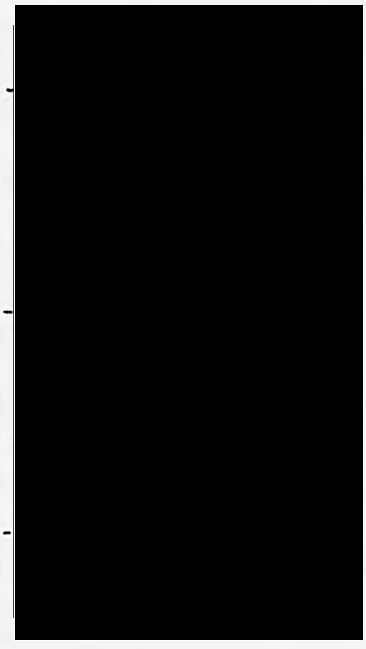


Figure 2.9. ß- Keratin

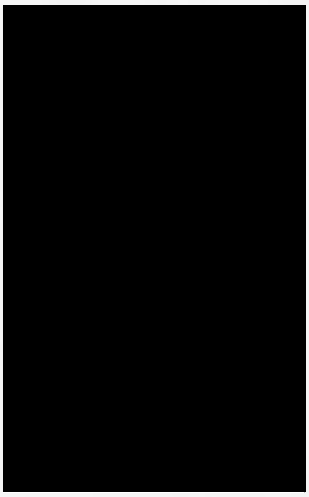


Figure 2.10

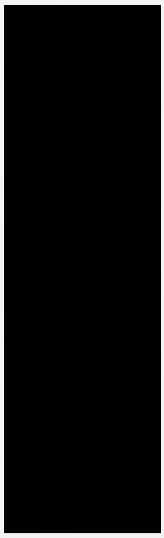
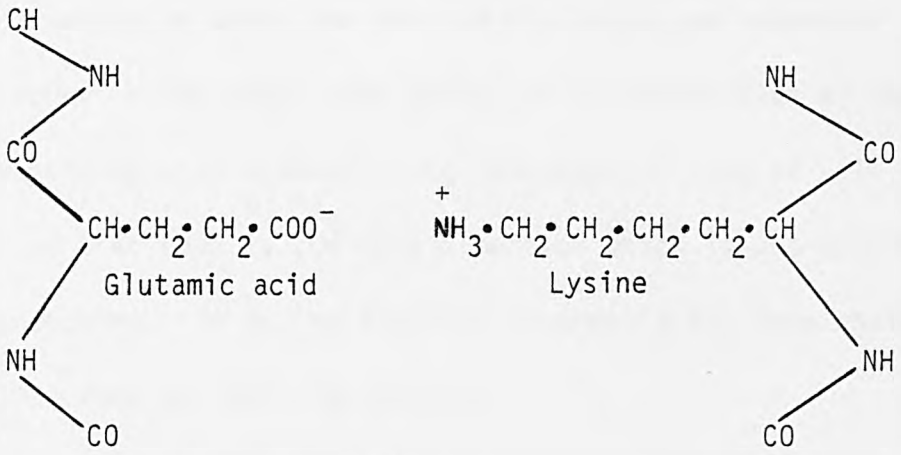


Figure 2.11. Helical cable structure.

In the helical structure hydrogen bonds are formed between carbonyl and secondary amino groups in adjacent turns of the coil, as shown by the dotted lines in Fig. 2.8. In the stretched beta form the hydrogen bonds become intermolecular instead of intramolecular (see dotted lines in Fig. 2.9). Mechanical tension causes the rupture of the hydrogen bonds in the alpha helix, and this may be assisted by the action of chemicals as well as by the application of heat. The rupture of these hydrogen bonds is the necessary preliminary to the uncoiling of the molecule for transformation from the alpha to the beta form.

Apart from hydrogen bonds there may also be bonds of a chemical nature between adjacent molecular chains. Where carboxyl and amino groups are situated opposite each other there is a possibility of salt linkages being formed as shown in (3):



(3)

Ionic bonding can also originate from interactions between the $-NH_3^+$ and $-CO_2^-$ groups present at the ends of the polymer chains, as shown in Fig. 2.12.

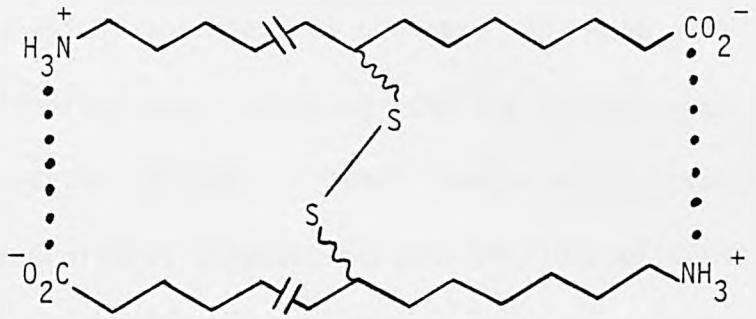
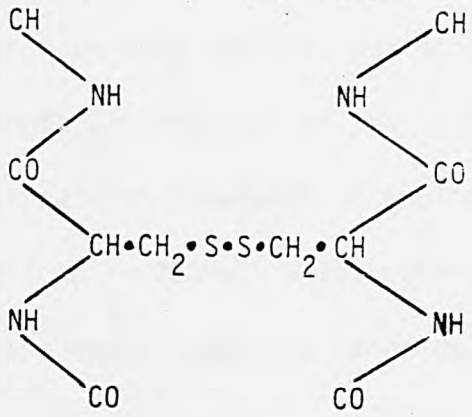


Figure 2.12. Ionic bonding in wool.

A unique feature of keratin is the existence of cystine linkages (4) which covalently bind polypeptide (protein) chains at intervals by bridges.



(4)

The distances by which the peptide skeletons are separated from each other in the orientated positions is represented by the spots corresponding with 0.98nm in Fig. 2.6 and 2.7, and it will be observed that this is the only dimension which is not altered on stretching. It is the distance separating the main chains to allow room for the side chains.

2.3 YELLOWING OF WOOL

2.3.1. Introduction

The exposure of wool keratin to sunlight results in a number of physical and chemical changes. The most sensitive change is a discolouration resulting from the formation of yellow photo-degradation products. Other changes which become evident after more prolonged exposure include the loss of tensile strength and abrasion resistance and altered dyeing properties. Since the enhanced yellowing by sunlight is so easily observed and has such serious commercial consequences, it has been the subject of most of the investigations into sunlight damage to wool.

The degree of discolouration induced in wool by exposure to sunlight depends on many factors, one of the most important being the wavelength distribution of the light. Yellowing is caused by the ultraviolet component of sunlight, i.e. the wavelength range from 380nm down to 300nm. Wavelengths below 290-300nm are filtered out by the ozone layer and hence do not reach the earth.

All other parameters being equal, the lower the wavelength in the U.V., the greater the extent of yellowing [18]. In contrast, irradiation with wavelengths above 380nm into the visible region causes bleaching to occur, the effect being maximal between 420-450nm [19, 20]. Photobleaching is most noticeable in yellow wool, because of the greater absorbance of blue light and hence the initial rate is more rapid. When wool is exposed to sunlight or some other mixed radiation source, yellowing and bleaching occur simultaneously, the overall effect being determined partly by the relative energies of the U.V. and blue light components, and partly by the initial colour of the wool. So for a given radiation source, yellow wool would photobleach whereas white wool would only yellow. Much more extensive yellowing will occur in summer than in winter months and yellowing can be reduced by exposure behind window glass. The above effect is summarised in Figure 2.12.

Oxygen plays a vital role in the sunlight yellowing of wool, and a considerable quantity of oxygen is actually consumed during ultraviolet irradiation [21]. Irradiation of dry wool in the absence of oxygen produces a green discolouration, believed to be due to the formation of thiyl free radicals. On exposure to atmospheric oxygen these radicals decay and the green colour of the wool turns yellow [18]. The presence of water vapour enhances yellowing, thus wet wool yellows much more rapidly than dry wool [22], (see Fig. 2.13).

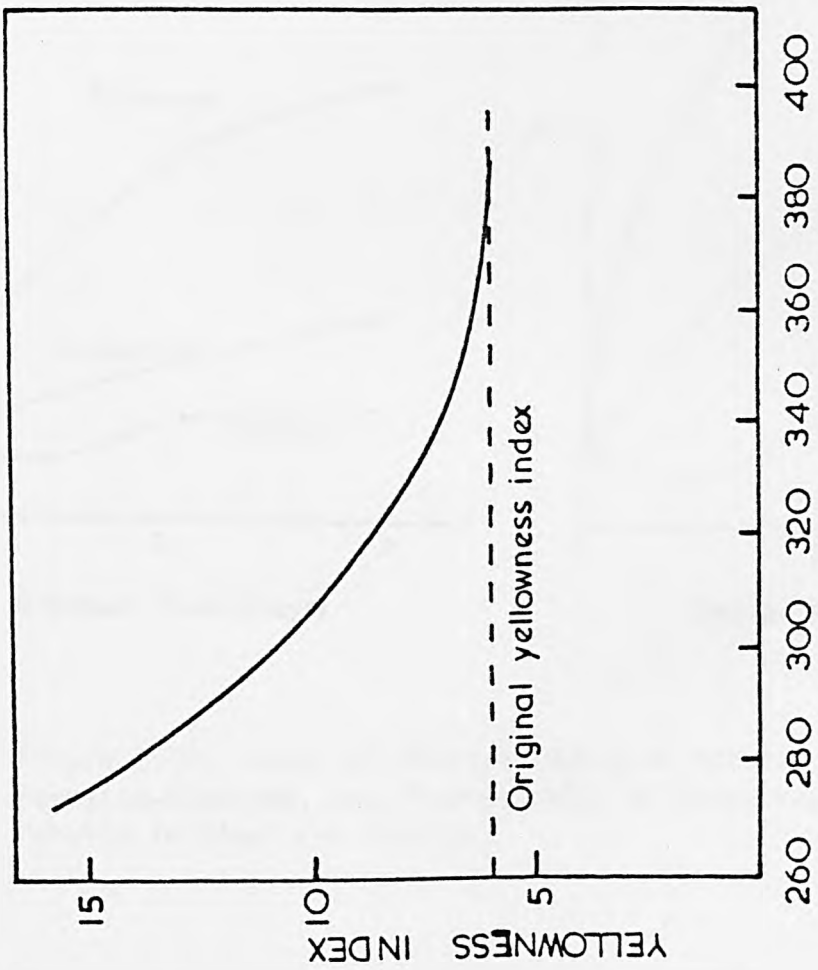


Figure 2.12. Photoyellowing vs. Wavelength

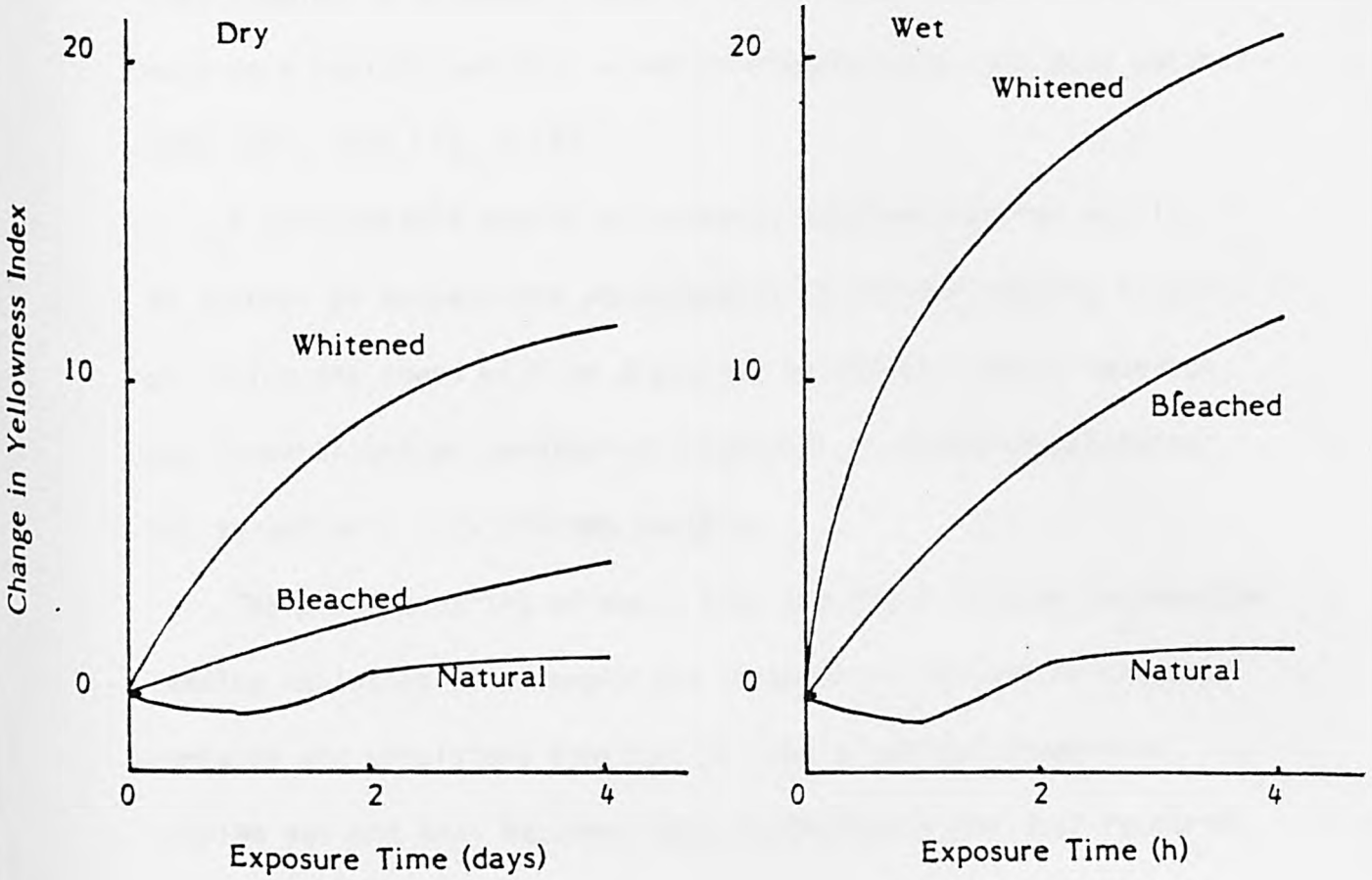


Figure 2.13. Rates of Photoyellowing of Natural, Peroxide-bleached, and Fluorescently Whitened Wool Fabrics in Simulated Sunlight.

In its natural state wool is not a white fibre and to match the whiteness of other textile fibres it is quite often necessary to bleach it, either oxidatively with alkaline hydrogen peroxide or reductively with hydrosulphite. Unfortunately wool that has been chemically bleached, especially by alkaline peroxide, yellows much more rapidly and by a wider wavelength band than does natural wool [22], (see Fig. 2.13).

A considerable amount of research has been carried out in an attempt to explain the photochemical reactions leading to wool yellowing and these will be discussed in detail. Other research has concentrated on developing treatments to minimise yellowing but as yet with only limited success.

The phototendering of wool, i.e. the light-induced degradation leading to losses in strength and abrasion resistance in carpets, curtains and upholstery fabrics, is also a serious commercial problem but one that has been most neglected by the wool research laboratories. A possible reason for this neglect is that phototendering is not nearly as obvious as photoyellowing and requires much longer times of irradiation to produce measurable changes. However it is known that phototendering is essentially due to both disulphide bond breakdown and main chain peptide bond breakdown [23, 24]. Some limited success has been achieved in reducing the rate of phototendering by using U.V. absorbers to screen the fibre from the damaging ultraviolet light [24].

2.3.2. Ultraviolet Absorbance

The ultraviolet absorption spectrum of a merino wool fibre, measured on a microspectrophotometer, is shown in Fig. 2.14 [21]. Between wavelengths of 250nm and 300nm the absorption is due essentially to the presence of the amino acids tyrosine and tryptophan with minor contributions from cystine and phenylalanine. Knowing the absorbance curves of these amino acids in polymer films and the amino acid composition of wool, it is possible to calculate an absorption curve due to the amino acid components of wool. This curve is shown in Fig. 2.14. It can be seen that in the 250 - 290nm wavelength region the agreement between the estimated and measured absorbances of keratin is within 10%, which is satisfactory in view of the errors involved in amino acid analysis, the heterogeneity of wool fibres and the computation of estimated absorbances.

At wavelengths above 290nm the wool fibre has a much higher absorbance than can be accounted for by amino acid absorption, indicating the presence of other species which absorb strongly in this wavelength region. The species accounting for this enhanced absorption have not yet been identified although it has been proposed that it could be due to the photodecomposition products of wool as a result of sunlight exposure during growth [25] or to the presence of natural pigment precursors [26].

Although the absorption in this wavelength region is relatively small compared with that occurring at wavelengths below 300nm,

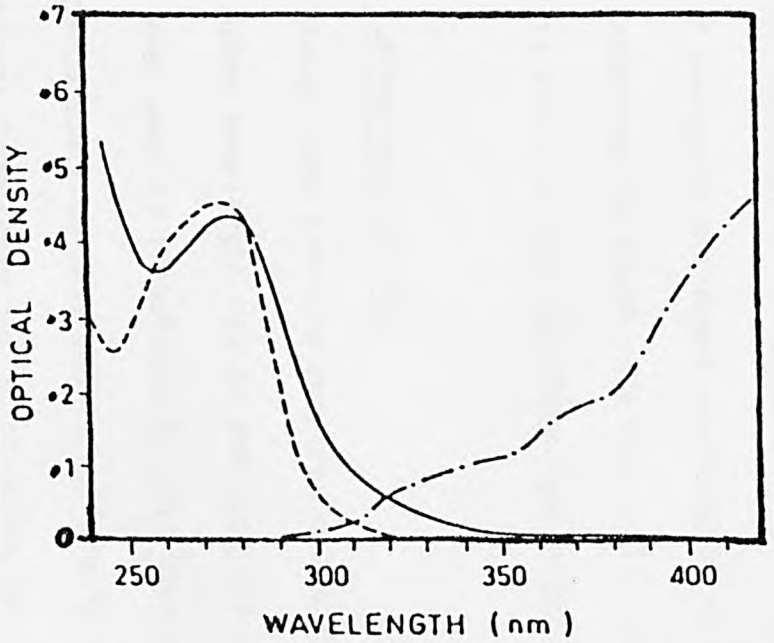


Figure 2.14. The ultraviolet absorption spectrum of a 6μm radial section of merino-wool keratin (———), the absorption spectrum calculated from the amino-acid composition (-----), and the relative spectral intensity of Sydney south light at noon(-.-.-.-) [21].

the radiance in the 300-380nm region of sunlight is much greater than in the wavelength region below 300nm, hence the absorption of sunlight by wool in the upper ultraviolet region must be taken into consideration when discussing the photochemistry of wool. This fact is well illustrated by the action spectrum for wool yellowing in Fig. 2.15 [18]. The spectrum does not correspond with the absorption spectrum of wool but shows a gradual increase in yellowing with decreasing wavelengths below 380nm. If, however, this action spectrum is corrected to correspond with the relative amounts of energy at different wavelengths in a typical sunlight spectrum reaching the Earth (see Fig. 2.14) the maximum yellowing is found to occur in the 340-350nm wavelength region.

2.3.3. Luminescence of Wool

Although both tyrosine and tryptophan absorb strongly in the 280-300nm wavelength region and both are capable of fluorescing, when wool is irradiated in this wavelength region the fluorescence spectrum observed is not simply the summation of the emissions from these two amino acids but is largely attributable to emission from tryptophan [27]. This phenomenon has been found to apply to a wide range of tryptophan-containing proteins and has been extensively reviewed by Konev [27] and Longworth [28]. The UV excitation and fluorescence emission curve at 77K are shown in Fig. 2.16 [18].

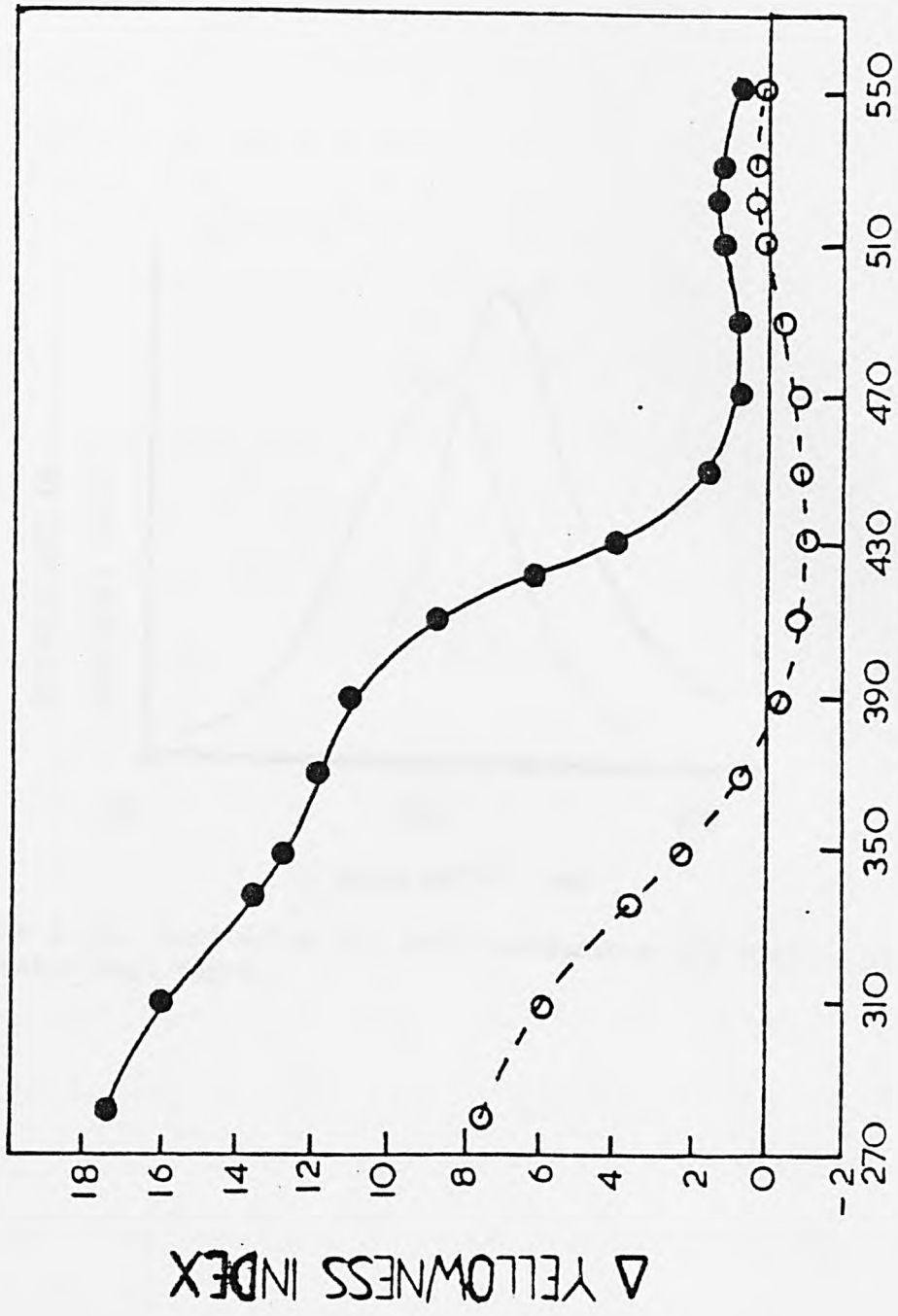


Figure 2.15. Action Spectra for Yellowing of F.W.A. treated [-●-] and Untreated Wool [-O-] [18].

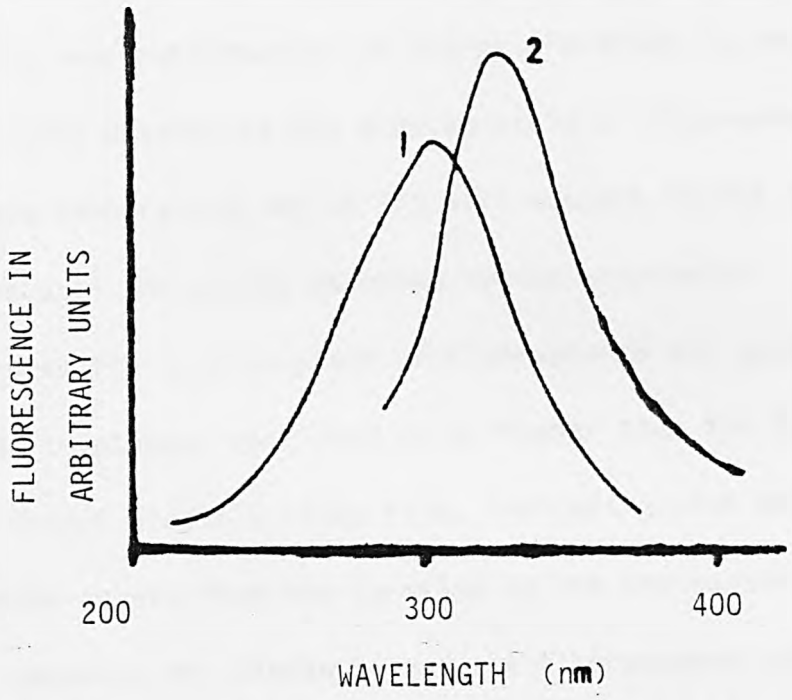


Figure 2.16. Excitation (1) and fluorescence (2) spectra of untreated wool fabric.

Initially it was suggested that the lack of significant emission from tyrosine was due to quenching within the protein of the singlet excited state of tyrosine. However, the fulfilment of the donor-acceptor conditions necessary for energy migration from tyrosine to tryptophan provides an alternative explanation and considerable evidence is now available to show that this type of energy transfer occurs in many polypeptides and proteins [28].

To seek confirmation of energy migration in wool, Ghiggino et al.[29] determined the quantum yield of fluorescence of wool at room temperature and at 77K with respect to the quanta absorbed by the wool and quanta absorbed by the tryptophan. At both temperatures the quantum yield of fluorescence per quantum absorbed by the tryptophan was found to be higher than for tryptophan in poly (vinyl alcohol) (PVA) film, indicating that excitation energy transfer occurs from the tyrosine to the tryptophan.

Based on his findings that the fluorescence quantum yields of a number of tyrosine and tryptophan derivatives and peptides in solution were significantly lower than the corresponding values for the free amino acids, Cowgill [30] proposed that the peptide bond could be quenching the fluorescence of soluble proteins. However, some recent work [31] has shown that the quantum yield of fluorescence of tyrosine and tryptophan derivatives and peptides in PVA film are the same as the free amino acids. Thus it would appear unlikely that the peptide bond causes significant fluorescence quenching in wool keratin.

There is, however, evidence that, in common with other cystine-containing proteins, the disulphide bonds of wool cause considerable fluorescence quenching. Reduction of the disulphide bonds in wool by treatment with tributylphosphine was found to double the original fluorescence [32, 33] indicating that the presence of the disulphides was responsible for at least 50% quenching of wool's fluorescence. Furthermore Bhatnagar and Gruen [32] found that subsequent alkylation of the thiol groups formed further enhanced the fluorescence. On the other hand Leaver [33] found that subsequent alkylation removed the enhancement achieved by the reduction process and attributed this loss of fluorescence to conformational changes in the fibre as a result of the chemical treatment.

If we accept that 50% of wool fluorescence is quenched by the cystine component, then the true fluorescence should be double that which is normally measured. The evidence presented by Ghiggino et al. [29] for energy migration then becomes even more compelling.

Following the recognition that the fluorescence of wool was due essentially to emission from its tryptophan component, it was soon recognised that the room temperature phosphorescence of wool was also essentially due to tryptophan. Konev [27] examined the decay kinetics of this phosphorescence and found both an intense short-lived component which he attributed to direct emission from the tryptophan triplet, and a longer-lived weak component which he proposed was due to recombination of a photoejected electron

with the tryptophan radical cation.

Ghiggino et al. [29] also examined the room temperature decay kinetics of wool phosphorescence (excitation at 280nm) and found that under normal atmospheric conditions wool decayed by first-order kinetics with a lifetime of 0.08s, but under dry nitrogen its lifetime was 0.45s and decayed at an apparent second-order rate. They concluded that under dry nitrogen the rate-determining step is a radiationless tryptophan triplet-triplet quenching, while in the presence of oxygen (and some moisture) the triplet state of tryptophan is quenched by oxygen with the possible formation of singlet oxygen.

By studying the excitation wavelength dependence of the phosphorescence of wool keratin Nicholls and Pailthorpe [21] found two quite distinct phosphorescent species (Fig. 2.17). With an excitation wavelength of 290nm the phosphorescence emission (emission peak 435nm, lifetime at room temperature in dry N_2, 0.45s) was attributed to the triplet state of tryptophan based on the good agreement of its spectral line shape with the emission from tryptophan in a PVA film under the same conditions. When excited by 350nm radiation a second phosphorescent species (emission peak 500nm, lifetime in N_2 at 20°C, 0.16s) was detected. The source of this emission is not yet clear but it could be the same species which caused the enhanced absorption of the wool in the 330-360nm range. The emission from wool excited with radiation between 290nm and 350nm is a combination of these two emissions. It is

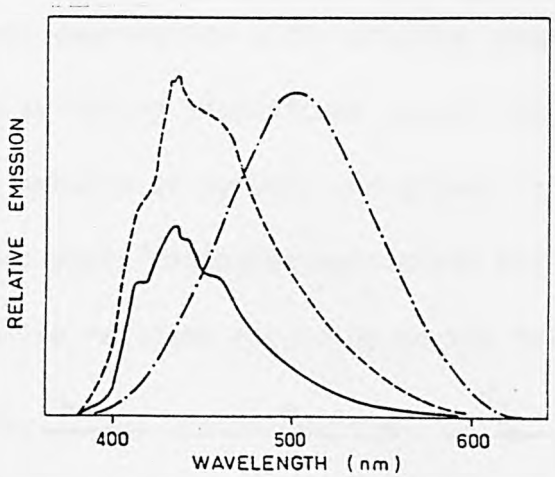


Figure 2.17. Triplet-state emission spectra at room temperature [21]. ----- Tryptophan in a dry PVA film for λ_{ex} = 280nm. ——— Wool keratin for λ_{ex} = 290nm. -.-.-.- Wool keratin for λ_{ex} = 350nm.

of interest to note that although the donor-acceptor conditions appear to be fulfilled, there is no apparent energy transfer from tryptophan in its triplet excited state to the other phosphorescent species.

More recently Leaver [34] has re-examined the room temperature phosphorescence decay curves of wool under dry nitrogen and has proposed that for excitation at 290nm the emission is a combination of two exponential decay curves with lifetimes of 0.18s and 1.45s. The former, he attributed to the species with a maximum absorption in the 330-350nm region, while the long-lived species was attributed to the triplet state of tryptophan.

2.3.4. Photochemical Reactions

Exposure of wool keratin to sunlight causes both side chain and main chain photo-oxidation. Amino acid analysis of the hydrolysates of irradiated wool has shown that tryptophan, histidine and cystine

suffered the most degradation with tyrosine, phenylalanine and methionine also suffering significant losses [35]. The identification of small amounts of pyruvic and glyoxylic acids [36] in the protein hydrolysate indicates main chain breakdown at the glycine and alanine residues according to the following mechanism [36]:

$$-CO-NH-CH_2-CO-NH- \xrightarrow{h\nu} -CO-NH-\dot{C}H-CO-NH- \xrightarrow{O_2} -CO-NH-\underset{\underset{O-O\bullet}{|}}{CH}-CO-NH-$$

$$\xrightarrow{RH} -CO-NH-\underset{\underset{OOH+R\bullet}{|}}{CH}-CO-NH- \longrightarrow -CO-NH_2+HCO-CO-NH-$$

The subsequent identification of a number of other α- keto acids in the hydrolysates of irradiated wool [37] suggests that main chain breakdown is not confined to the glycine and alanine residues but can occur adjacent to many other amino acid residues.

The above proposed reactions leading to main chain breakdown involve the formation of free radicals. The presence of free radicals in U.V. irradiated wool can be readily detected by electron spin resonance spectroscopy (e.s.r.) but due to a lack of distinct hyperfine structure of the resulting spectra they are sometimes difficult to identify. A typical e.s.r. spectrum obtained from wool following irradiation at room temperature with a narrow band of energy at 310nm wavelength is shown in Fig. 2.18 and consists essentially of an intense singlet line (A) at g=2.00 and an asymmetric low field signal (B_1 and B_2). This low field signal is readily identified with the thiyl radical $R-CH_2-S\bullet$, formed either by the homolytic fission of the disulphide bond of cystine or by the

rearrangement of a cystinyl radical anion (formed by trapping an electron) [38], e.g.

$$R\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}R \xrightarrow{h\nu} R\text{-}CH_2\text{-}S\overset{+\bullet}{S}\text{-}CH_2\text{-}R + e^-$$

$$R\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}R + e^- \longrightarrow R\text{-}CH_2\text{-}S\overset{-\bullet}{S}\text{-}CH_2\text{-}R \longrightarrow R\text{-}CH_2S^- + {}^{\bullet}S\text{-}CH_2\text{-}R$$

This disulphide bond breakdown, together with the main chain breakdown, is mainly responsible for the phototendering of the wool.

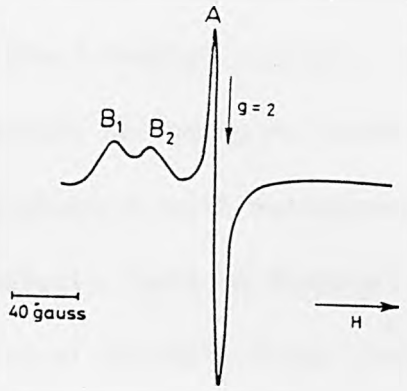


Figure 2.18. Typical e.s.r. curve of wool irradiated at 310nm at room temperature in the absence of oxygen.

The singlet line spectrum (A) was originally designated by Gordy and Shields [39] to be a combination of a glycyl radical $-NH-\dot{C}H-CO-$ and an alanyl radical $-NH-\dot{C}(CH_3)-CO-$, which agrees with the chemical evidence following the isolation of pyruvic and glyoxylic acids [36]. However Barkakaty and Keighley [40] have queried this designation after showing that this singlet line spectrum can be resolved into a doublet and a quartet. It has been proposed that the doublet was due to an unpaired electron associated with the peptide carbonyl oxygen, $\diagdown\!\!\diagup C = O\bullet$, and the quartet with an unpaired electron located on the γ-carbon of

proline. At present there is no chemical evidence to support this designation.

These designations of free radicals in u.v. irradiated wool do not include radicals associated with the strongly absorbing and photolabile tryptophan residues, despite the fact that a free radical, readily detected by e.s.r., is formed by the irradiation of aqueous solutions of tryptophan [41]. This free radical has been identified as the 3-indolyl radical, formed by the rearrangement of the 1-indolyl radical following N-H bond fission [41] and/or photoejection of an electron with subsequent deprotonation [42]. However, no such radicals could be detected when tryptophan was irradiated in poly(vinyl alcohol) films [43]. Amouyel et al. [44] have shown that during the u.v. irradiation of tryptophan in aqueous solution both photoejection of electrons and N-H bond fission contribute to radical formation at wavelengths less than 275nm (which corresponds to the photoionisation energy threshold for tryptophan in water) but at higher wavelengths only N-H bond fission contributes. The photoionisation energy threshold for tryptophan in isopropanol is less than 250nm [44], a value which might be expected to apply to poly (vinyl alcohol) films, hence no photo-ejection of electrons would be expected from tryptophan in this polymer for radiation with wavelengths in excess of 250nm. Some tryptophyl radical formation by N-H bond fission would be expected to occur in the polymer film following u.v. irradiation but apparently not to a sufficient extent to form radicals in detectable quantities.

Thus radical pathways would appear to play a very minor role in the photodegradation of tryptophan in poly (vinyl alcohol) films with radiation of wavelength above 300nm, and by deduction in wool under the same radiation conditions.

When wool is exposed to radiation with wavelengths in excess of 360nm, enhancement of the naturally occurring e.s.r. signal occurs [45]. This signal, which is stable to air and has a quite different e.s.r. spectrum to that attributed to radicals produced by radiation of wavelengths less than 325nm, has yet to be identified. Since this spectrum is very similar in shape and stability to that obtained from pigmented wool [26], it has been suggested that it may be formed from some pigment precursor in the unpigmented wool. In fact it could be due to the same unidentified species which has been detected in the u.v. absorption and phosphorescence spectra of wool [21]. As an alternative explanation, Smith [46] has proposed that this stable radical is due to the presence of a ferric ion-keratin complex in the wool. Considerable research has been carried out to explain the photochemical reactions leading specifically to wool yellowing and a number of theories have resulted from this effort. Meybeck and Meybeck [36] suggested that wool yellowing was due to the visible light absorbed by the α-keto acids formed by the photochemical decomposition of the polypeptide chain. However, Holt and Milligan [37] have shown that whereas the irradiation of dry wool causes an increase in carbonyl groups, their rate of production is approximately the same for unbleached

and bleached fabrics, yet these fabrics yellow at markedly different rates. Furthermore, irradiation of the same fabrics in water resulted in only a slight increase in carbonyl group formation although extensive yellowing occurred. They concluded that it was unlikely that the formation of carbonyl groups was responsible for wool yellowing.

Hoare [47] proposed that the inherent yellowness of wool was due to the presence of mobile electrons which could be readily photo-excited into conduction bands. Additional yellowing by u.v. irradiation, alkali and heat was probably due to an increase in the number of mobile electrons following the introduction of additional unsaturated groups into the fibre. The fact that e.s.r. studies could find no evidence for electrons in conduction bands in irradiated wool [45] and that yellow compounds can be isolated from photoyellowed wool by enzymatic digestion [48] cast doubt on this proposal.

The most widely held theory of wool yellowing proposes the photodecomposition of the tryptophan residues to form yellow products. Considerable evidence is now available to support this proposal.

Tryptophan absorbs approximately a quarter of the radiation in the 290-310nm waveband. It is the amino acid most susceptible to yellowing by sunlight, both in aqueous solution [49] and in polymer films [43]. The rate of decomposition during sunlight irradiation is shown in Fig. 2.19 [50]. A strong correlation was found for the rates of yellowing of 29 different keratin samples

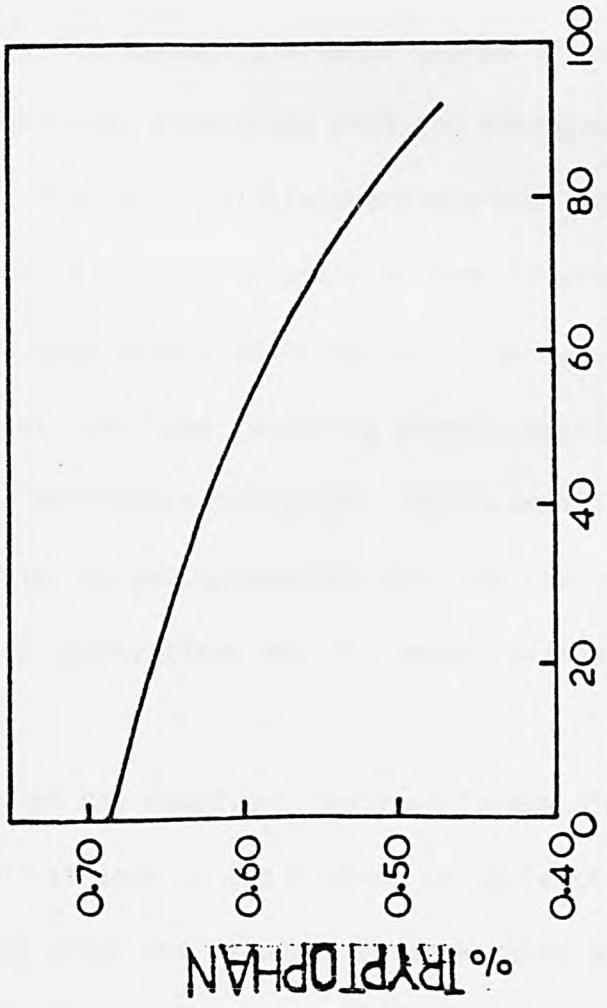


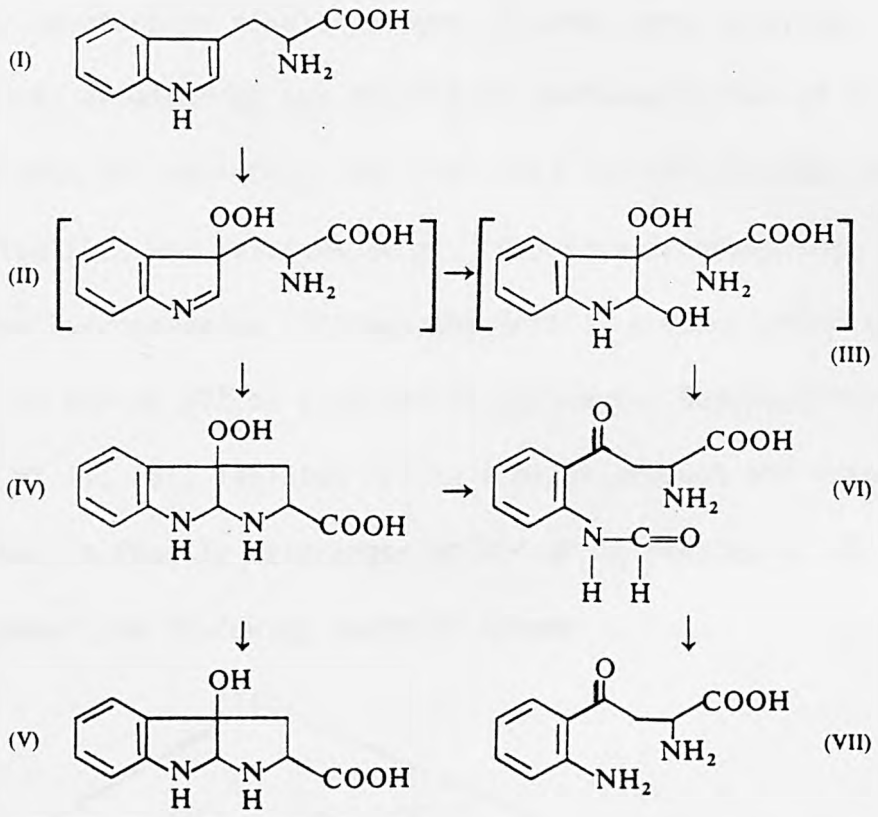
Figure 2.19. Change in Tryptophan Content After Irradiation [50].

with their initial concentration of tryptophan and with the destruction of tryptophan [51]. Correlations of yellowing with the destruction of other amino acids were less significant. The incorporation of tryptophan derivatives into wool causes a significant increase in yellowing [52]. Holt and Milligan [48] were able to isolate some yellow products of photodegraded wool by enzymatic digestion but only one product, kynurenine, a recognised oxidation product of tryptophan, could be identified. Furthermore they were able to show that when the tryptophan residues in wool were made radioactive, the yellow photoproducts were likewise radioactive, thus directly implicating tryptophan as a major source of yellowing.

To avoid the problems associated with the isolation of peptide-bound photoproducts from an insoluble substrate such as wool, the photodegradation in aqueous solution of free tryptophan and its derivatives has been widely investigated. Two types of studies have been carried out, one type involving direct photolysis, the other by means of a photosensitising dye. Most sensitising dyes used have been singlet oxygen generators [53] and the photo-oxidation of tryptophan and its derivatives was the result of the reactions with singlet oxygen.

The complexity of the reactions involved in the direct photolysis of tryptophan is illustrated by the finding of at least nine degradation products after the exposure of an aerated aqueous solution of tryptophan to a flash photolysis lamp [54]. The short duration of the flash (15μs) ensured that most of the products,

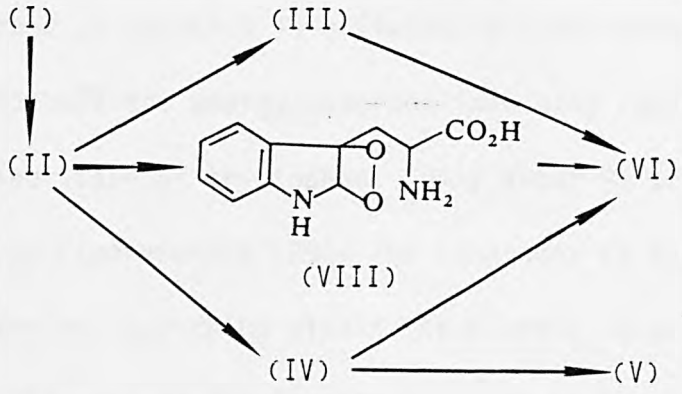
which have not yet been identified, were essentially primary reaction products. Following prolonged irradiation of tryptophan (I), N-formylkynurenine (NFK, VI) [55,56], kynurenine (VII) [57] (both intensely yellow coloured compounds), 3a-hydroperoxypyrrolidinoindole (PPI, IV) [58] and 3a-hydroxypyrrolidinoindole (HPI,V) [58] have been isolated and identified. Sun and Zigman [58] have proposed that the short-lived intermediate II (indolenine hydroperoxide) is involved in the formation of products IV and V according to the following scheme:



Such a reaction scheme would apply to the situation where the α- amino group of tryptophan is free, allowing the lone pair on the nitrogen to attack the indole in position 2. However when

tryptophan is incorporated into a protein chain the ring closure in converting II to IV would be restricted by both steric factors and the loss of activity of the nitrogen bound into an amide group. This loss of activity probably accounts for the reduced rate of yellowing of tryptophan in aqueous solution following acetylation of the free amino group [59].

If we accept the theory, to be expounded later, that singlet oxygen is involved in the direct photoyellowing of wool, then the dye-sensitised photo-oxidation studies of tryptophan, where the major reactant is singlet oxygen, becomes very relevant. The products obtained by dye-sensitised photo-oxidation of tryptophan have included NFK (VI) [60], PPI (IV) [61] and HPI (V) [62, 63]. Both Savige [62] and Nakagawa et al. [63] have proposed that an indolenine hydroperoxide (II) was the initial product which either converts to NFK or HPI by alternative pathways. Subsequently Nakagawa et al. [61] isolated PPI as a major product and found that either it readily rearranges to NFK or it reduces to HPI. They proposed the following reaction scheme:



This reaction scheme is very similar to that proposed by Sun and Zigman for direct photolysis [58] and the restrictions noted for protein-bound tryptophan in that case would also apply here. The formation of PPI and ultimately HPI from protein-bound tryptophan in wool must therefore be considered to be highly improbable. However the intensely yellow coloured NFK would be a photoproduct, formed, not via the intermediate PPI, but by other pathways, e.g. via the energetically unfavourable dioxetane (VIII) [61]. The fact that kynurenine (VII), formed by the removal of the labile formyl group from NFK, has been isolated from photoyellowed wool [48] indicates that such pathways do exist.

While these findings clearly implicate tryptophan residues in the photochemical yellowing of wool, they give very little indication of the primary photochemical reactions involved, apart from the fact that free-radical reactions are not involved to any extent.

Wool absorbs energy at wavelengths below 320nm by virtue of the amino acids tyrosine and tryptophan; however, the energy absorbed by tyrosine is efficiently transferred to tryptophan, so that all the energy absorbed initially resides in the singlet excited state of tryptophan. Only about 8% of this energy is lost by fluorescence [29]; the remainder is dissipated by internal conversion, quenching within the protein, e.g. by disulphides [32, 33], and by intersystem crossing to the triplet state. A phosphorescence quantum yield of 0.13 for the tryptophan emission

peak of wool at 77K indicates that considerable intersystem crossing to the tryptophan triplet state occurs [21].

For wavelengths above 320nm, energy absorption is due to an absorbing species yet unidentified. Its singlet excited state apparently undergoes similar deactivation pathways to that of the tryptophan singlet state, including intersystem crossing to the triplet state, which at 77K has a phosphorescence quantum yield of 0.06 [21]. Thus when wool is exposed to sunlight two distinct triplet state molecules are formed concurrently. In both cases their rates of decay are of first order in the presence of oxygen and are accelerated by the presence of moisture [21]. It has been found that at least ten molecules of oxygen are consumed for every molecule of tryptophan destroyed, irrespective of whether the wool is irradiated with wavelengths below 320nm or above 340nm [21].

To account for these results, it has been proposed [21] that the triplet excited states of tryptophan and the unidentified species absorbing at 340nm react with ground state oxygen to produce singlet excited oxygen by energy transfer.

$$^3T^* + {}^3O_2 \longrightarrow S_o + {}^1O_2$$

The singlet excited oxygen then diffuses through the wool keratin to react with the amino acids histidine, tryptophan and methionine - the three amino acids known to react with singlet oxygen [64] - and possibly with the unidentified absorbing species. The fibre

swelling caused by the presence of moisture enhances the rate of diffusing of both ground state and singlet excited state oxygen.

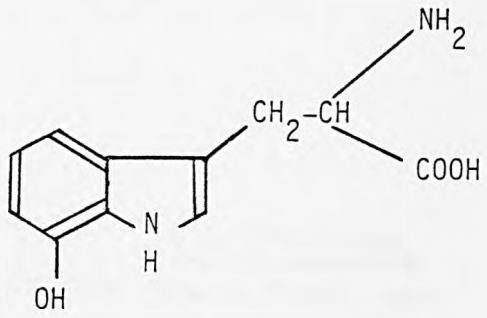
To confirm this proposal it would be necessary to show the formation of singlet oxygen in irradiated wool and that wool reacts with singlet oxygen to produce yellow discolouration, both of which are experimentally difficult. It has been found, however, that when sodium azide, a known singlet oxygen quencher, is applied to wool, the rate of tryptophan degradation and rate of oxygen consumption are both reduced [21]. Furthermore, by passing oxygen over irradiated wool fabric, $6\mu Mg^{-1}h^{-1}$ of singlet oxygen was detected [21].

Further indirect evidence comes from the fact that wool that has beenchemically bleached, e.g. by alkaline peroxide, is much more susceptible to photoyellowing than unbleached wool [22] and also has a much greater phosphorescence [34]. Thus this increased phosphorescence, resulting in a greater yield of singlet oxygen, could account for the increased yellowing. The increased phosphorescence in bleached wool has yet to be identified, but could be due to the oxidation to more phosphorescent products of certain amino acids, especially tryptophan, or other substances in the wool. Alternatively, the oxidation of cystine would reduce the cystine quenching of the excited states in wool, thus enhancing the normal phosphorescence.

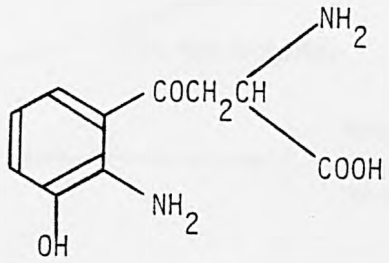
Many publications concerned with the sunlight yellowing of wool make such statements as 'most of the light absorbed by wool

is due to tryptophan residues' and others that 'the most damaging wavelengths are those in the 290-310nm wavelength region of the spectrum'. However, if the action spectrum of wool yellowing [18] is corrected for the energy distribution in sunlight, the maximum yellowing is found to occur in the 340-350nm wavelength region, the wavelength at which the unidentified absorbing species has its absorption peak. It would therefore appear that when wool is exposed to light, as distinct from laboratory ultraviolet sources, yellowing is dependent more on the absorption by the 340nm absorbing species than that of its amino acid components. Irrespective of which species is the major source of phosphorescence, subsequent yellowing is probably due to the oxidation of tryptophan by singlet oxygen. At this stage it is worth noting that the excitation spectrum of the delayed fluorescence of Rhodamine B on wool does not correspond to the tryptophan absorption curve but to the absorption curve of the unknown phosphorescent substance which absorbs maximally at 340nm wavelength [65]. Thus identification of this absorbing species, the behaviour of its excited states and its reactivity with singlet oxygen would help to understand more fully the reactions leading to wool yellowing.

Most of the above work implicates tryptophan as the precursor of the yellow pigment formed after wool is irradiated with u.v. light. Lennox et al. [18] suggest that oxidation can take place at the benzene ring of tryptophan to give a hydroxytryptophan:



which on rupturing of the indole ring would yield 3-hydroxykynurenine



which is the yellow pigment isolated frombutterfly wings [66,67].

The yellow colour of wool arising naturally during growth of the fibre can also be partly due to the formation of deeply coloured products and polymers related to melanin [68,69]. Melanin exists in two distinct forms, eu-melanin (present in karakul wools - grey, dark brown and black) and phaeo-melanin (yellow, red brown and red).

Eu-melanin is formed by the following sequence of reactions [70,71]:

Tyrosinase catalysed oxidation of side chain tyrosine residues and ring closure to give dopachrome (5,6-dioxoindole -2- carboxylic acid) is followed by reduction and decarboxylation to 5,6-dihydroxy-indole which undergoes oxidative polymerisation to form eu-melanin (see Fig. 2.20).

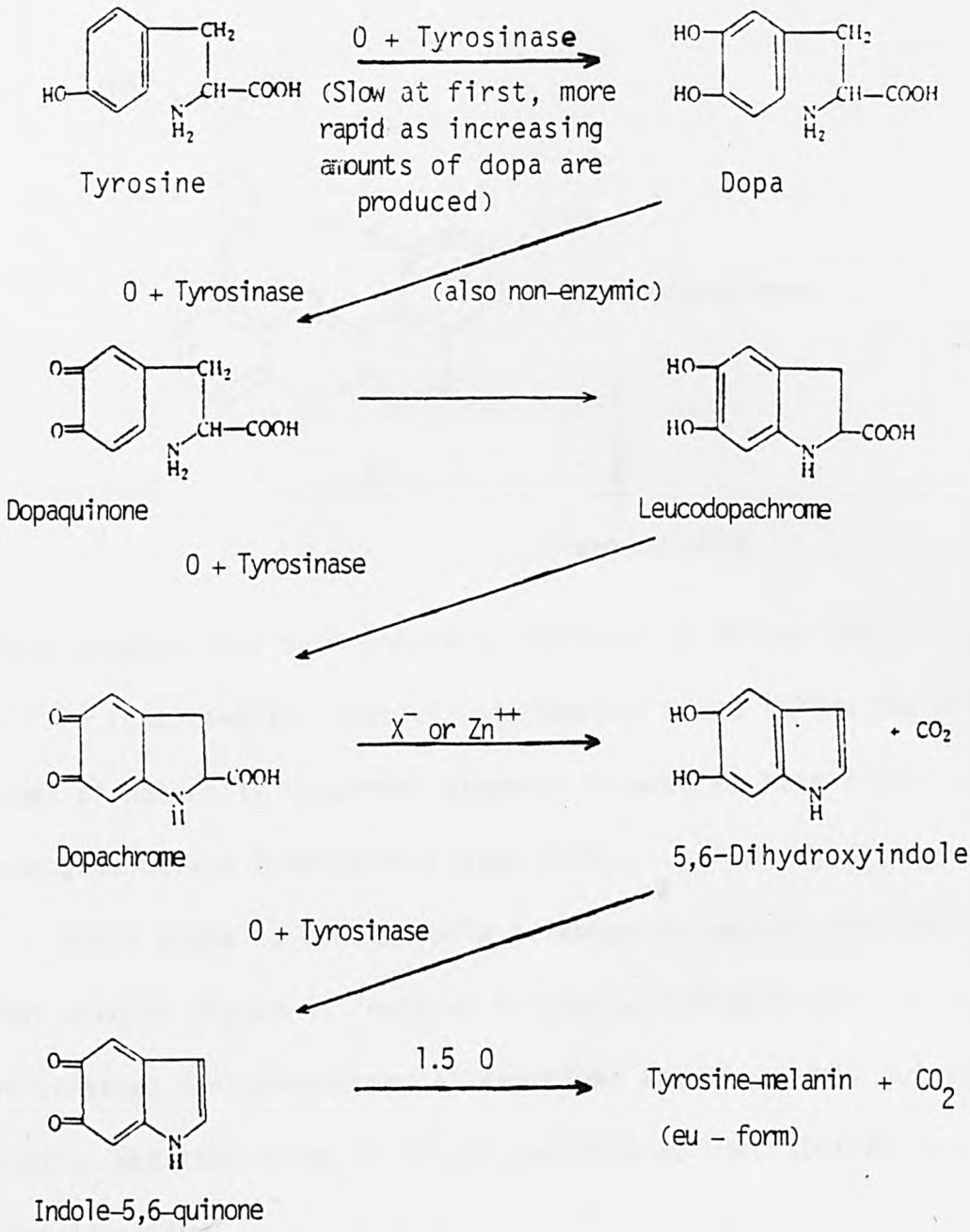
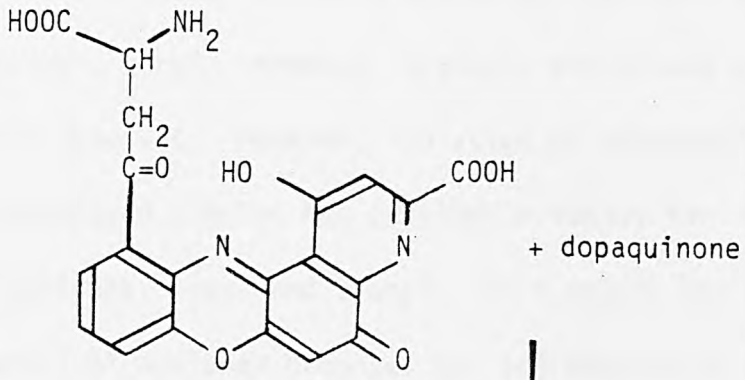


Figure 2.20. Postulated mechanism of formation of melanin (eu-type).

Phaeo-melanin is formed from tryptophanyl residues via the intermediate 3-hydroxykynurenine which forms the yellow pigment xanthommatin (5):



+ dopaquinone

(5)

Phaeo-melanin

These aspects have been admirably reviewed by Stoves [72].

It is noteworthy that the biochemical route to the two different types of naturally occurring pigments in wool or hair fibres arise from tyrosine and tryptophan respectively.

While there is considerable evidence to support the theory that singlet oxygen is involved in the yellowing of wool it must be stressed that photochemical reactions are invariably quite complex and that other reactions may also be contributing to the discolouration.

2.3.5. Thermal Yellowing

Air-dried wool is degraded and yellowed by heat above 100°C but the magnitudes of these changes are small and, unless accelerated by the presence of water and alkali, present no commercial problems.

From a practical and mechanistic viewpoint thermal yellowing is of two types - dry and aqueous - alkaline - and the latter will be dealt with in the next section.

In the case of dry wools heated at 100-150°C slow breakdown of cystinyl, seryl, threonyl, arginyl, prolyl and tryptophyl residues has been observed. However, isolation of degradation products from hydrolysed samples has provided evidence for loss of two other residues, lysyl and alanyl. As a result the scheme outlined in Figure 2.21 has been proposed for degradation of several of the residues thought to be involved in thermal yellowing. The observations that water promotes yellowing whereas the exclusion of oxygen does not inhibit it completely are in accord with this scheme. Further, silk, which contains little or no cystinyl residues, does not yellow as readily as wool.

2.3.6. Alkali Yellowing

When wool, particularly wet wool, is heated in the presence of alkali, significant yellowing occurs. Hydrothermal yellowing, that is thermal yellowing in aqueous media under neutral conditions, can also be included under this heading if water is considered to function as a mild alkali.

It appears that degradation of cystinyl residues to α - aminoacrylic or α- iminopropionic acid residues is a major source of yellowing with alkali although similar degradations of seryl and threonyl residues have been implicated (Fig. 2.22). The

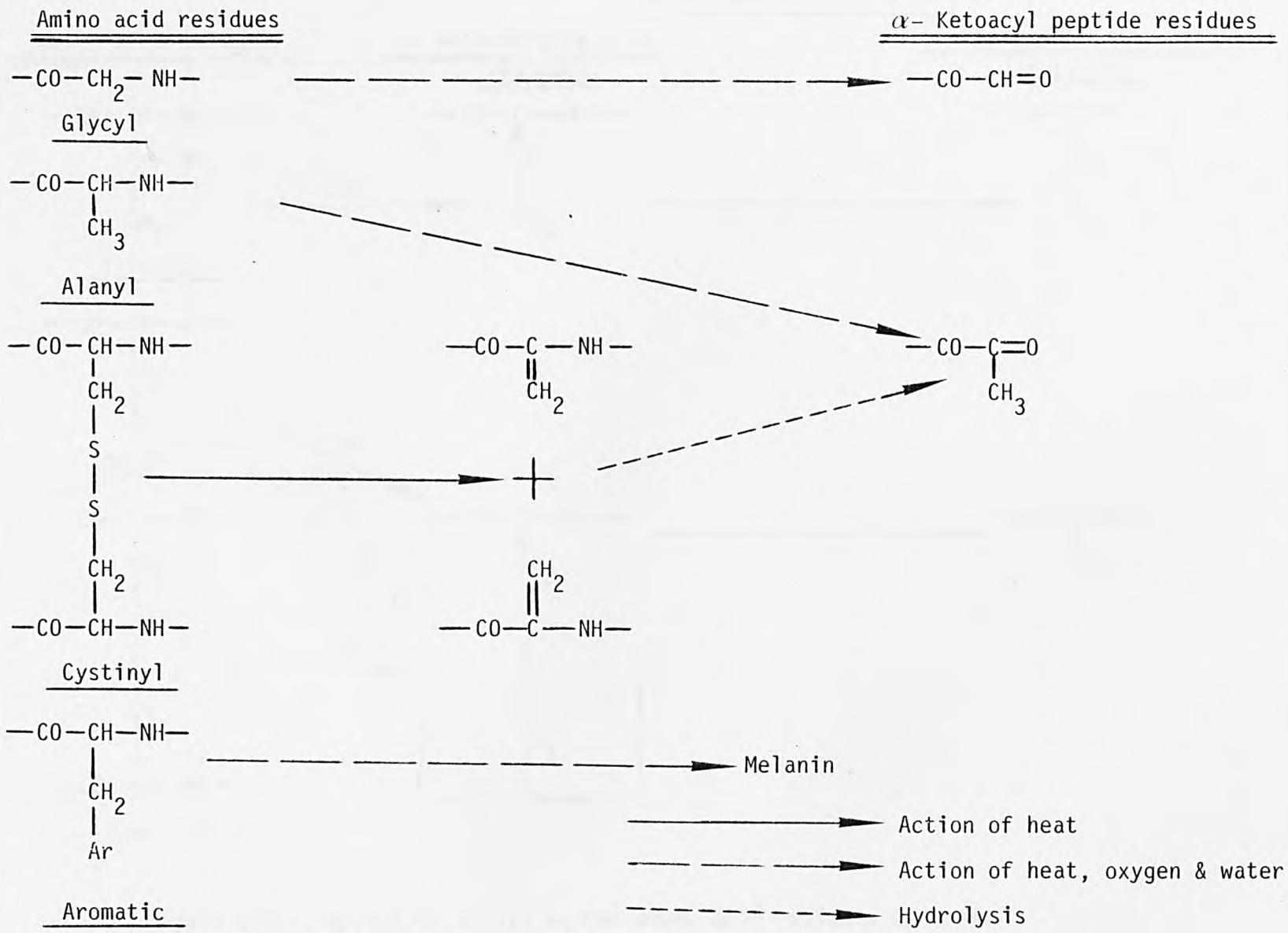


Figure 2.21. Action of heat on the amino acid residues of wool.

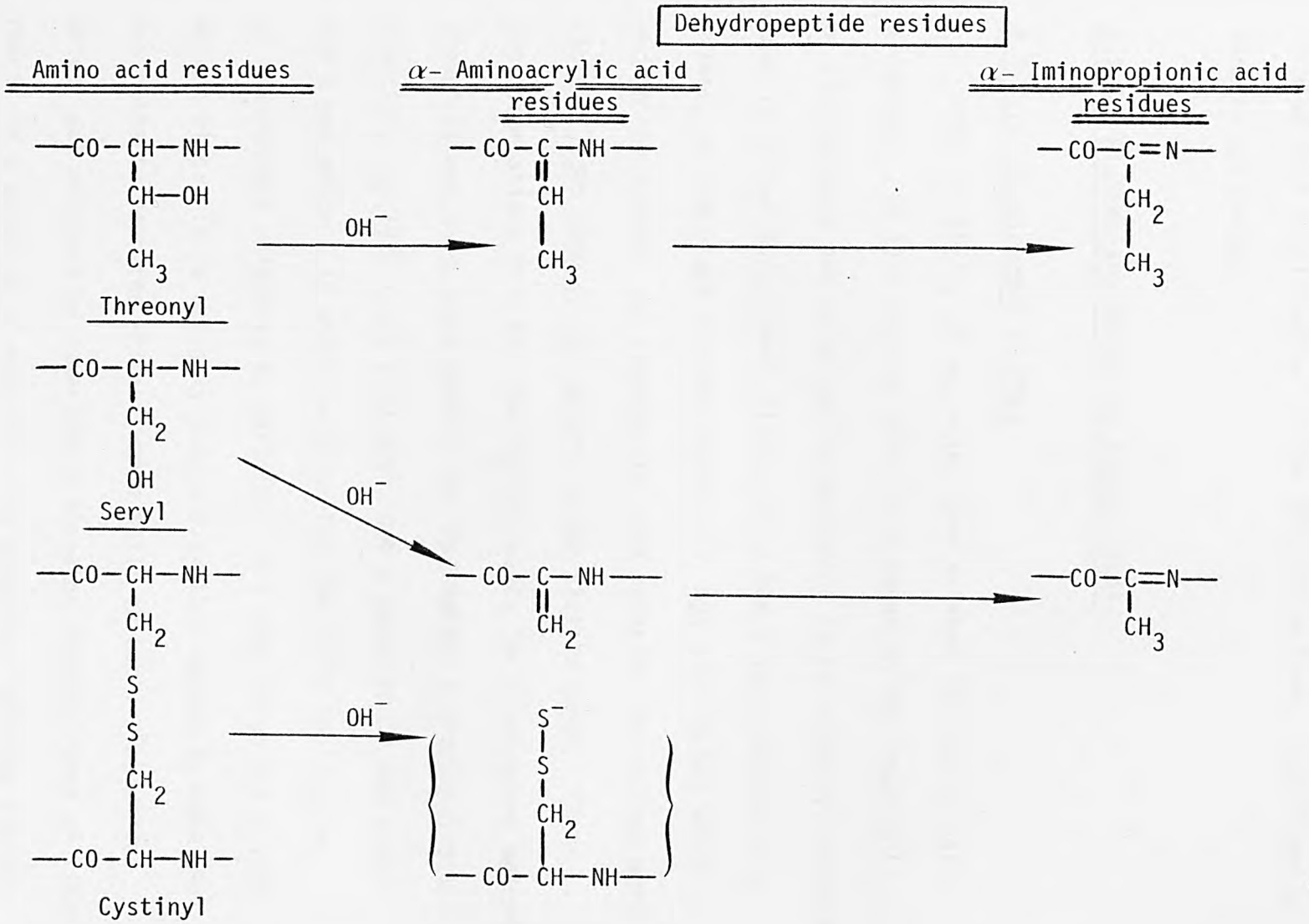


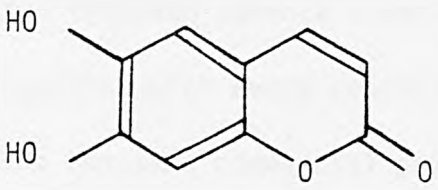
Figure 2.22. Action of alkali on the amino acid residues of wool.

involvement of cystinyl residues is further substantiated by the finding that stabilisation of the disulphide bonds significantly reduces yellowing.

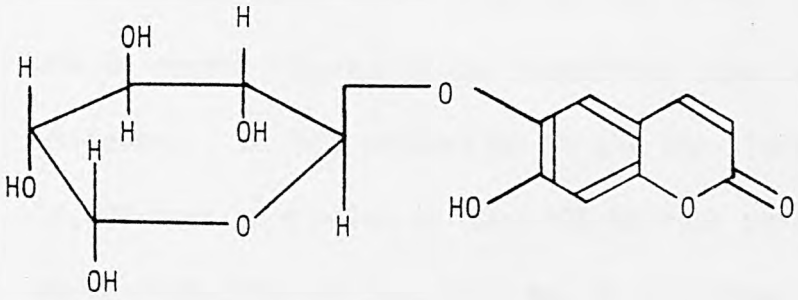
2.3.7. Fluorescent Whitening Agents (FWAs)

2.3.7.1. Development of FWAs

FWAs, in theory at any rate, have existed for nearly half a century. In 1921 Lagorio [73], in a paper on the importance of fluorescence and polarisation phenomena in colorimetry, observed that in colour measurement fluorescence has a very considerable effect, on the black content especially, but also on the white and on the colour; and fluorescing dyed material can reflect more visible light than it can absorb in the visible range. These two observations form the theoretical basis for fluorescent whiteners; though it was some years before the idea became a practical proposition. In 1929 Krais [74] published a paper on 'A new black and a new white', in which he described the first application of fluorescent whitening to textiles. His conclusion has a very modern ring: 'It is actually possible by this method to make what has hitherto been the whitest white still whiter'. The white effect was obtained by immersing a piece of viscose rayon or linen fabric in a solution of esculin. The treatment left the fibres, after drying, considerably whiter than before. The fluorescent portion of the esculin is 6,7-dihydroxy coumarin (6).



(6)



(7)

Esculin occurs in nature as aglucoside (7), that is, etherified with a sugar group, for example in extract of horse chestnuts. There were certain shortcomings attached to this, the first fluorescent whitening process. Since esculin has no affinity for cellulose, the white effect disappeared when the fabric was rinsed in cold water, and also when the fabric was exposed to light it very quickly yellowed. There was no further progress in fluorescence whitening for some years, although it is interesting that patents were taken out on a great variety of compounds for other uses without their fluorescence whitening properties being recognised. Some of these are still being produced and marketed in considerable quantities.

2.3.7.2. Fluorescent whiteners in industry

The industrial development of fluorescent whiteners began about 1940. Since then it has gone from strength to strength, until now it is difficult tokeep count of the enormous number of products available. It is enough to say that there are about

two thousand patents covering a vast number of compounds and systems, together with every conceivable application. There are about two thousand commercial products on the market from about two hundred manufacturing or distributing firms. Unfortunately there are no recent figures on the industrial importance of fluorescent whiteners. In 1965 production in the USA alone [75] was about 14,000 tons at a value of some 150 million Swiss francs (£15m). World production in that year may be estimated at 220-250 million Swiss francs (£22-£25m). These figures are likely to be very much higher today, considering the growing demand for maximum white effects in the race to be 'whiter than white'.

2.3.7.3. Mode of action

In the raw (undyed) state, articles such as textiles, paper and plastics, which are composed primarily of organic materials, absorb some of the daylight which strikes them (Portion A - Fig.2.23), particularly in the blue range (400-480nm). This lends them a more or less pronounced yellow appearance (Case a - Fig. 2.23).

This yellow tint, which is generally attributable to coloured impurities, can be reduced by chemical bleaching but not completely eliminated. An attempt is made with textiles to compensate for this yellow appearance by blue tinting, the blue dye absorbing the excess yellow portion to produce a relative increase in the reflected blue portion. This makes the object appear whiter. The correction, however, is only obtained at the expense of light

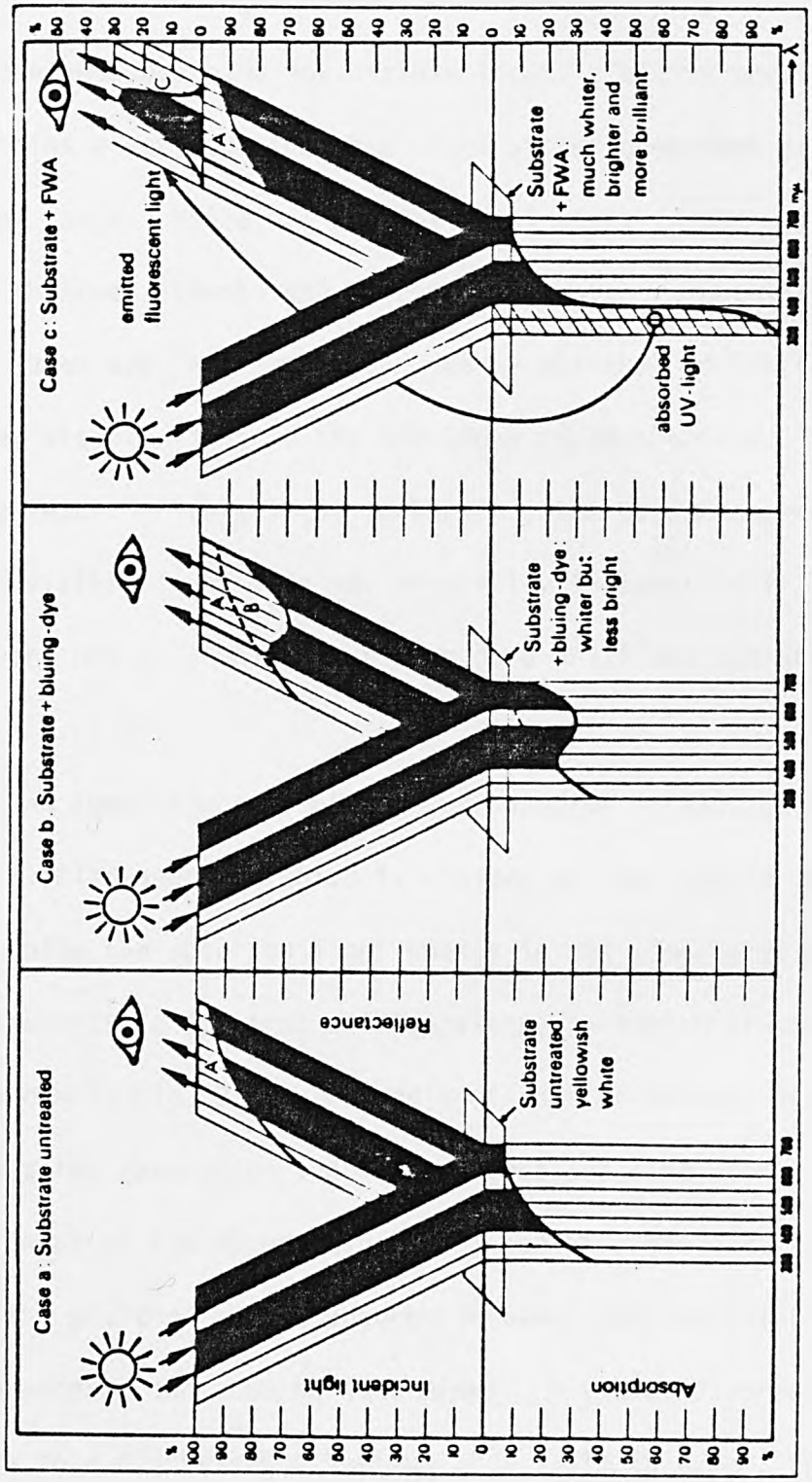


Figure 2.23. Absorption and reflectance of daylight on white surfaces.

reflected (Portions A and B, case b - Fig. 2.23).

Fluorescent whiteners, though, are substances which can provide the blue-violet light needed to compensate the yellow, without themselves absorbing any visible light. This is possible as daylight contains a very considerable ultra-violet component in the 300-400nm range. While not absorbing any light in the visible portion, the whitener absorbs the UV-component which is not visible to the human eye, and remits the energy absorbed in the form of longer-wave, visible light in the 400-500nm range (Portion C - Fig.2.23). As a result of this light conversion, the object apparently reflects more visible light than was originally incident on it, and therefore appears not only whiter but also more brilliant and brighter (Case C - Fig. 2.23).

An ideal fluorescent whitener absorbs in that part of the ultraviolet spectrum which is closest to the visible spectrum and emits the absorbed light energy in the short-wavelength region of the visible spectrum as fluorescent radiation of various hues as shown in Fig. 2.24. According to one of Stokes' laws (- spectral curves for absorption and fluorescence are like mirror images) the shape of the fluorescence band can be predicted from the shape and the position of the absorption band, and the colour of the fluorescence can thus be determined. A violet fluorescence corresponds to a fluorescence maximum at λ = 415 to 429nm, for λ = 430 to 440nm it is blue and for λ = 441 to 466nm it is greenish-blue. In addition to the localization of the maximum, the relative

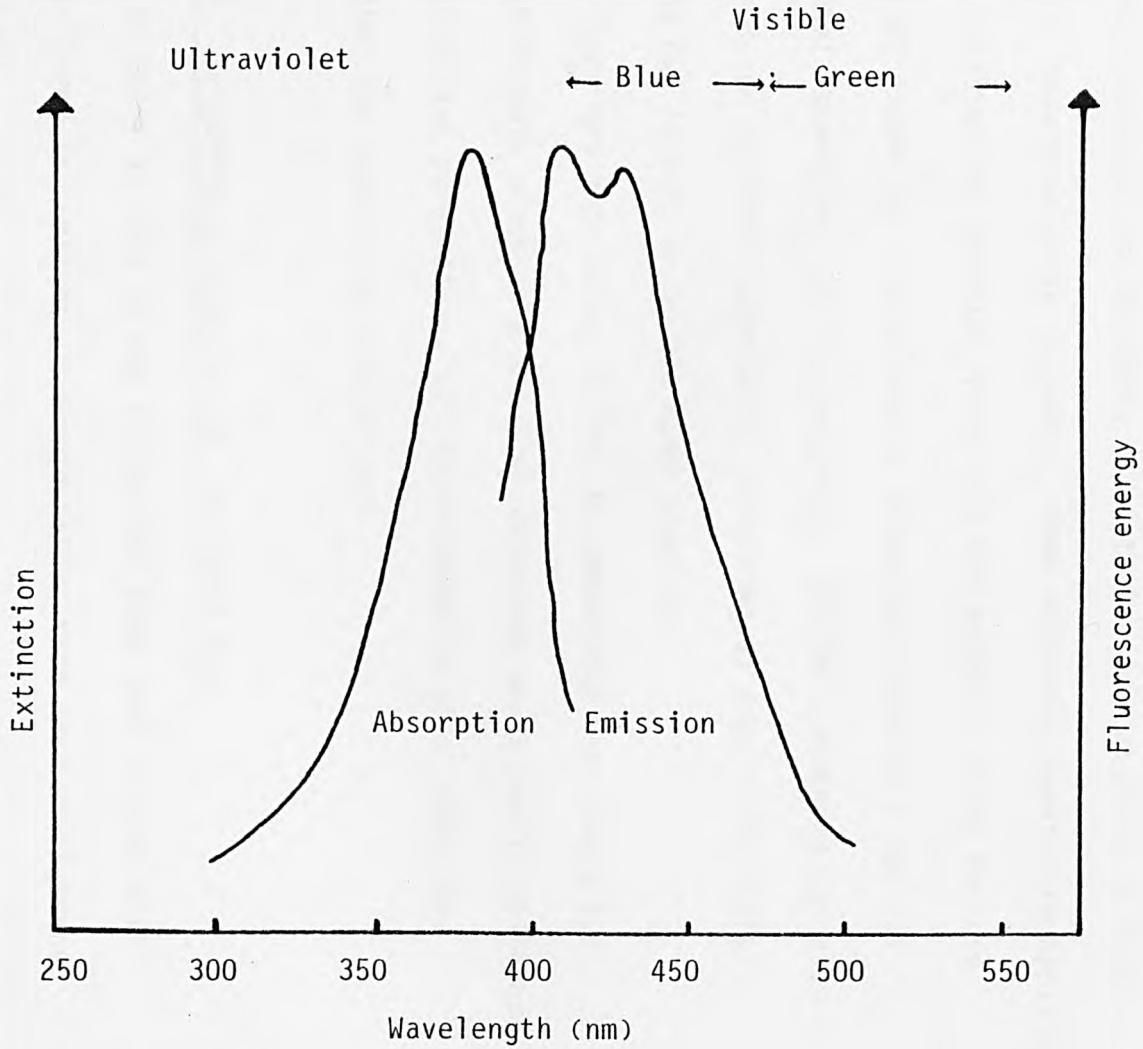


Figure 2.24. Absorption and emission spectra of a typical FWA.

distribution of the emitted light within the emission band is also important. The peak of fluorescence lies within very narrow limits of wavelengths (about 10nm). An ideal FWA should possess a strong absorption at λ = 350nm, maximally shifted to λ = 400nm; the absorption band should drop steeply into the visible region (Fig. 2.24) and the absorbed energy must be transformed to fluorescence as quantitatively as possible, so that the quantum yield of fluorescence should be close to unity. When whitening agents are applied to substrates the quantum yields are not equal to unity because of other competing intermolecular processes, causing a partial or total "quenching" of fluorescence. Similar quenching can also be produced by other substances, occurring as impurities either in the FWA itself, or in the dyed substrate.

There are many types of FWAs in commercial use (Table 2.3), but three main groups are used for whitening wool; namely stilbenes, coumarins and pyrazolines, all substituted to give solubility in water and reactivity towards wool.

2.3.8. Yellowing of Fluorescent Whitened Wool

As early as 1956 it was recognised that wool treated with FWA yellowed in sunlight at a much faster rate than unbrightened wool [76], (see Fig. 2.13). The action spectrum for the yellowing of brightened wool clearly shows that the energy absorbed by the FWA is responsible for this accelerated yellowing [18] (see Fig. 2.15). Possible causes include the decomposition of the FWA to give yellow

Table 2.3. Basic chemical structures of FWAs with the most important fields of application of their derivatives. (p.87-88) removed for copyright reasons

products and the photosensitised yellowing of the wool by the FWA.

Detailed studies of the photodecomposition of several types of FWA on wool have shown that when brightened wool is irradiated the brightening agents are degraded at a much slower rate than the rate of wool yellowing, and virtually no yellow substances could be found among the FWA decomposition products [77]. Whereas the presence of oxygen is required for the rapid photoyellowing of whitened wool [18], its presence has little effect on the rate of decomposition of many of the FWA which accelerate the yellowing [78]. Furthermore, the rate of photoyellowing of wool containing FWA of varying photostability is independent of the whitener used [79]. Thus it is apparent that the photodecomposition of the FWA does not contribute to the accelerated photoyellowing of brightened wool.

Considerable evidence now exists to show that the FWAs are promoting wool yellowing by acting as photosensitisers. Such evidence includes the findings that histidine and tryptophan residues in wool are degraded much faster in the presence of FWA [80], and that whitened wool in which most of the tryptophan residues have been chemically modified, yellows at a significantly lower rate than whitened untreated wool [81].

The possibility that this photosensitisation is due to direct energy transfer from the excited state of the whitener to the wool must be discounted because of the various energy levels involved.

Other possible photosensitising reactions involve either hydrogen or electron transfer between the triplet FWA molecule and substrate, followed by reaction of the resulting substrate radical with ground state oxygen, or interaction of the triplet FWA molecule with oxygen to produce singlet excited oxygen. Since the presence of oxygen is essential for photosensitisation to occur and since the chemical changes which occur in the photosensitised wool are very similar, but more pronounced, to those occurring in non-brightened wool, where the singlet oxygen mechanism has been proposed, the latter of these two possibilities seems the more probable.

The fact that photosensitisation results in significant increased degradation of the amino acids histidine, tryptophan and methionine - those most susceptible to singlet oxygen attack [64] - supports this conclusion. Reactions involving electron or hydrogen transfer would be far less selective in regard to the amino acids involved. Further support for this mechanism has come from some recent work by Leaver [82]. By measuring the fluorescence of single wool fibres under conditions where the fluorescence of tryptophan could be distinguished from that of the whitener, he found that FWA, which had previously been shown not to bind closely to the tryptophan residues, could directly sensitise the photo-oxidation of tryptophan and that up to seven molecules of tryptophan were degraded for each molecule of whitening agent destroyed. Furthermore, both the rate of yellowing of brightened wool and the rate of tryptophan degradation could be significantly restricted by introducing sodium

azide into the fibre. Thus singlet oxygen seems to be playing a major role in the FWA photosensitised yellowing of wool. The yellow products responsible would be essentially the same as those produced by sunlight exposure of unbrightened wool, viz. N-formyl-kynurenine and kynurenine.

2.3.9. Processes for the Prevention of Photoyellowing

No satisfactory commercial process for the prevention of photoyellowing of wool has yet been devised although much work has been directed to this end. In general, chemical modification of the aliphatic side-chains of wool, for instanceacetylation of lysyl amino and tyrosyl hydroxyl groups and esterification of glutamyl and aspartyl carboxyl groups, does not protect wool against sunlight yellowing. Neither does fission of the cystinyl disulphide bonds, by reduction, and blocking of the resultant thiol groups by methylation; this appears to indicate that cystinyl residues are not necessarily involved in wool yellowing.

The one chemical modification which does reduce yellowing is conversion of the lysyl amino groups to thioureido groups by reaction with methyl isothiocyanate [83] in dimethylformamide. However, this is not a feasible commercial treatment and the level of protection achieved is not high.

Wool has been impregnated with many chemical substances in an effort to protect it against photoyellowing (one study involved 270 compounds) but only a handful have shown any promise. One

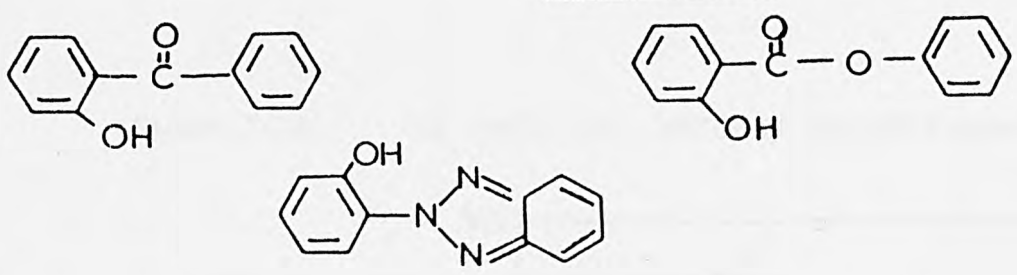
class of compounds, known as "ultra-violet screens" is used commercially to protect synthetic fibres and plastics against ultra-violet degradation. They absorb ultra-violet light and dissipate the energy harmlessly but their effectiveness on wool is not sufficient for commercial exploitation. Similarly low levels of protection are found with miscellaneous compounds such as alkylorthophosphate esters, tetraalkyl titanates, zinc stearate, alicyclic amines, thiourea and various reducing agents such as sodium hydrosulphite and thiourea dioxide. The structures of some of these compounds are shown in Fig. 2.25.

There are two methods which give good protection but for reasons discussed below these do not seem to be feasible commercially.

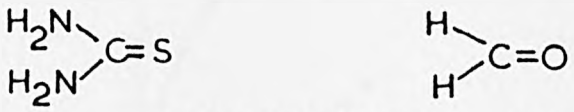
The first involves impregnation with either a mixture of thiourea and formaldehyde or a condensate of these two chemicals followed by thermal curing [84]. As can be seen from Fig.2.26, the treatment is especially effective in retarding the yellowing of wet wool. Similarly good protection is obtained if the treatment is applied to wool previously peroxide bleached and/or fluorescently whitened. In these cases the order of treatment is important, best effects being obtained with thiourea-formaldehyde as an after-treatment. Using fairly high levels of thiourea (up to 10% on the weight of the fibre) and formaldehyde (up to 20% on the weight of the fibre), lightfastness ratings for fluorescent-whitened wool, tested in the dry state, can be raised from 3 to 5 or more; this represents a 4-fold increase in the lightfastness. However, the process has

Figure 2.25. COMPOUNDS WHICH RETARD PHOTOYELLOWING

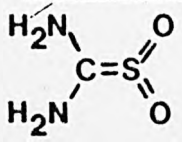
U.V. SCREENS



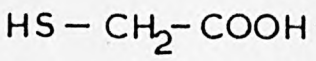
THIOUREA-FORMALDEHYDE



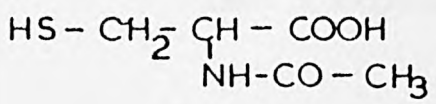
THIOUREA DIOXIDE



THIOGLYCOLLIC ACID



RELATED COMPOUNDS



THPC

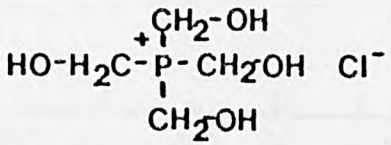
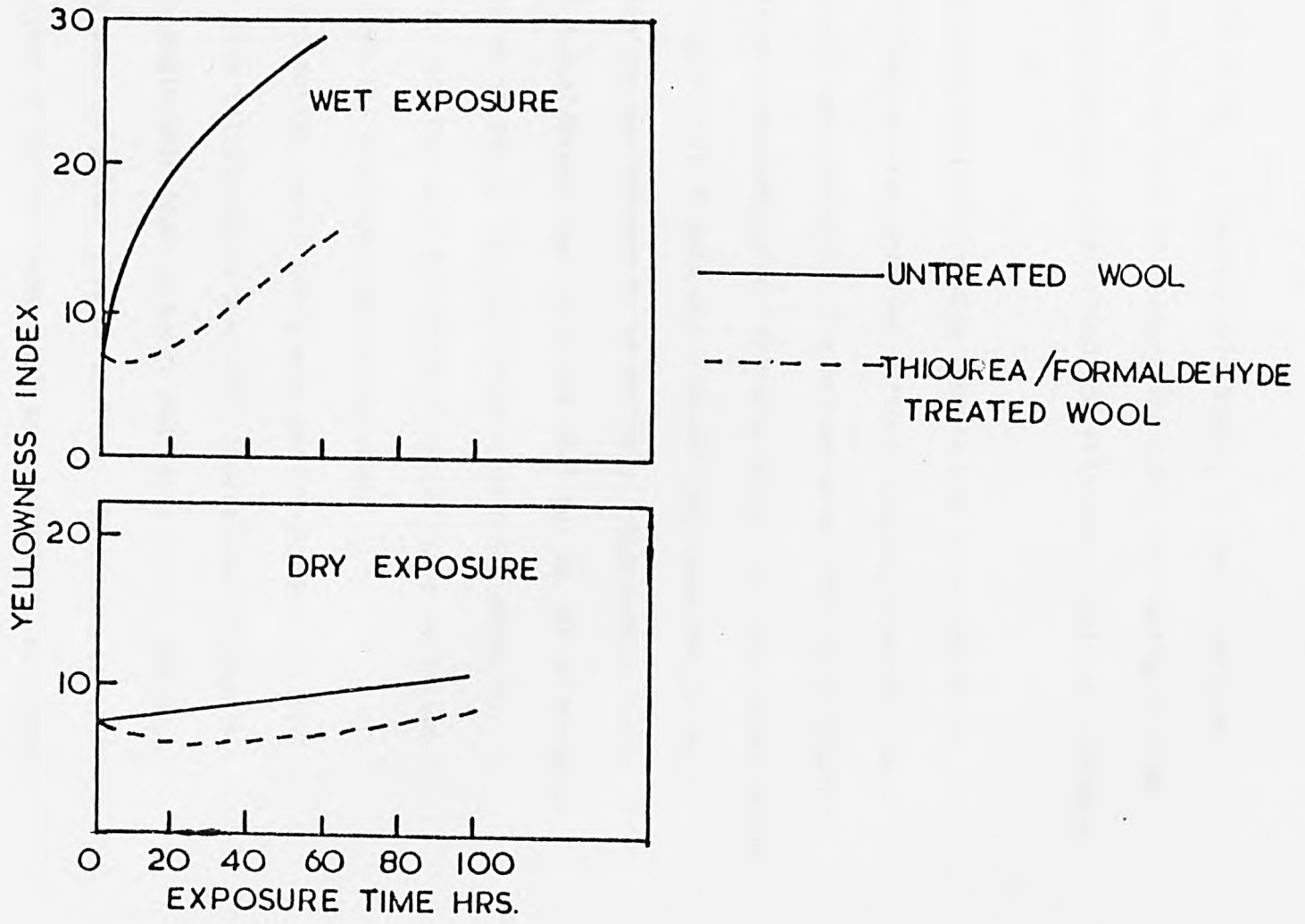


Figure 2.26. THE PROTECTIVE EFFECT OF THIOUREA/FORMALDEHYDE RESINS [84].



several deficiencies. A considerable amount of the protection is lost on the first (but not subsequent) wash, the fading of some dyes in pastel depths is accelerated and effective levels of treatment are costly.

Many thiourea derivatives have been tested in an effort to find a more effective treatment but without success, even for compounds of greater washfastness. The mechanism of protection remains obscure, neither formaldehyde nor thiourea alone, nor urea-formaldehyde resins show significant effects and ultra-violet absorbing or anti-oxidant mechanisms are thought to be unlikely. Apparently, both thiourea and formaldehyde react with the wool but as yet no evidence for modification of any of the light-sensitive amino acids has been obtained. Further work is needed to establish a mechanism and then perhaps the treatment can be improved.

The second method, which gives even better protection, is impregnation with thioglycollic acid [85] and related compounds. Those which contain both free carboxyl and thiol groups are most effective but some sulphides give protection. Compounds containing sulphur in higher oxidation states such as sulphoxides, sulphones, sulphuric and sulphonic acids afford no protection. Unless low levels of thioglycollic acid are used, excessive photobleaching occurs with natural wool and lightfastness ratings are poor for this reason rather than because of yellowing. For peroxide bleached and/or fluorescent-whitened wool yellowing still occurs although at a reduced rate. The drawbacks of this treatment are that it

sensitizes the wool to both alkali yellowing and metal staining and the protective effect is not fast to washing. Treatment with certain metal salts overcomes the first deficiency but no solution has been found for the others.

The mechanism of protection by these thiols and sulphides is obscure but has been attributed to their ability to act as anti-oxidants and to destroy hydroperoxides which otherwise could have initiated reactions leading to coloured products. In view of the sensitisation to bleaching of natural wool, it is possible that the apparent protective effect is in part due to bleaching of the yellow products of irradiation.

Tetrakishydroxymethyl phosphonium chloride (THPC) was shown by Milligan et al. [86] to protect white wool from yellowing especially in the wet state but the effect was not durable to washing. In an attempt to overcome the latter problem sulphonated triphenylphosphine was prepared, found to be substantive to wool but was only effective in reducing photoyellowing at high add-ons (15% of weight fibre) [86].

All of the methods described above have been aimed at preventing yellowing by modification of the wool itself or by impregnation with a chemical agent which will prevent degradation and yellowing. However, as stressed earlier, the overwhelming commercial problem is the accelerated yellowing of wool in the presence of fluorescent whitening agents. If stable fluorescent whitening agents, which do not sensitise but whiten the wool at an acceptable level, could

be found it is doubtful whether methods of preventing yellowing would be required.

2.3.10. REFERENCES

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CHAPTER 3

FLUORESCENT WHITENING OF WOOL WITH PHTHALIMIDES

3.1 INTRODUCTION (also see Chap.2, pp.80-91)

There are many types of fluorescent whitening agents (FWAs) on the market today [1]. Their main applications are in the fields of textile, paper, plastic and detergent industries [2]. The commonly used FWAs for wool are either stilbenes, pyrazolines or coumarins. These whiten the wool to an acceptable level but are found to lead to photoyellowing. The yellowing occurs at a much faster rate than untreated or peroxide bleached wool [3]. There is considerable evidence to suggest that the FWAs are promoting wool yellowing by acting as photosensitisers. This may be via singlet oxygen, produced by interaction of the triplet FWA molecule with ground state oxygen [4, 5]. The excited oxygen produced by this manner can cause similar chemical changes as those occurring in non-whitened wool [6] and this may explain the pronounced yellowing of FWA treated wool. It has also been observed that the FWAs photo-decompose either in solution or on wool leading to the formation of photoproducts which do not fluoresce in the visible region [1]. This additional degradation also leads to a loss of the whitening of the fabric. Fluorescent whitened wool samples show a lightfastness rating of 3 obtained by the British Standard Method against blue standards in the Xenotest 150.

This chapter describes a series of fluorescers, viz, the phthalimides, which have been synthesised, their fluorescence quantum yields have been determined and some have been applied to wool. Some phthalimides have been shown to possess very high

quantum yields of fluorescence (close to unity) but when applied to wool show lightfastness rating typical to that of any other conventional FWA.

3.2 Experimental (All absorptions occurring in the i.r. spectrum of a compound are not quoted.)

A * below indicates that the compound (or the product from the reaction) has not been reported in the literature.

3.2.1. Preparation of 4-nitrophthalic anhydride [7].

4-Nitrophthalic acid was refluxed in an excess of acetic anhydride for 45 minutes. The reaction mixture was concentrated under reduced pressure to give a brown oil which turned into crystals upon cooling. Pale brown crystals were obtained after filtering the wet crystalline substance and drying it over sodium hydroxide pellets (Yield, 93%).

Analysis

Melting point: 94 - 107°C (Lit. m.p. = 114°C (sharp)[8]).

IR (nujol mull): 1843 and 1775 cm^{-1} (due to C=O stretches in the anhydride).

3.2.2. Preparation of 4-hydroxyphthalic anhydride [9].

4-Hydroxyphthalic acid was heated in a Kugelrohr to approximately 200°C under reduced pressure. The white solid product was collected in the cold end of the bulb tube and dried in a

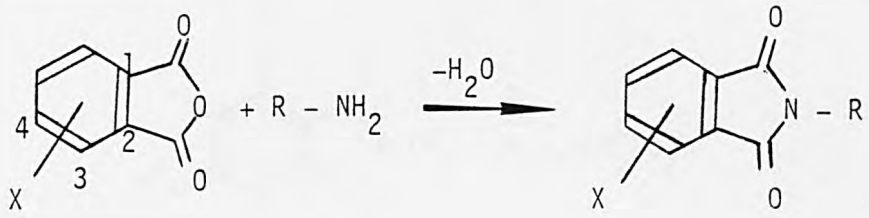
vacuum dessicator.

Analysis

IR (nujol mull): 1750 and 1840 cm^{-1} (due to C=O stretches in the anhydride).

3.2.3. Method of preparation of phthalimides 1 - 5.

The general method employed is summarised by the following equation:-



For X = 3-NO_2, R = $\text{-}(CH_2)_5CO_2H$(1),

$\text{-}(CH_2)_3CH_3$(2), - Ph(3).

When R = $\text{-}(CH_2)_5CO_2H$, X = 4 - NO_2(4) or

4 - OH(5)

Equimolar quantities of the appropriate phthalic anhydride and amine were fused at a melt between 180 - 200°C in a Woods metal bath for 1 - 2 hours. A reflux condenser was connected to the reaction vessel for reactions with compounds having R as $\text{-}(CH_2)_3CH_3$ and -Ph. The water formed during the condensation reaction was evaporated by maintaining the reaction vessel (but without the condenser equipped to it) on the metal bath (at 180 - 200°C). The water that was formed for the reactions with the compound having R as $\text{-}(CH_2)_5CO_2H$ escaped the vessel whilst the condensation

was in progress. The liquid which remained solidified upon cooling and was recrystallised using ethanol - water mixture.

Analysis

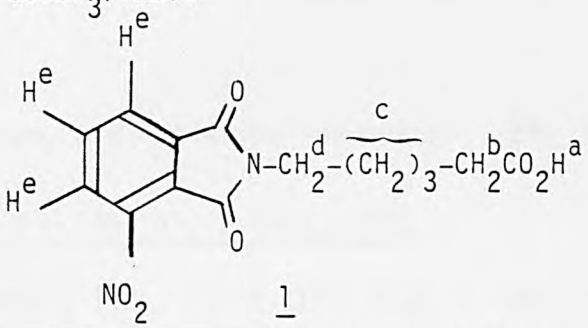
X = 3-NO_2, R = $(CH_2)_5CO_2H$(1), [Yield, 77%].*

Melting point : 152 - 154°C

Elemental analysis : Calculated for $C_{14}H_{14}N_2O_6$:

C, 54.90; H,4.61; N, 9.15. Found : C, 55.07; H, 4.64; N, 9.01.

Proton nmr ($CDCl_3$/TMS):



1

1.16 - 2.00δ (m,6H, due to c protons), 2.32δ (t, 2H, due to b protons), 3.68δ (t, 2H, due to d protons), 7.68 - 8.08δ (m, 3H, due to e protons), but no signal was observed for the carboxylic proton (a).

IR (nujol mull) : 1692 cm^{-1} (C=O stretch of -COOH), 1720 cm^{-1} and 1776 cm^{-1} (due to C=O absorptions in -CO-N-CO-) and 2500 - 3260 cm^{-1} (O-H stretch).

Mass spectrum, m/e (relative intensity) : 306 ($M^{+\cdot}$, 0.6%), 262 ($-CO_2$, 2.5%), 205 (100%).

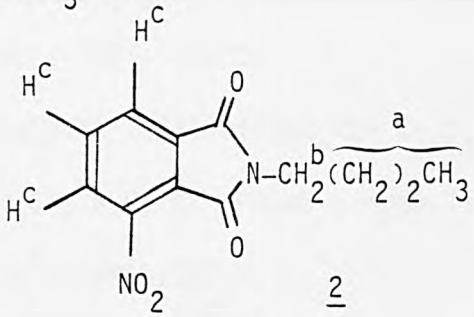
X = 3-NO_2, R = $(CH_2)_3CH_3$ (2), [Yield, 46.5%].

Melting point : 67 - 70°C (Lit. m.p. = 71 - 72°[10]).

Elemental analysis : Calculated for $C_{12}H_{12}N_2O_4$:

C, 58.06; H, 4.87; N, 11.29. Found : C, 58.18; H, 5.10; N, 11.00.

Proton nmr ($CDCl_3$/TMS) :



2

0.84 - 1.84δ (m, 7H , due to a protons), 3.73δ (t,2H, due to b protons) and 7.80 - 8.20δ (m, 3H, due to c protons).

IR (nujol mull) : 1700 and 1768cm^{-1} (due to C=O absorptions in -CO-N-CO-).

Mass spectrum, m/e (relative intensity) : 248 ($M^{+\cdot}$, 11.9%), 231(100%).

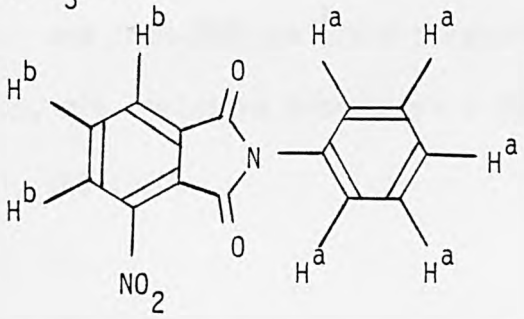
X = 3-NO_2, R = -Ph (3), [Yield, 77%]

Melting point : 138 - 141°C (Lit. m.p. = 136-137°C [11]).

Elemental analysis : Calculated for $C_{14}H_8N_2O_4$:

C, 62.69; H, 3.01; N,10.45. Found : C, 62.41; H, 3.16; N, 10.56.

Proton nmr ($CDCl_3$/TMS):



3

7.34δ (single resonance, 5H, due to a protons) and 7.72 - 8.14δ (m, 3H, due to b protons).

IR (KBr disc) : 1730 and 1777cm^{-1} (due to C=O absorptions in -CO-N-CO-)

Mass spectrum, m/e (relative intensity) : 268 ($M^{+\cdot}$, 38.7%), 75(100%).

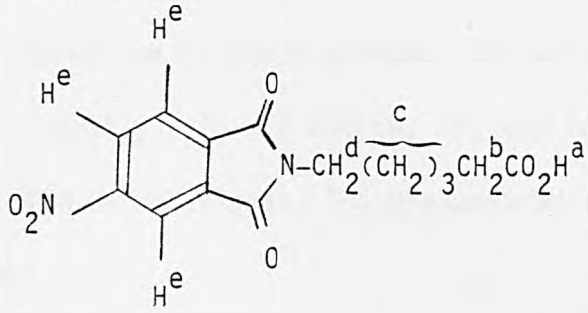
X = 4-NO_2, R = $(CH_2)_5CO_2H$(4), [Yield, 55%]*

Melting point : 107 - 125°C.

Elemental analysis : Calculated for $C_{14}H_{14}N_2O_6$:

C,54.90; H,4.61; N,9.15. Found : C,55.11; H,4.62; N,9.27.

Proton nmr (d^6 - DMSO/TMS):



4

1.00 - 1.84δ (m,6H, due to c protons), 2.22δ (t, 2H, due to b protons), 3.68δ (t, 2H, due to d protons), 8.08 - 8.92δ (m, 3H, due to e protons), but no signal was observed for the carboxylic proton (a).

IR (nujol mull) : 1700 and 1770cm^{-1} (due to C = O absorptions in -CO-N-CO-) and 2400-3300 cm^{-1}(O-H stretch).

Mass spectrum, m/e (relative intensity) : 306 ($M^{+\cdot}$, 2.6%), 262 (-CO_2, 0.3%), 205 (100%).

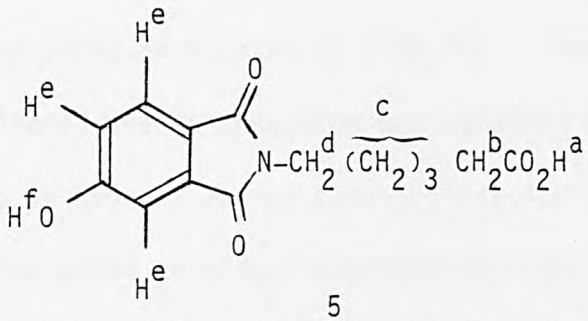
X = 4-OH, R = $(CH_2)_5CO_2H$ (5)*

Melting point : 150 - 156°C.

Elemental analysis : Calculated for $C_{14}H_{15}NO_5$:

C,60.64; H,5.45; N,5.05. Found : C,60.55; H,5.47; N,5.09.

Proton nmr (d^6 - DMSO/TMS) :



5

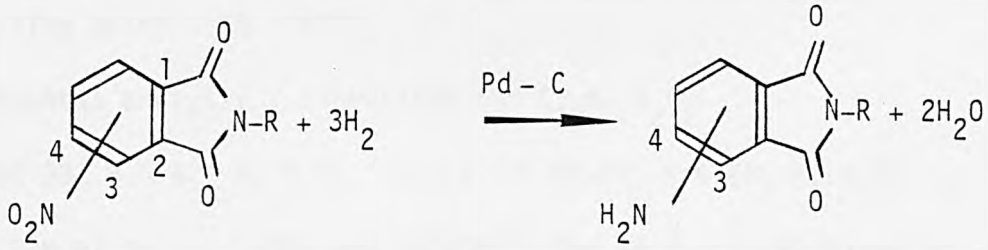
1.00 - 1.80δ (m,6H, due to c protons), 2.24δ (t,2H, due to b protons), 3.60δ (3H, a triplet due to the d protons (2H) which superimposed the broad $-OH^f$ signal), 7.16 - 7.92δ (m, 3H, due to e protons) and 10.40 - 12.40δ (broad signal, 1H, disappeared on D_2O shake, due to a proton).

IR (KBr disc) : 1700 and 1770cm^{-1} (due to C = O absorptions in -CO-N-CO-).

Mass spectrum, m/e (relative intensity) : 277 ($M^{+\cdot}$, 3.7%), 233 ($-CO_2$, 1.8%), 176 (100%).

3.2.4. Method of preparation of aminophthalimides 6 - 9.

The method employed is summarised by the following equation:-



When $-NH_2$ is in the 3- position R = $\leftarrow CH_2)_5CO_2H$ (6), $\leftarrow CH_2)_3CH_3$(7), -Ph(8) and when it takes up the 4- position R = $\leftarrow CH_2)_5CO_2H$(9).

A solution of the appropriate nitrophthalimide (1 - 4, see above) in the appropriate solvent (ethanol in the case when R = $\leftarrow CH_2)_5CO_2H$ and -Ph and glacial acetic acid when R = $\leftarrow CH_2)_3CH_3$)

was hydrogenated using Pd-C catalyst (10% Pd on charcoal). The mixture was filtered when the reaction was complete [the product 8 was insoluble in ethanol and so a hot filtration was necessary (more ethanol was added to dissolve completely the insoluble compound)]. The solvent was evaporated from the filtrate to leave a solid [the product 8 was recrystallised from ethanol].

Analysis

Phthalimide 6.*

Melting point : 138 - 143°C.

Elemental analysis : Calculated for $C_{14}H_{16}N_2O_4$:

C,60.86; H,5.84; N,10.14. Found : C,60.96; H,5.90; N,9.94.

IR (nujol mull) : 3340 and 3460cm^{-1} (due to N-H stretchings of the primary amine group).

Phthalimide 7, [Yield, 78%].*

Melting point : 50 - 59°C.

Elemental analysis : Calculated for $C_{12}H_{14}N_2O_2$:

C,66.03; H,6.47; N,12.84. Found : C,65.85; H,6.68; N,12.60.

IR (nujol mull) : 3340 and 3470cm^{-1} (due to N-H stretchings of the primary amine group).

Phthalimide 8, [Yield, 80%].

Melting point : 179 - 191°C (Lit. m.p. = 186 - 7°C [12]).

Elemental analysis : Calculated for $C_{14}H_{10}N_2O_2$:

C,70.58; H,4.23; N,11.76. Found : C,70.64; H,4.17; N,11.88.

IR (KBr disc) : 3360 and 3480cm^{-1} (due to N-H stretchings of the primary amine group).

Phthalimide 9.*

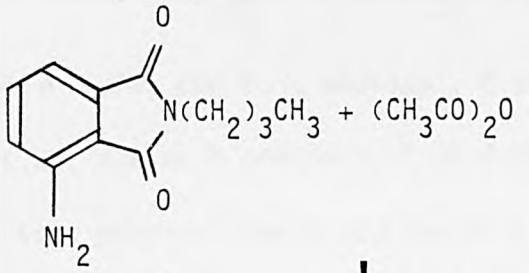
Elemental analysis : Calculated for $C_{14}H_{16}N_2O_4$:

C,60.86; H,5.83; N,10.13. Found : C,60.82; H,5.87; N,10.08.

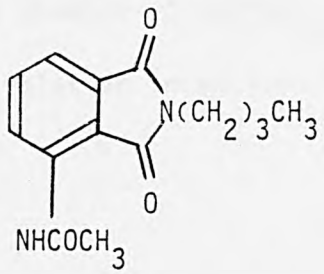
IR (nujol mull) : 3348 and 3458cm^{-1} (due to N-H stretchings of the primary amine group).

3.2.5. Acetylation of N-butyl - 3 - aminophthalimide (7).*

This acetylation reaction is depicted by the following equation:



7



10

7(0.4g, 1.8mmol) in an excess of acetic anhydride (20ml, 21.64g, 0.21 mol) was refluxed overnight. The cooled refluxed mixture was then added to 300 ml water and left to stand overnight at room temperature. The brown crystalline product, 10, was filtered and recrystallised using ethanol-water mixture (yield, 19%).

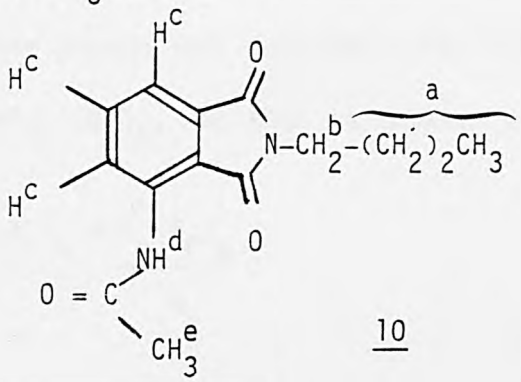
Analysis

Melting point : 100 - 106°C.

Elemental analysis : Calculated for $C_{14}H_{16}N_2O_3$:

C,64.60; H,6.20; N,10.76. Found : C,64.55; H,6.16; N,10.50.

Proton nmr ($CDCl_3$/TMS) :



10

0.80-1.92 δ (m,7H, due to a protons), 2.32 δ (s,3H, due to e protons), 3.76 δ (t,2H, due to b protons), 7.56-7.96 δ and 8.92-9.08 δ (m, 3H, due to c protons) and 9.80 δ (broad single resonance, 1H, due to d proton).

IR (nujol mull) : 1635cm^{-1} (due to C=O stretch in $-NHCOCH_3$) and 3380cm^{-1} (due to N-H stretch in $-NHCOCH_3$).

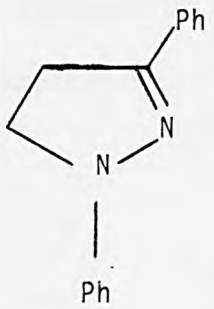
Mass spectrum, m/e (relative intensity) : 260 ($M^{+\cdot}$, 17%), 175 (100%).

3.2.6. Fluorescence Quantum Yield Measurements

Fluorescence spectra of degassed(by flushing with a stream of dry argon for approximately 5 minutes) solutions of the phthalimides (made up to an optical density of 0.1 in either methanol or ethanol at the excitation wavelength) were obtained at room temperature using a Perkin-Elmer MPF-4 fluorescence spectrophotometer. Quantum yields of fluorescence, $(\emptyset_f)_b$, of the phthalimides were determined relative to that from a degassed solution of 1,3 - diphenyl - 2 - pyrazoline, 11[$(\emptyset_f)_a$ = 0.23 in methanol [13]]. Quantum yields were calculated by comparison of the integrated areas (A) bounded by the uncorrected spectra of the phthalimide with the corresponding areas for the pyrazoline, recorded under similar conditions. The quantum yield, $(\emptyset_f)_b$, was then calculated with the equation

$$(\emptyset_f)_b = \frac{A_b(\emptyset_f)_a}{A_a}$$

where A_a and A_b are the integrated areas under the uncorrected fluorescence spectra of the pyrazoline and the phthalimide, respectively.* Quantum yields derived from a range of settings were averaged out.



11

(Synthesised as in ref. [14]).

* See Appendix C

The fluorescence quantum yield of 11 in ethanol (0.47) was obtained by comparing the integrated areas of the fluorescence spectra of 11 in methanol and ethanol (optical density = 0.1 in each solvent at the excitation wavelength; spectra taken under similar conditions). The following equation was then used in determining the fluorescence quantum yield:

$$(\emptyset_f)_{ethanol} = \frac{A_{ethanol}(\emptyset_f)_{methanol}}{A_{methanol}}$$

where $(\emptyset_f)_{ethanol}$ and $(\emptyset_f)_{methanol}$ are the unknown and known fluorescence quantum yields of 11 in ethanol and methanol, respectively, and $A_{ethanol}$ and $A_{methanol}$ are the integrated areas bounded by the uncorrected spectra of 11 in ethanol and methanol, respectively.

3.2.7. Wool bleaching

Wool serge fabric (botany serge, 2/2 twill of weight 200g/m^2) was bleached overnight by a pad-batch method (room temperature), padding on (100% wet pick-up) 80g/l hydrogen peroxide (100 volumes, about 30% w/v H_2O_2), 5g/l formic acid (95-97%) and 10g/l Lissapol N (ICI) [15]. This was followed by rinsing in cold water and drying in air at room temperature.

3.2.8. Application of aminophthalimides 6 and 9 to wool

3.2.8.1. Method 1 : Exhaustion onto wool serge fabric (buffered to pH 7).

0.5 and 1.0% of weight fabric (o.w.f.) phthalimide (1g each of 6 and 9 were dissolved innet volumes of 25ml and 10ml acetone, respectively. The appropriate volumes to correspond with the required amounts of phthalimides were added to the baths).

3.0% o.w.f. thiourea dioxide.

1.0% o.w.f. ethylenediaminetetraacetic acid, disodium salt, dihydrate.

1.0% o.w.f. Lissapol N.

Exhaustion was carried out using liquor ratios of 30:1 at pH 7 (the pH was adjusted with sodium bicarbonate). The temperature was raised to 80°C at 1°C/min. and maintained for one hour, followed by rinsing in cold water and drying at room temperature.

3.2.8.2. Method 2 : Exhaustion onto H_2O_2 bleached wool.

1.0% o.w.f. phthalimide (1g each of 6 and 9 were dissolved in net volumes of 25ml and 10ml acetone, respectively. The appropriate volumes to correspond with the required amount of phthalimides were added to the baths).

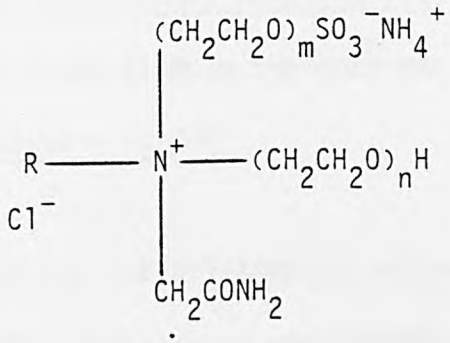
1.0% o.w.f. ethylenediaminetetraacetic acid, disodium salt, dihydrate.

1.0% o.w.f. Lissapol N.

1.0% o.w.f. Albegal B, 12 (Ciba-Geigy).

Exhaustion was carried out on pre-bleached wool samples using

liquor ratios of 30:1 at pH 4 (4% o.w.f. sodium acetate plus required acetic acid). The temperature was raised to 80°C at 1°C/min. and maintained for one hour, followed by rinsing in cold water and drying at room temperature.



$R \approx C_{18}H_{37}-$

$m + n = 7$

12

Albegal B (structure reproduced from ref.16).

3.2.9. Determination of % exhaustion of aminophthalimides 6 and 9 onto wool.

Liquor baths were made up using methods 1 and 2 in which 1.0% o.w.f. phthalimides 6 and 9 were present. The optical densities (after dilution by the same factor, of small equal volumetric fractions of the bath liquors at the beginning and after exhaustion) were measured on a Pye-Unicam SP8-100 spectrophotometer at the wavelength of maximum absorption for each phthalimide. The initial level of each bath liquor was maintained throughout the exhaustion period

by making small additions of water to compensate for loss due to evaporation. It was thus possible to determine the percentage exhausted onto the wool fibre by either method for each phthalimide.

3.2.10. Yellowness Index (Y.I.) Measurements

The yellowness index formula employed was the following:

$$Y.I. = \frac{100\ (1.316X - 1.164Z)}{Y}$$

where X, Y and Z and the CIE tristimulus values obtained from the Macbeth Micromatch reflectance spectrophotometer. The lower the yellowness index value the whiter is the wool colour.

3.2.11. Light fastness measurements

Dry wool samples were exposed to Grey Scale 4 using the Xenotest (original quartz Hanau) according to the ISO standard procedure (ISO 105-B02 : 1978).

3.2.12 Thin Layer Chromatography

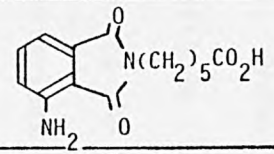
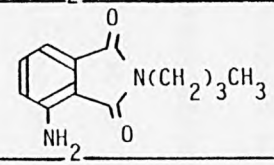
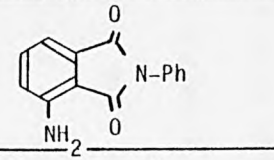
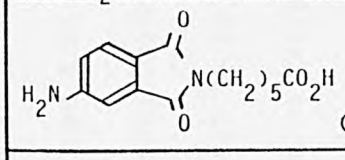
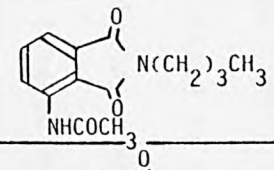
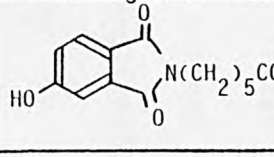
The purified aminophthalimides (6-9) were subjected to thin layer chromatography on silica gel sheets using ethyl acetate as the developing solvent. The developed plates were viewed under a UV lamp having a maximum output at 375nm.

3.3 RESULTS AND DISCUSSION

The fluorescence quantum yields of the synthesised phthalimides 1-10 were determined as described in the experimental section. The nitrophthalimides 1-4 show no fluorescence at all and are probably quenched by the presence of the nitro group. The values obtained for the other phthalimides are quoted in Table 3.1. Phthalimides bearing the amino group in the 3 position of the benzene ring (6-8) show strong fluorescence quantum yields with values close to unity. This meant that phthalimides of these types are probably very photostable. With the electron releasing groups (-OH or $-NH_2$) in the 4- position (phthalimides 5 and 9) the quantum yields drop tremendously. Acetylation of 7 led to a compound (10) with a low quantum yield. The sodium salt of 5 (in a strong aqueous NaOH solution with a pH of 13) also shows a very low quantum yield of fluorescence (≈ 0.02; measured relative to 1, 3-diphenyl-2-pyrazoline in methanol under similar conditions).

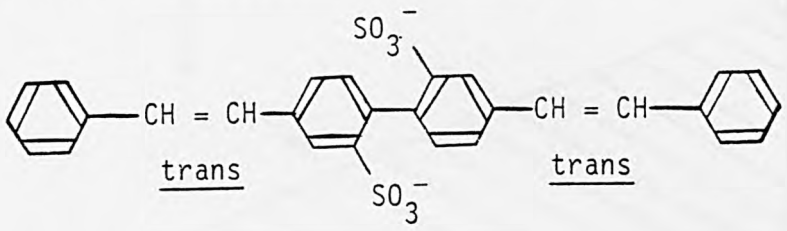
The phthalimides (5-10) were all hydrophobic and some were highly coloured (6-9). TLC analyses were carried out on these highly coloured solids to observe if the colouration were due to any minor impurities present despite elemental analyses being correct. The coloured major spots on the plates were highly fluorescent whereas other non-coloured spots were weakly fluorescent. Thus, we concluded that the compounds are intrinsically coloured and are highly fluorescent. The impurities are not coloured and

TABLE 3.1. Absorption and Fluorescence Data for Substituted Phthalimides in Ethanol or Methanol (Room Temp.).

Phthalimide	ABSORPTION λmax(nm)	EXCITATION WAVELENGTH (nm)	SOLVENT	FLUORESCENCE		
				λmax(nm)	YIELD[d]	COLOUR
 (6)	393	400	Ethanol	478	~1	Turquoise
 (7)	392	390	Methanol	483	~1	Turquoise
 (8)	398	370	"	485	0.95	Turquoise
 (9)	386	370	"	527	0.25	Green
 (10)	345	360	"	423	0.17	Blue
 (5)	335	370	"	–	0.00	None

d. Determined relative to 1,3-diphenyl-2-pyrazoline (see experimental section).

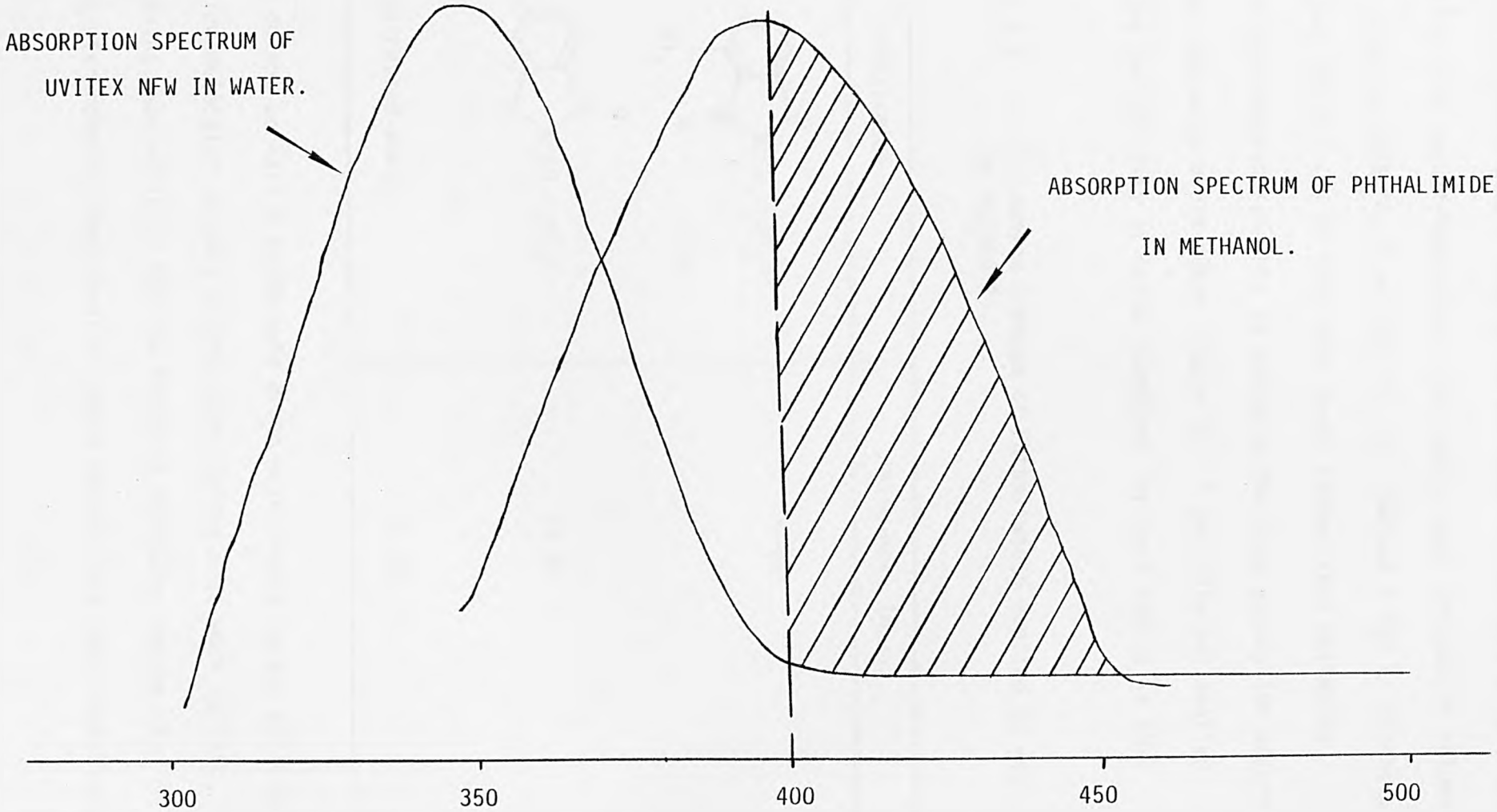
are only weakly fluorescent. Further evidence to support the above conclusion was obtained from their UV spectra which all showed strong absorption in the visible region. UV absorption spectra for a typical brightener (Uvitex NFW, Ciba-Geigy, 13) which has no absorption above 400nm and for phthalimide 8 are shown in Figure 3.1. The shaded area under the absorption spectrum of phthalimide 8 accounts for the colour observed in the compound.



13

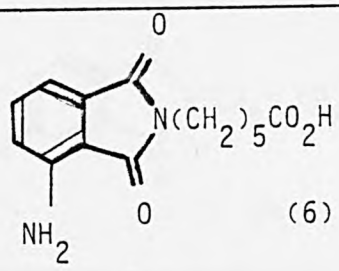
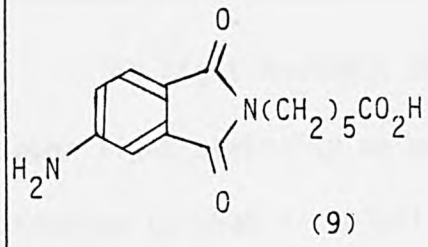
Phthalimides 6-8 possess very high fluorescence quantum yields. Despite their fluorescent colour (turquoise; a blue colour would be necessary to whiten wool fabric [17]), one of these (6) was applied to wool (see experimental section) in the hope of observing a high lightfastness (>3; this value may be observed as quantum yield implies that all the light that is absorbed is emitted as fluorescence). Phthalimide 9 was also applied to wool using similar application methods (these compounds were first dissolved in acetone and then added to the baths as they were water insoluble). Application of these phthalimides using Method 1 gave very poor exhaustion values (9% for phthalimide 6 and 23% for 9). Values as high as about90% should have been obtained [18]. Method 2 was tried in which Albegal B was present in the liquor bath. The presence

FIGURE 3.1


ABSORPTION SPECTRUM OF
UVITEX NFW IN WATER.
ABSORPTION SPECTRUM OF PHTHALIMIDE 8
IN METHANOL.
300
350
400
450
500
WAVELENGTH (nm)

of this compound should lead to an increase in the rate of exhaustion and thus give good exhaustions. Yet again, poor exhaustion values were obtained (36% for 6 and 33% for 9). Method 2 led to strongly coloured fabrics (as if they were dyed) rather than whitening and an explanation for this is probably the high absorption occurring in the compounds above 400nm (Table 3.2).A possible explanation for the low affinity of these compounds for wool fabric is that

TABLE 3.2 Yellowness indices of Phthalimides applied to wool (by Method 2).

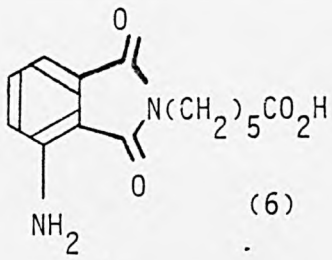
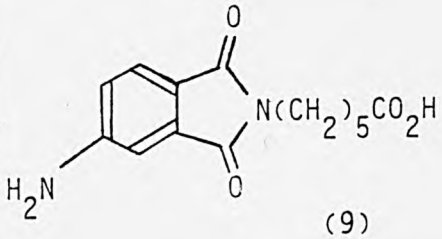
Phthalimide	Yellowness Index
 (6)	40.66
 (9)	43.97
Untreated wool	21.88

they do not contain a sulphonate group which would impact affinity. The low molecular weights of the phthalimides also work against obtaining good affinity for the fibre (a molecular weight of ~500 with correct functionality would result in a high exhaustion

value even at a high pH of the liquor bath).

Light fastness measurements were carried out on dry wool samples obtained using Method 2 and are quoted in Table 3.3.

TABLE 3.3 Light fastnesses of Phthalimides applied to wool (by Method 2).

Phthalimide	Light Fastness
 (6)	2-3
 (9)	3

The light fastness of compound 6 implicates that it may have poor light stability on wool surface and behaves in a different fashion to that in solution (quantum yield of fluorescence = 1 in ethanol indicates it is very photostable in solution). Compound 9 gave a light fastness as expected.

3.4. CONCLUSION

The statement that high fluorescence quantum yields observed with some of the phthalimides should lead to high light fastnesses when applied to wool is not true. The phthalimides behave in

a similar fashion to other conventional FWAs [18]. The poor affinity observed with phthalimides 6 and 9 towards wool fibre should also be the case for the other fluorescent compounds and good fixations can only be obtained by increasing their molecular weights and introducing correct functionality into them.

Appendix C

Fluorescence Quantum Yields ($\emptyset_f$) of Phthalimides

$\emptyset_f$ is defined as the following ratio:

$$\frac{\text{Rate of emission of photons}}{\text{Rate of absorption of photons}}$$

but rates of emission and absorption of photons are immeasurable. The following describes how relative quantum yields of fluorescence can be determined.

If a solution of c moles/litre of a compound with a fluorescence quantum efficiency of $\emptyset_f$, and a path length of 1 cm is placed in a monochromatic light beam of incident intensity I_o (quanta/sec) for which the compound has a molar extinction coefficient ϵ, the total fluorescence intensity I_f (quanta/sec) from the solution will be given by

$$I_f = I_o (1-10^{-\epsilon c l}) \emptyset_f \qquad (e)$$

For dilute solutions ($\epsilon c l \lesssim 0.02$), for which only a small fraction of the incident light is absorbed, (e) reduces to

$$I_f = 2.303\, I_o \epsilon c l \emptyset_f \qquad (f)$$

If relative fluorescence-intensity measurements are made in the same cell and monochromatic beam of incident light, using dilute solutions of a standard compound (1) of known fluorescence quantum efficiency $(\emptyset_f)_1$ and some compound (2) whose fluorescence efficiency $(\emptyset_f)_2$ is to be determined, it follows from (f) that $(\emptyset_f)_2$ can be calculated from

$$(\emptyset_f)_2 = \frac{(I_f)_2 \epsilon_1 c_1}{(I_f)_1 \epsilon_2 c_2} (\emptyset_f)_1 \qquad (g)$$

If the concentrations of the solutions are regulated so that

$\epsilon_1 c_1 = \epsilon_2 c_2$, then uncertainties due to reflections at windows, etc., are eliminated, and (h) applies.

$$(\emptyset_f)_2 = \frac{(I_f)_2(\emptyset_f)_1}{(I_f)_1} \qquad \text{(h)}$$

The relative numbers of quanta emitted/second as fluorescence by the standard and the compound to be studied are required to calculate $(\emptyset_f)_2$.

The following steps are necessary in determining the fluorescence quantum yield of a compound by the relative method:

1. Dilute solutions ($\epsilon cl = 0.1$) of the standard fluorescent compound (1) of known $\emptyset_f$ and the compound (2) under study are prepared such that $\epsilon_1 c_1 = \epsilon_2 c_2$.
2. The fluorescence emission spectra of both solutions are determined, using a constant slit width of the spectrograph and a constant intensity of the exciting monochromatic light. These spectra give the response of the detecting photomultiplier recorded as a function of the frequency of the emitted fluorescence.
3. The areas under the fluorescence emission spectra curves of the two solutions are found, these are proportional to the total quanta fluoresced, $(I_f)_1$ and $(I_f)_2$.
4. The fluorescence quantum efficiency of the compound under study $(\emptyset_f)_2$ can be calculated from these data by using h with the ratio $(I_f)_2/(I_f)_1$ = (area under the fluorescence emission curve of compound (2) under study) / (area under

the curve for the standard compound).

Several precautions should be taken in measuring fluorescence quantum efficiencies to eliminate errors due to oxygen quenching, photodecompositions, etc.

1. Equation g applies only for small values of ϵcl. If solutions are chosen such that $\epsilon_1 c_1 \neq \epsilon_2 c_2$, an optical density of about 0.02 (at the exciting wavelength at the depth of solution from which fluorescence is viewed) should not be exceeded, or an error greater than 4% is introduced. The use of solutions of equal optical densities avoids this complication.
2. Solutions to be used for fluorescence measurements should be free from dirt, lint, or other light-scattering particles.
3. Because of the common occurrence of oxygen quenching of fluorescence, it is necessary to degas the solutions (by flushing with dry argon for five minutes).
4. The exposure times should not be extended unnecessarily, since products of the photodecomposition of the reactant will build up and may contribute significantly to the observed fluorescence or possibly act as efficient quenchers of the reactant's fluorescence.

3.5. REFERENCES

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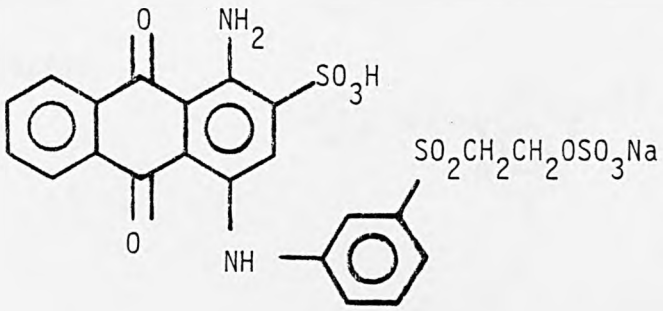
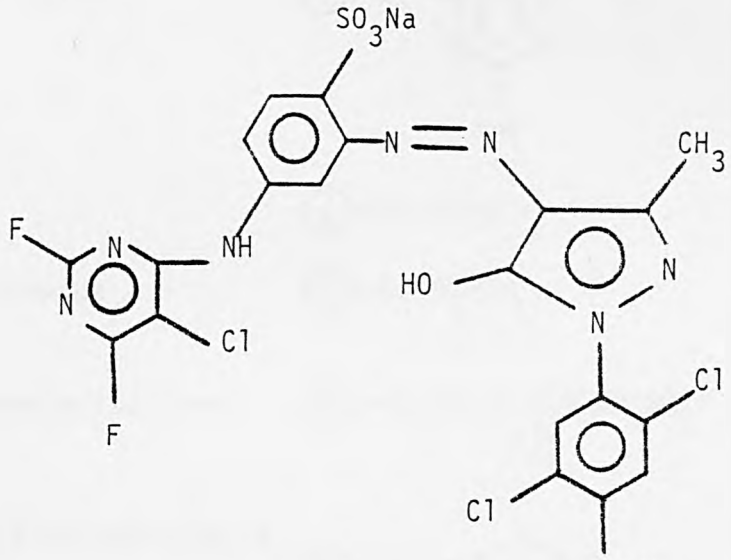
CHAPTER 4

THE EFFICIENCY OF REACTION BETWEEN REACTIVE FLUORESCENT WHITENING AGENTS AND WOOL

4.1. INTRODUCTION

The use of reactive dyes is widespread in the textile industry for dyeing cotton and wool [1]. The structures of the various reactive functional groups in many of the commercially available reactive dyes which can be applied to wool are shown in Table 4.1 [2]. The formulae of some dyes possessing such reactive groups are:





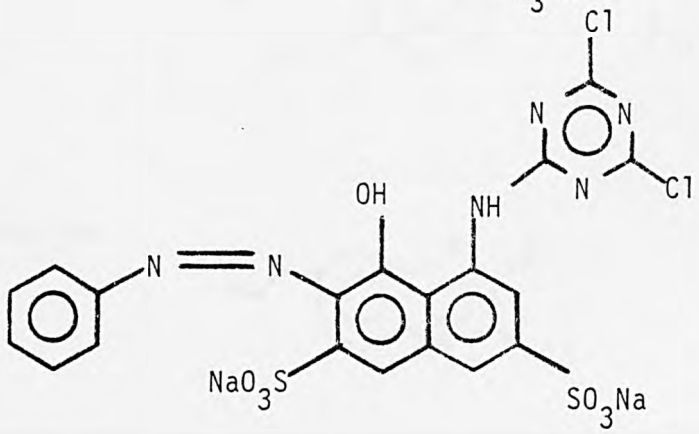
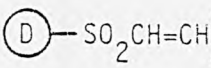
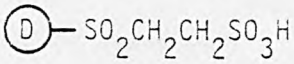
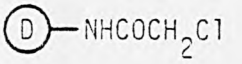
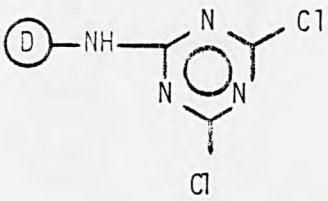
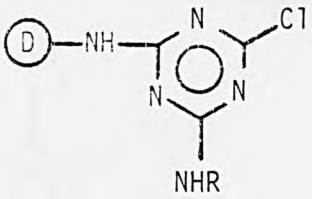
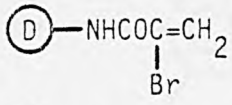
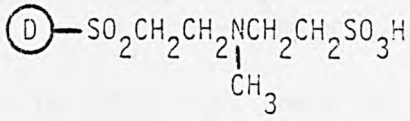
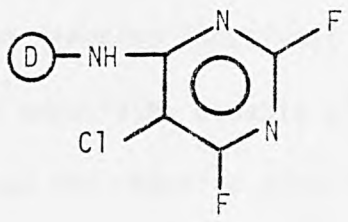
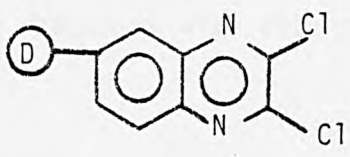
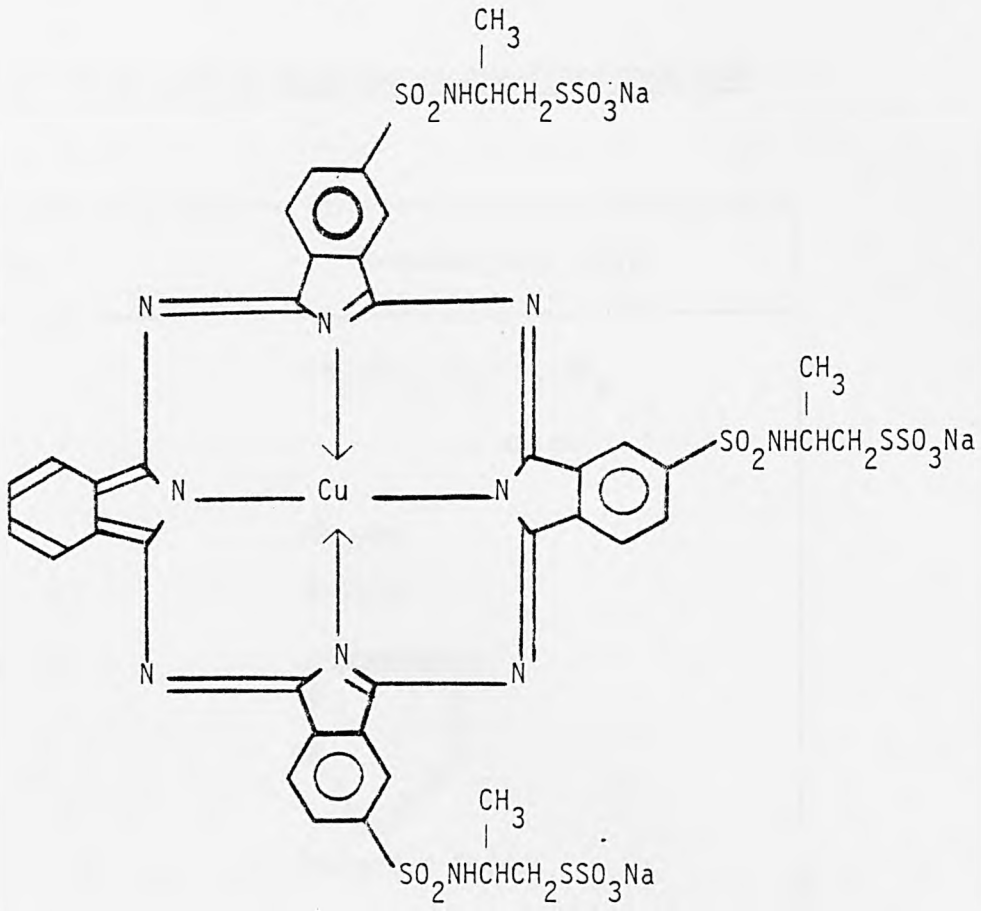
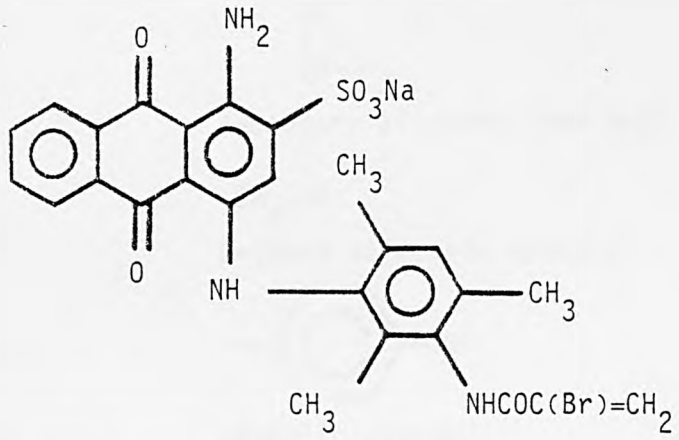


Table 4.1 Functional groups in reactive dyes for wool

Reactive functional group	Structure	Dye class
Vinyl sulphone		Solidazol N
ß-Sulphatoethylsulphone		Remazol/Remalan
Chloroacetamide		Cibalan Brilliant
Dichlorotriazine		Procion M
Monochlorotriazine		Procion H Cibacron
Acrylamide		Procilan
α-Bromoacrylamide		Lanasol
Methyltaurinoethylsulphone		Hostalan
Monochlorodifluoropyrimidine		Verofix Reactolan Drimalan F
2,3-Dichloroquinoxaline		Levafix E

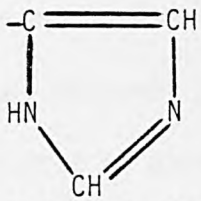
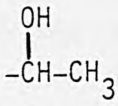
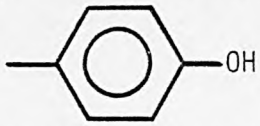
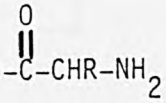
a. (D) represents the chromophoric residue.





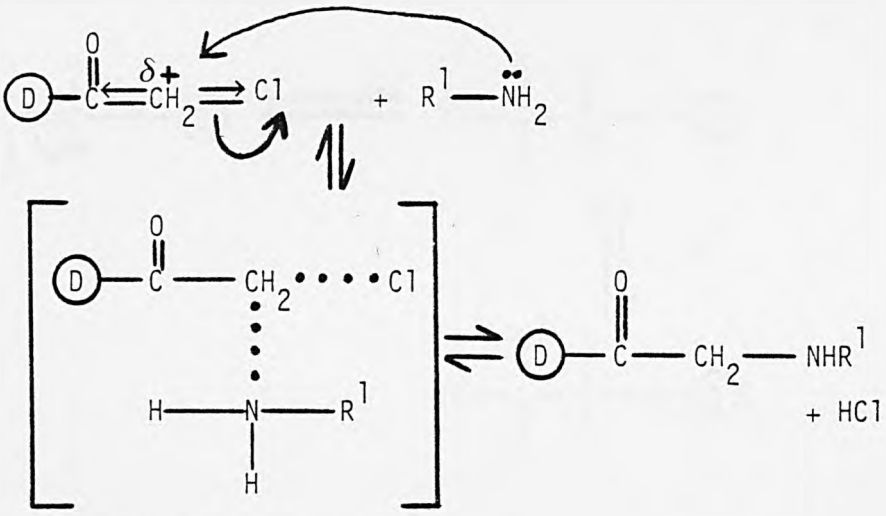
Application of such dyes to substrates leads to the formation of covalent bonds between the reactive functional groups present in the dyes and sites in the substrates capable of acting as nucleophiles. Table 4.2 shows the reactive side chains in wool (in descending order of importance) capable of acting as nucleophiles towards covalent bond forming reactions with reactive dyes [2].

Table 4.2 Side chains involved in dye-fibre reaction

Amino acid	Reactive side chain
Lysine	$-CH_2-CH_2-CH_2-CH_2-NH_2$ ϵ- amino
Cysteine	$-CH_2-SH$ ß-thiol
Histidine	 imidazole imine
Threonine	 secondary aliphatic hydroxyl
Serine	$-CH_2-OH$ primary aliphatic hydroxyl
Tyrosine	 phenolic hydroxyl
Methionine	$-CH_2-S-CH_3$ mercapto ether
N-terminal amino	 α-amino

Reactive groups commercially available in reactive dyes used for wool dyeing can be divided into two types, (i) those which react by nucleophilic substitution reactions, and (ii) those which react by the Michael addition reaction. These reaction types are discussed below:

(i) Nucleophilic Substitution Reactions These reactions can best be described by the attraction of an electron-deficient carbon atom for the free lone pair of electrons on the nucleophile. Usually this reactive centre on the carbon atom is activated by electron-withdrawing groups adjacent to it (usually SO_2 or C = O). The reactive carbon atom is also attached to a leaving group, usually halogen, sulpho or quaternary nitrogen. For example, such a system may be described by the reaction of a chloroacetyl dye with an organic amine R^1NH_2:



transition state

(where (D) represents the chromophoric residue)

Since both the reactants are involved simultaneously in covalence change this mechanism is termed bimolecular and denoted

by S_N2.

Nucleophilic substitution reactions can also proceed by a unimolecular S_N1 mechanism which is a two-stage process:-

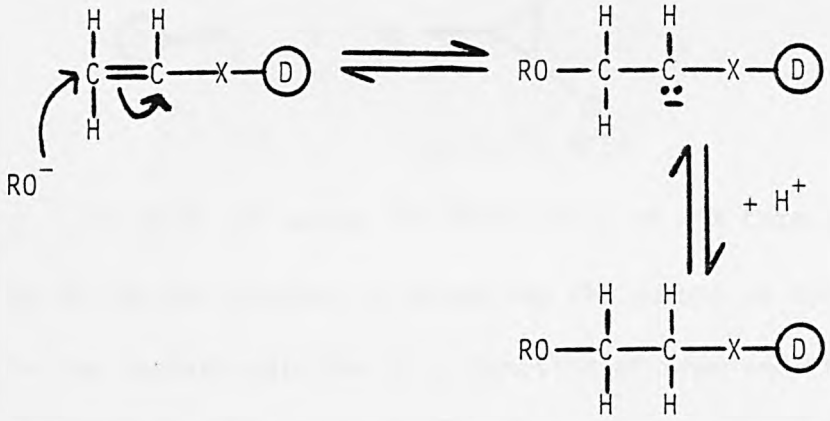
(1) Ionization $\quad R - X \longrightarrow R^+ + :X^-$ (slow)

(2) Carbonium ion reaction $\quad R^+ + :\bar{N}u \longrightarrow R - Nu$ (rapid)

nucleophile

Reactive dyes with halogen groups can be considered to react with nucleophiles exclusively by the S_N2 mechanism.

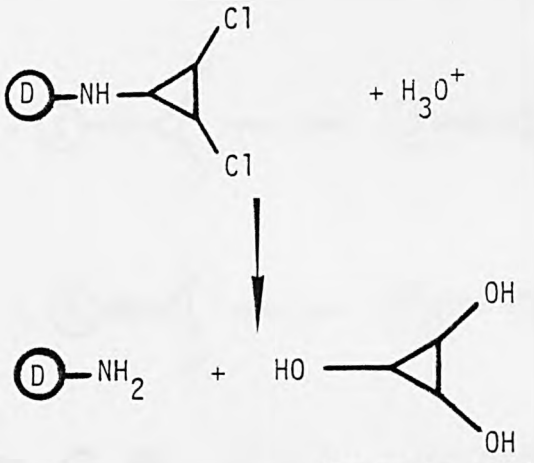
(ii) Michael Addition Reaction The general reaction of dyes containing polarized, unsaturated carbon-carbon double bonds with nucleophiles can be considered to be a 1, 2 trans-addition. The double bond is necessarily activated by the presence of electron-withdrawing substituents such as carbonyl or sulphonyl; a typical scheme may be written thus:



where X = C=O, O = S=O.

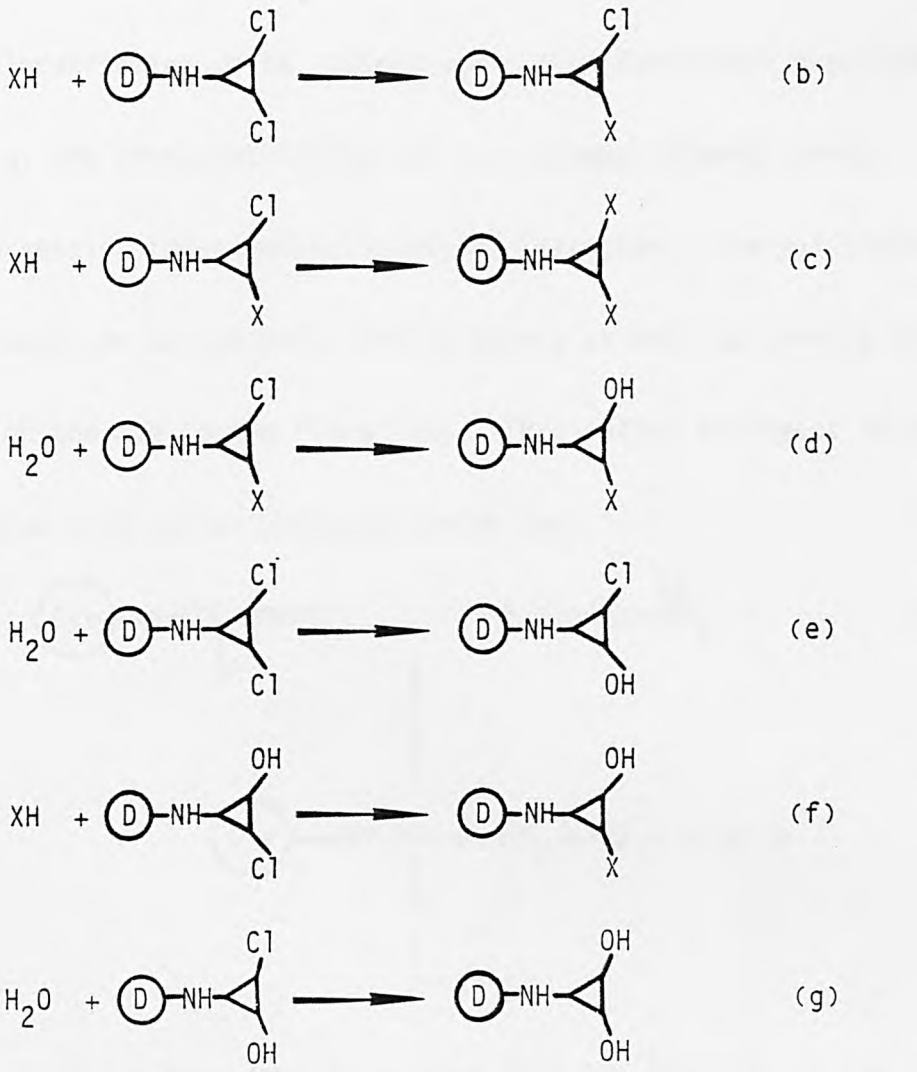
Reactive dyes can be successfully applied to wool from aqueous solution by exhaustion or a pad-batch process. Under the application conditions of the dye hydrolysis of the reactive group

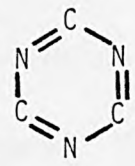
via nucleophilic attack by the water of the dye liquor is a side reaction apart from the vital bond forming reactions between the dye and wool fibre. Scheme 4.1 illustrates such reactions occurring in the system fibre/dichlorotriazine dye/water. The hydrolysis side reactions (e) and (g) have been studied in detail by Horrobin [3] who observed that reaction (e) is promoted in both alkaline and acid conditions, but reaction (g) only proceeds under acidic conditions. One other important hydrolysis reaction which may occur in acidic solutions with certain dichlorotriazine dyes is shown below:



Cyanuric acid

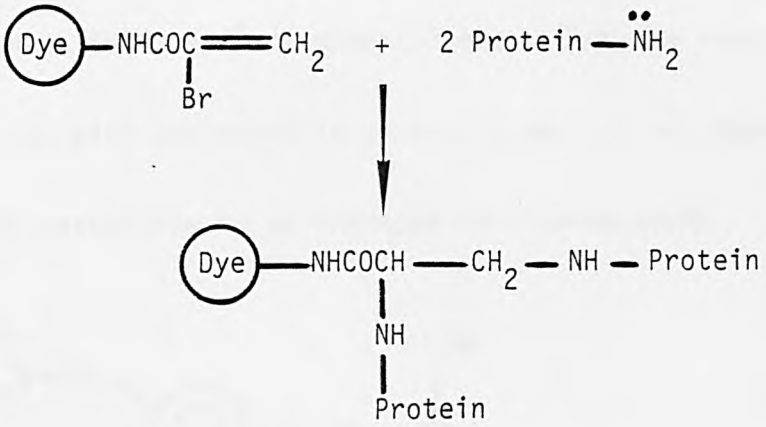
In order to assess the efficiency of dye take-up it is usual to follow the process by measuring the amount of dye present in the dyebath solution as a function of time and thereby determining the amount of unabsorbed dye remaining at the end of the dyeing process. Solvent extraction of the unfixed dye on the fibre is also usually carried out with acid or neutral pyridine solutions [4-7] or urea/surfactant mixtures [5,6,8,9].



where ◁ ≡  and XH ≡ fibre nucleophile

Scheme 4.1

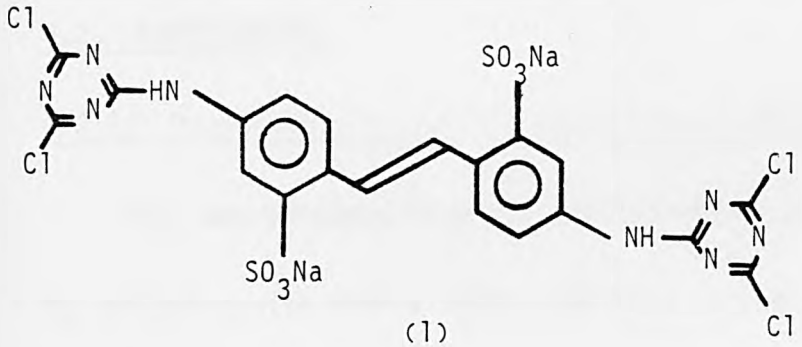
Many dyes carry more than one reactive group, e.g. two monochlorotriazine units. Others carry multifunctional reactive groups, e.g. the dichlorotriazine and α- bromoacrylamido groups, each possessing two potential sites for reaction. The multifunctional groups can act as crosslinking agents as well as serving to covalently link the dye to the fibre [10]. This latter statement is illustrated below with an α- bromoacrylamido dye:



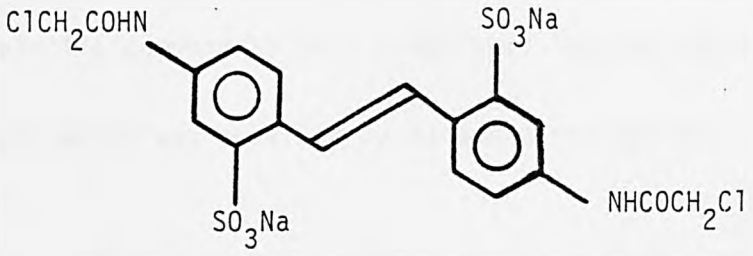
It has been tacitly assumed that the majority of the reactive groups in the dye molecule have reacted when such dyes are covalently attached to the fibres. This chapter presents adequate evidence to show that, in the case of wool, the method of application determines the extent to which the bonds are established and show that many of the reactive groups may only be reacting with water or other nucleophiles.

The strategy used for the work was to synthesise the potential

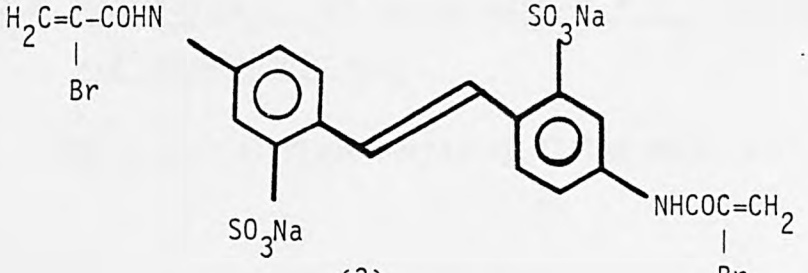
fluorescence whitening agents (1) - (3). The compounds are hardly fluorescent since they each contain halogen atoms. It is well known that the presence of halogen atoms leads to fluorescence quenching via the heavy atom effect [11,12, also see Chapter 1, pp.20–25]. In some cases the fluorescence quenching is attended by enhanced intersystem crossing. Replacement of the halogen atoms by hydroxyl or amino groups will lead to fluorescence typical of a substituted 4,4′-diaminostilbene. Thus the reaction of (1) - (3) with nucleophilic groups in wool or via hydrolysis will be detectable by an increase in fluorescence.



(1)



(2)



(3)

A further test was to brominate wool. Application of conventional fluorescent whitening agents (e.g. Photine-HV and Blankophor BA) to wool treated in this way should lead to little observable fluorescence from the fluorescent whitening agent (FWA) due to the external heavy atom effect [13,14, also see Chapter 1, pp.20-25], i.e. the halogen atoms present in the wool will quench the fluorescence of the FWA. Restoration of the fluorescence should be achievable by reduction of the halogenated wools thereby removing the halogen atoms.

4.2. EXPERIMENTAL

4.2.1. Preparation of 2,3-dibromopropionyl chloride

This was obtained from its corresponding carboxylic acid by refluxing with excess oxalyl chloride in benzene for 3 hours. The reflux condenser was equipped with a silica gel drying tube. The volatile components were evaporated leaving the product as a liquid which was purified by vacuum distillation.

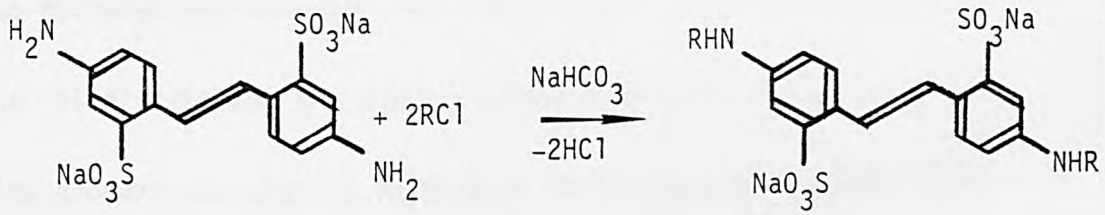
4.2.2 Preparation of the sodium salt of 4,4′-diaminostilbene-2,2′-disulphonic acid

Into a conical flask partially filled with water, diamino-

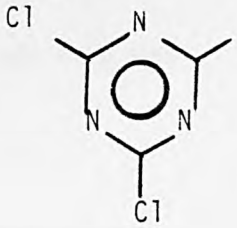
stilbenedisulphonic acid was introduced forming a paste which was constantly stirred. After the addition of an equimolar quantity of solid sodium carbonate the suspension was stirred and heated to the boil until the disodio-diaminostilbenedisulphonate completely dissolved.

4.2.3. Method of preparation of stilbenes (1), (2) and (4).

The general method employed is summarised by the following equation:-



(1) R =



(2) R = $ClCH_2CO-$

(4) R = $CH_2BrCHBrCO-$ (see above for the preparation of 2,3-dibromopropionyl chloride).

The electrophilic reagent (RCl in acetone) was slowly added dropwise to the well stirred aqueous solution of the sodium salt of diaminostilbenedisulphonic acid (prepared as above) which

was kept between 0-5°C. The pH of the reacting mixture was maintained between 6 and 7 by adding solid sodium bicarbonate. The electrophilic reagent was added in excess until the reaction mixture gave a negative result to a suitable amine test [h] (an intermediate probably the monosubstituted derivative possessing free amino groups was precipitated in the case of R=$ClCH_2CO$- and $CH_2BrCHBrCO$- under the reaction conditions employed. More water was therefore added and the reaction continued at room temperature). The product was salted out with solid sodium chloride and filtered (no salt was required in the case of R=$ClCH_2CO$-). The filter cake was thoroughly washed with acetone and dried (the product was soluble in acetone in the case of R=$CH_2BrCHBrCO$- and so it was washed with ether). For (4) the free acid was obtained via this procedure.

h. Amine test

A small quantity of the reaction mixture was dissolved in dilute hydrochloric acid. The mixture was cooled to 0-5°C with ice and excess sodium nitrite was then added forming the diazonium salt. Excess nitrite was destroyed by addition of sulphamic acid. If any red azo dye was formed on the addition of the above

cold solution to a cold solution of ß-naphthol in sodium hydroxide (pH 10) then it indicated that free amine was present in the original reaction mixture.

Analysis of compounds (2) and (4)

R=$ClCH_2CO-$ (2)

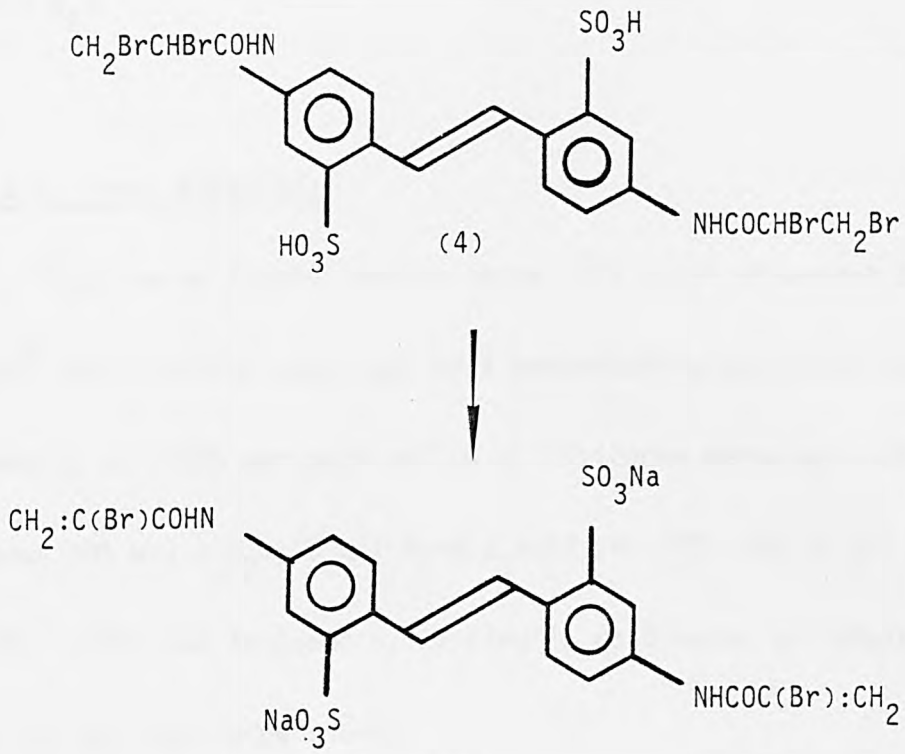
An infrared spectrum of the nujol mull included a band at 1675 cm^{-1} (amide carbonyl). The NMR spectrum with tetramethylsilane as a reference in dimethylsulphoxide - d_6 showed signals at 4.28δ (2H, singlet, methylene protons), 7.60-8.20δ (4H, multiplet, aromatic and vinyl protons) and 10.60δ (1H, sharp single peak, amino proton) which disappeared on shaking with D_2O.

R=$CH_2BrCHBrCO-$ (4)

This material also showed the amide carbonyl band at 1670 cm^{-1} in its infrared spectrum (nujol mull). The NMR spectrum with tetramethylsilane as a reference in dimethylsulphoxide - d_6 showed signals at 3.92-4.28δ (2H, multiplet, methylene protons), 4.84-5.12δ (1H, multiplet, methine proton), 7.72-8.52δ (4H, multiplet, aromatic and vinyl protons), 10.68δ (1H, sharp single peak, amino proton) and 11.08δ (1H, sharp single peak, $-SO_3H$). The latter two signals disappeared on shaking with D_2O.

4.2.4. Preparation of stilbene (3) from compound (4).

This conversion is depicted by the following equation:



(3)

Solid sodium bicarbonate (2 mol equivalent) was added to the starting stilbene (1 mol equivalent) in water. To this was added 0.04% (of the theoretical yield of product)hydroquinone (inhibitor of acrylate polymerisation) and triethylamine (4 mol equivalent) and the final mixture was stirred overnight at room temperature. The product was salted out with solid sodium chloride and filtered. The filter cake was thoroughly washed with ether and dried.

NMR spectroscopy with tetramethylsilane as reference in dimethyl-sulphoxide - d_6 showed signals at 6.36 δ (1H, doublet, J = 4Hz),

6.88 δ (1H, doublet, J = 4Hz), 7.60-8.32 δ (multiplet, 4H) and 10.52 δ (1H, sharp single peak) which disappeared on shaking with D_2O.

4.2.5. Wool bleaching

Wool serge fabric (botany serge, 2/2 twill of weight 200 g/m^2) was bleached overnight by a pad-batch method (room temperature), padding on (100% wet pick-up) 80 g/l hydrogen peroxide (100 volumes, about 30% w/v H_2O_2), 5 g/l formic acid (95-97%) and 10 g/l Lissapol N [15]. This was followed by rinsing in cold water and drying in air at room temperature.

4.2.6. Application of reactive FWAs 1-3 to wool

4.2.6.1. Method 1: Exhaustion onto wool serge fabric (buffered to pH 7)

0.0, 0.25, 0.50 and 1.00% of weight fabric (o.w.f.) reactive FWA.
3.0% o.w.f. thiourea dioxide.
1.0% o.w.f. ethylenediaminetetraacetic acid, disodium salt, dihydrate.
1.0% o.w.f. Lissapol N.

Exhaustion was carried out using liquor ratios of 30:1 at pH 7 (the pH was adjusted with sodium bicarbonate). The temperature was raised to 80°C at 1°C/min. and maintained for an hour, followed by rinsing in cold water and drying at room temperature.

4.2.6.2. Methods 2-4

Wool serge fabric and bleached wool (see above) were pad-batch treated overnight at room temperature with the following different liquors. This was followed by rinsing in cold water and drying in air at room temperature.

Method 2

0 and 5 g/l reactive FWA

300 g/l urea

1 g/l acetic acid

10 g/l Lissapol N

10 g/l ethylenediaminetetraacetic acid, disodium salt, dihydrate.

Method 3

As method 2 but included 10 g/l sodium metabisulphite.

Method 4

0 and 5 g/l reactive FWA

30 g/l thiourea dioxide

10 g/l ethylenediaminetetraacetic acid, disodium salt, dihydrate.

20 g/l sodium bicarbonate

10 g/l Lissapol N

% pick-up of liquor, on padding, was approximately 80% o.w.f.

4.2.7. Determination of % exhaustion of FWA-1 onto wool serge fabric

A liquor bath was made up using method 1 in which 0.50% o.w.f. FWA-1

was present. The optical densities, after dilution by the same factor, of small equal volumetric fractions of the bath liquor at the beginning and after exhaustion were measured on a Pye-Unicam SP8-100 spectrophotometer at the wavelength of maximum absorption (345 nm). The initial level of the bath liquor was maintained throughout the exhaustion by making small additions of water to compensate for loss due to evaporation. It was thus possible to determine the percentage exhausted onto the wool fibre.

4.2.8. Determination of % fixation of FWA-1 by cold pad-batch methods (2-4) onto wool serge fabric

Wool serge fabric was padded with the appropriate liquor. An immediate strip was cut off and FWA-1 was extracted from it using copious quantities of cold water. The strip was then allowed to dry at room temperature and weighed. The remaining padded wool was allowed to batch for 20 hours after which the above procedure was repeated. The two solutions, so obtained, were made to equal volumes and the optical density of each was measured on a Pye-Unicam SP8-100 spectrophotometer at the wavelength of maximum absorption. It was thus possible to determine the % fixation using the following formula [16]:

$$\text{Fixation (\%)} = 100 - \frac{100\ (OD_t)}{(OD_o)}$$

where OD_t = optical density of the extract froml g of treated wool after a batching time of t hours

and OD_o = optical density of the extract from 1 g of treated wool immediately after padding (t = 0).

4.2.9. Reaction of FWA treated wool with:-

(iii) Morpholine The fabric was stirred for 30 minutes, at room temperature, in an aqueous solution of morpholine in which Lissapol N was also present. This was followed by rinsing the treated fabric with cold water and drying at room temperature.

(iv) Water The fabric, after wetting with aqueous Lissapol N solution and rinsing with cold water, was left to stand for 20 minutes in boiling water. This was followed by rinsing with cold water and drying at room temperature.

(v) Sodium carbonate The fabric was pad-batch treated overnight at room temperature with 20 g l^{-1} anhydrous sodium carbonate and 10 g l^{-1} Lissapol N, followed by rinsing in cold water and drying at room temperature.

4.2.10. Application of Photine-HV (Hickson and Welch Ltd) and FWA-2 to pre-bleached wool by the conventional industrial method

Liquor baths containing 1% o.w.f. above named brighteners, 15% o.w.f. sodium dithionite and 1% o.w.f. Lissapol N were applied to H_2O_2 bleached fabric using liquor ratios of 30:1. The temperature was raised to 80°C at 1°C/min. and maintained for one hour, followed by rinsing in cold water and drying at room temperature.

4.2.11. Bromination of wool

60 g wool serge fabric was soaked for 30 seconds at room temperature in bromine water (48g neat Br_2 in 2 l water) which was set to pH 2 by adding hydrobromic acid. The brominating liquor also contained some Lissapol N. This was followed by rinsing in cold water and drying in air at room temperature.

4.2.12. Application of Blankophor BA(Bayer) and Photine-HV to wool serge fabric and brominated wool

These commercial FWAs were applied using the cold pad-batch method (2, see above).

4.2.13. Reduction of brominated wools treated with commercial FWAs

The FWA treated brominated fabrics were reduced by stirring them for a few minutes at room temperature in an aqueous saturated solution of sodium sulphite in which Lissapol N was present. This was followed by rinsing with cold water and drying at room temperature.

4.2.14. Fluorescence spectra

Fluorescence spectra of degassed (by flushing with dry argon for five minutes) solutions of the reactive FWAs 1-3 (made up to an optical density of 0.1 in either methanol or water at the excitation wavelength of 370 nm) were obtained using a Perkin-Elmer MPF-4 spectrofluorimeter. Fluorescence spectra of fabric

samples were obtained as described previously [17] except that the samples were placed at 50° to the incident beam to avoid scattering.

4.2.15. Diffuse reflectance spectra

Reflectance spectra of fabric samples were obtained as described previously [17] except that barium sulphate was used as a reference in this case.

4.2.16. Photographs

Photographs were taken by placing the wool fabrics on black cardboard and exposing them to an ultraviolet lamp (Gelman Instrument Company, model No: 51438) having a maximum output at 375 nm .

4.2.17. Yellowness Index (Y.I.) Measurements

The yellowness index formula employed was the following:

$$YI = \frac{100\ (1.316\ X - 1.164\ Z)}{Y}$$

where X, Y and Z are the CIE tristimulus values (D65/10° illuminant/observer) obtained from the Macbeth Micromatch reflectance spectrophotometer. The lower the yellowness index value the whiter is the wool.

4.2.18. Light fastness measurements

Dry wool samples were exposed to blue standards 2 and 3 using the Xenotest machine (Hanau) according to the ISO standard procedure (ISO 105-B02: 1978).

4.3. RESULTS

The FWAs 1-3, which are non-fluorescent in aqueous and methanolic solutions, were applied to wool in a number of ways (Methods 1-4). The method of application determines the extent to which these FWAs are fluorescent.

(vi) Exhaustion

FWAs 1-3 were significantly taken up (Table 4.3) using this method of application (Method 1) and all were strongly fluorescent (Photograph 4.1 and Tables 4.3-4.5). FWA 2 led to the most effective whitening and its performance was compared with a commercial FWA-Photine-HV. These were applied in the usual way to peroxide bleached wool [15% o.w.f. sodium dithionite and 1% o.w.f. FWA at 80° for 1 hr]. Yellowness indices showed that Photine-HV (0.18) gave a much whiter fabric than FWA 2 (7.38) but the light fastness of FWA 2 (assessed as a 3) was slightly better (light fastness of Photine-HV treated fabric was 2-3).

(vii) Pad-batch method

Pre-bleached wool serge was treated with the reactive (di-chlorotriazine) FWA-1 by pad-batch method 2 and subsequently rinsed in cold water and dried. Several after treatments, which were designed to bring about increased nucleophilic substitution reactions with the fibres, were carried out on this treated fabric involving:

- boiling water
- aqueous sodium carbonate
- aqueous morpholine

The precise after treatment conditions are described in the experimental section of this chapter.

Diffuse reflectance spectra obtained from these samples are shown in Fig. 4.1. This figure clearly indicates that the treatment method is successful in applying significant amounts of agent since UV light is strongly absorbed at about 350 nm (curve 'a') in marked contrast to the bleached control fabric without FWA-1 (curve 'e'). This result was further confirmed by measuring theuptake of FWA-1 following pad-batch application (Table 4.3).

There is a clear marked effect on the diffuse reflectance spectrum following the after treatments designed to promote further nucleophilic substitution reactions of FWA-1 with the fibre (Fig. 4.1). There is a shift from strong absorption in the 350 nm region to increased reflectance due to the onset of fluorescence. In reality these increased reflectance values are due to visible emission in the region of 450 nm excited by absorbed UV light (about 350 nm) - the photomultiplier tube in the particular optical arrangement employed does not discriminate between this emission and the reflected light [17, also see Chapter 8, p.258]. Using this evidence for increasing fluorescence following the after treatments it is evident from Figure 4.1 that fluorescence is

increased greatly by after treatment either in boiling water or aqueous sodium carbonate or aqueous morpholine - the latter after treatment achieving the greatest increase in fluorescence. These effects correspond with stepwise removal, by nucleophilic substitution reactions, of the quenching chlorine atoms from the triazine rings. Photograph 4.2 (middle bands) also demonstrates the above effects, showing the visual fluorescences perceived in the UV ($\lambda_{excitation}$ 375 nm) viewing cabinet.

Similar results were obtained for the reactive FWAs 2 and 3, when applied by the pad-batch methods. As shown in Photograph 4.3 and Figure 4.2 FWA-2 applied by pad-batch method 2 gave only a weak fluorescence, but application by pad-batch methods 3 and 4 showed increased fluorescence indicating more complete nucleophilic substitution of the quenching chlorine atoms. FWA-3 showed a very similar quenching/fluorescence behaviour to FWA-1 when applied by pad-batch method 2 and subjected to various basic after treatments. In the case of FWA-3 if covalent attachment to the fibre substrate occurs via replacement of only one of the bromine atoms, the presence of the remaining bromine should be sufficient to lead to efficient fluorescence quenching.

This latter statement is in accord with theory and experimental observations that the bromine atom is a far more potent quencher of fluorescence via the heavy atom effect than is chlorine [18, also see Chapter 1, pp. 20-25].

The extent to which the FWAs become affixed to the wool

using the pad-batch method is also determined by the addition of urea or sodium metabisulphite. Thus the inclusion of urea in method 2 led to a greater uptake of FWA-1 than method 4 (Table 4.3). In method 3, sodium metabisulphite was added to the pad liquor. As can be seen from Table 4.3, this method gave a greater fixation of FWA-1 than method 2. Also the use of urea alone in the pad liquor gave a fabric having a higher yellowness index for FWA-2 than when a combination of urea and sodium metabisulphite was used (Table 4.4 and Photograph 4.3). Brief outlines for these observed effects on adding urea or sodium metabisulphite to the pad liquors are given below (these outlines are based on reactive dyes but equally well hold for other reactive FWAs):

Urea Observations by workers at Ciba-Geigy [19], C.S.I.R.O.[20] and I.W.S. [16] have shown that urea, used at concentrations of up to 30% (weight/volume) in the dyebath, has a remarkable effect on the dyeing of wool by pad-batch procedures and by low temperature exhaustion methods. Dyeings carried out by these methods, in the presence of these high concentrations of urea, exhibited better penetration, better build-up and better levelness than similar dyeings made in the absence of urea.

In order to discover why high concentrations of urea promote the dyeing of wool at room temperature, numerous studies have been carried out on the interaction of dyes with urea solution [21-24] and on the interaction of this system with wool [22-25].

The effect of urea on lowering the aggregation number of

dyes in solution is well documented and sometimes, as with phthalocyanine dyes, it is possible to follow this effect spectrophotometrically. The absorption peak at shorter wavelength (618 nm) is rapidly superseded by the peak at longer wavelength (667 nm) with increasing urea concentration, indicating possible disaggregation. This effect is also produced by the addition of ethyl alcohol to aqueous solutions of phthalocyanine dyes, but higher concentrations of ethyl alcohol in solution do not promote wool dyeing at low temperatures to the same extent as do urea solutions. The role of urea in promoting the rate of reaction of wool with reactive dyes must also depend partly on its protein denaturing properties. For example, with ovalbumin of hen's eggs, five -SH groups per molecule can be titrated with such reagents as ferricyanide in 8M urea, but these -SH groups are completely undetected in the absence of urea. Asquith et al. [24] raise the interesting possibility that the hydrophobic 'keratin sheath' is removed by these high urea concentrations, thus facilitating the penetration of the fibre by the dye. The detection of increased fibre swelling due to these high urea concentrations has led to some contradictory results [22,24], but it now appears that high urea concentrations only swell processed or 'damaged' wool fibres [26].

Sodium metabisulphite The incorporation of sodium metabisulphite (10-20 g/l) in the pad liquor has been shown to offer the following advantages:

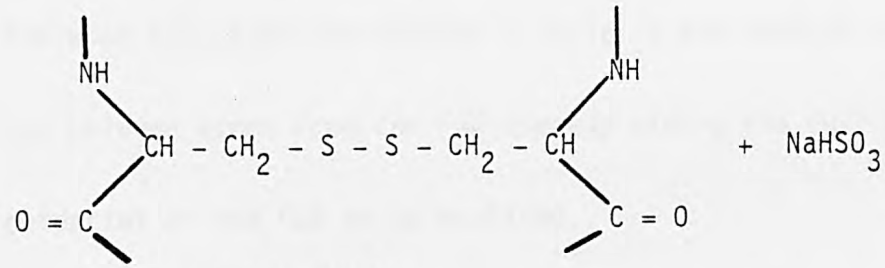
Reduction in batching time

Improved levelness

High yields when using dyes of lower reactivity, or high-molecular weight dyes.

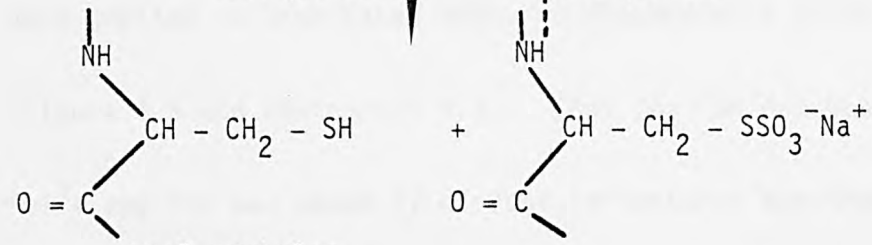
The effect of sodium metabisulphite on fixation rate is shown for a dye of high reactivity in Figure 4.3, and for a dye of moderate reactivity in Figure 4.4; in the latter case, there is a great increase in the rate of reaction. The reason for this great improvement, both in rate of reaction and in equilibrium fixation, lies in the well known chemical reaction between wool cystine and sodium metabisulphite:

$$Na_2S_2O_5 + H_2O \longrightarrow 2NaHSO_3$$



wool cystine

ca. pH 5.0 ↓ ca. pH 9.0 ↑



wool cysteine

The rupture of the cystine bond has two favourable effects on the cold dyeing of wool. Firstly, the fibre swells more readily, allowing easier and more rapid penetration by the diffusing reactive

dye (a factor which is especially important with high molecular weight dyes), and secondly the formation of the highly nucleophilic cysteine residue could account for a higher rate of reaction.

That the full potential of FWAs 1 and 3 to whiten the wool had not been attained by the cold pad-batch procedures was shown by the fact that treatment with cold aqueous sodium carbonate solution or morpholine at room temperature led to an increase in fluorescence. Boiling with water led to a marked yellowing (also for fabrics treated with FWA-2) as detectable by the eye, even though the fluorescence due to the FWA increased (the fabrics treated with FWA-1 did not yellow on boiling). Under these circumstances there is competition between thermal yellowing of the wool [15, also see Chapter 2, p.76] and removal of all the halogen atoms from the FWA thereby aiding the full fluorescence potential of the FWA to be realised.

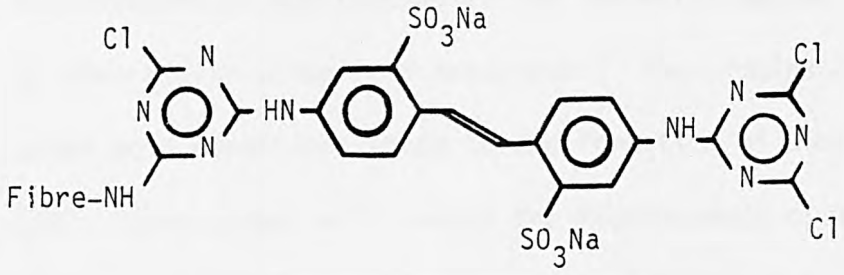
When the commercial FWAs (Photine-HV and Blankophor BA) were applied to brominated wool, no fluorescence could be detected (Figure 4.5 and Photograph 4.4). That the FWA had been successfully applied was shown by diffuse reflectance spectroscopy. Following reduction of the brominated wools by treating them with aqueous sodium sulphite solution, the wools exhibited fluorescence typical to that due to the FWA and also at a similar intensity (Photograph 4.4). The procedure of bromination followed

by reduction was not optimised hence fibre degradation was evident following the reduction procedure.

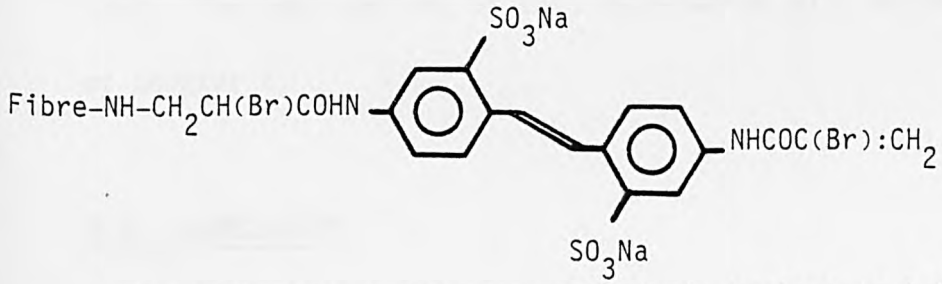
4.4. DISCUSSION

The particular pad-batch method of application exerts considerable influence on the extent of covalent reaction between the wool and reactive FWA. Although the FWAs had clearly been adsorbed onto the fibre, many of the reactive groups remained intact as shown by the lack of fluorescence caused by intramolecular fluorescence quenching by the halogen groups. The chloroacetyl derivative, FWA-2, when applied by pad-batch in the presence of urea (adjusted to pH5 with acetic acid) gave only a weak fluorescence indicating little replacement of the chlorine atoms; on the other hand, in the presence of urea and sodium metabisulphite, significant fluorescence was imparted indicating more extensive reaction under these conditions (Photograph 4.3). In this particular FWA there are only two quenching chlorine atoms per molecule and hence even if there is only a limited amount of reaction, observable fluorescence will develop. In contrast, FWA-1, which contains four chlorine atoms per molecule and FWA-3, which contains two bromine atoms per molecule (very potent quenchers of fluorescence), gave hardly any observable fluorescence when applied by any of the pad-batch methods. In the case of dichlorotriazine reactive dyes [27] analogous to FWA-1, it is known that the cold pad-batch methods described above give very significant covalent bonding

to the fibre. Thus, the non-fluorescent species present on the fibre following pad-batch application of FWA-1 is rich in chlorine and possibly may be



Once the remaining halogen groups had been removed, e.g. by boiling water, cold sodium carbonate solution or morpholine, the fluorescence developed (Photograph 4.2). A similar explanation may be advanced to describe the behaviour of the bis -α-bromoacrylamido FWA-mono-addition reactions probably leading to a non-fluorescent quenched form:



The after treatments are more likely to lead to halogen removal via nucleophilic attack by water or added amine in the case of morpholine or from increased nucleophilicity of amine and thiol groups in the fibre. Since the application of the FWAs by exhaustion is carried out in aqueous solution at 80°, it is not surprising that the reactive groups lose their halogen atoms but the question must remain as to how many of the reactive

groups have established covalent bonds between the FWA and the wool. That the method of fluorescence quenching is a useful test for determining whether reactive FWAs lose their halogen substituents on application by the pad-batch method is attested by the results using brominated wool. The bromination of wool under acid conditions leads to the formation of N-bromoamines [28]. Such groups will quench the fluorescence of applied commercial FWAs via the external heavy atom effect and this was indeed observed. Removal of the offending bromine atoms by reduction led to the restoration of the fluorescence of the FWA thereby validating the proposed rationale.

An interesting point to note from this work is that the reactive FWAs, when applied by pad-batch or exhaustion in the presence of thiourea dioxide, gave improved light fastness ratings (3+). The implications of this observation will be the subject of chapter 6.

4.5. CONCLUSION

The results illustrate the power of fluorescence quenching to assess the extent to which halogen containing reactive groups react under given application conditions. With multifunctional reactive FWAs it is clear that the cold pad-batch method does not lead to efficient reaction of all the reactive groups in the FWA with nucleophilic sites in the fibre. Subsequent after treatment further removes the reactive groups but it is not yet

possible to state whether reactions leading to covalent bonds with the fibre or hydrolysis is occurring. A similar situation exists for the application of these multifunctional compounds under high temperature long liquor batch techniques.

TABLE 4.3 Results for FWA 1

Method of Application*	% Fixation of FWA onto fabric	Fluorescence Yes/No	Yellowness Index (Y.I.)	Light Fastness (L.F.)	AFTER TREATMENTS					
					WATER		Na_2CO_3		MORPHOLINE	
					Y.I.	L.F.	Y.I.	L.F.	Y.I.	L.F.
1 (0.50% o.w.f., wool serge fabric)	70%	Yes	–	–	–	–	–	–	–	–
2 (0.50% o.w.f., bleached wool)	57%**	No	20.20	3	19.34	2–3	16.87	2–3	19.41	2–3
3 (0.50% o.w.f., bleached wool)	73%**	No	21.65	3	16.26	2–3	20.47	2–3	24.34	2–3
4 (0.50% o.w.f., bleached wool)	47%**	No	16.63	3+	15.86	3	14.71	3+	16.51	3+

* The quantity of FWA and the type of fabric used are indicated in parentheses.

** Measured on wool serge fabric

TABLE 4.4 Results for FWA 2

Method of Application*	Fluorescence Yes/No	Yellowness Index (Y.I.)	Light Fastness (L.F.)	AFTER TREATMENTS					
				WATER		Na_2CO_3		MORPHOLINE	
				Y.I.	L.F.	Y.I.	L.F.	Y.I.	L.F.
1 (0.25% o.w.f., wool serge fabric)	Yes	14.91	3+	–	–	–	–	–	–
1 (0.50% o.w.f., wool serge fabric)	Yes	10.64	2–3	–	–	–	–	–	–
1 (1.00% o.w.f., wool serge fabric)	Yes	10.67	3	–	–	–	–	–	–
2 (0.50% o.w.f., wool serge fabric)	Yes	20.60	3	–	–	–	–	–	–
3 (0.50% o.w.f., wool serge fabric)	Yes	15.45	3	–	–	–	–	–	–
4 (0.50% o.w.f., wool serge fabric)	Yes	13.54	3+	–	–	–	–	–	–
3 (0.50% o.w.f., bleached wool)	Yes	14.39	2–3	22.90	3	16.22	2–3	17.74	2–3
4 (0.50% o.w.f., bleached wool)	Yes	13.53	3	23.52	3	14.09	3	16.28	3

* The quantity of FWA and the type of fabric used are indicated in parentheses.

TABLE 4.5 Results for FWA 3

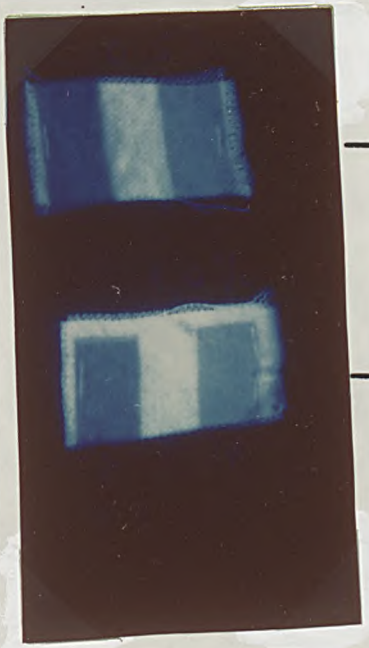
Method of Application*	Fluorescence Yes/No	Yellowness Index (Y.I.)	Light Fastness (L.F.)	AFTER TREATMENTS					
				WATER		Na_2CO_3		MORPHOLINE	
				Y.I.	L.F.	Y.I.	L.F.	Y.I.	L.F.
1 (0.50% o.w.f., wool serge fabric)	Yes	13.17	3+	–	–	–	–	–	–
1 (1.00% o.w.f., wool serge fabric)	Yes	12.64	3	–	–	–	–	–	–
2 (0.50% o.w.f., bleached wool)	No	23.33	3	29.63	3+	22.72	3	25.16	3

* The quantity of FWA and the type of fabric used are indicated in parentheses.

TABLE 4.6 Results for controlled fabrics (i.e. no FWA present in the liquor)

Method of Application*	Yellowness Index (Y.I.)	AFTER TREATMENTS		
		WATER Y.I.	Na_2CO_3 Y.I.	MORPHOLINE Y.I.
1 (wool serge fabric)	16.64	–	–	–
2 (bleached wool)	17.37	28.32	21.95	19.79
3 (bleached wool)	15.70	23.30	19.85	21.89
4 (bleached wool)	17.31	20.93	19.89	19.08

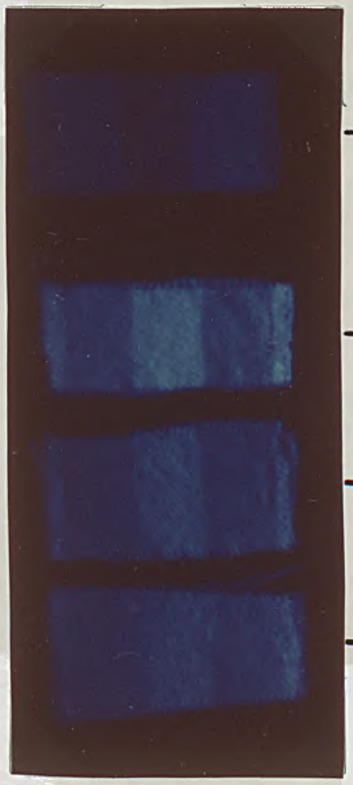
* The type of fabric used is indicated in parentheses.



PHOTOGRAPH 4.1

Fluorescence (middle bands only) of FWA 3 applied to wool serge fabric at (a) 0.5% and (b) 1.0% o.w.f. by Method 1.

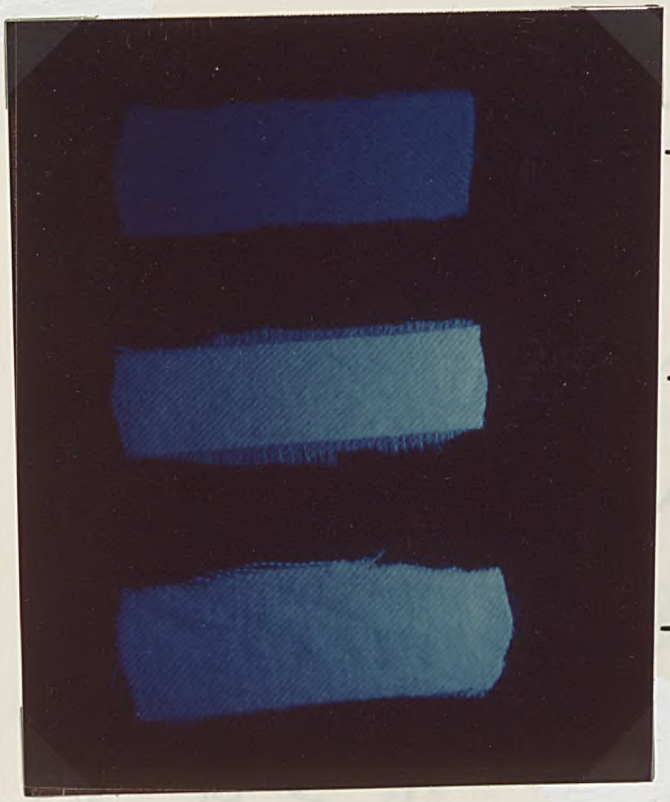
The side bands do not fluoresce as these portions of the fabrics were exposed to light during a light fastness measurement experiment.



PHOTOGRAPH 4.2

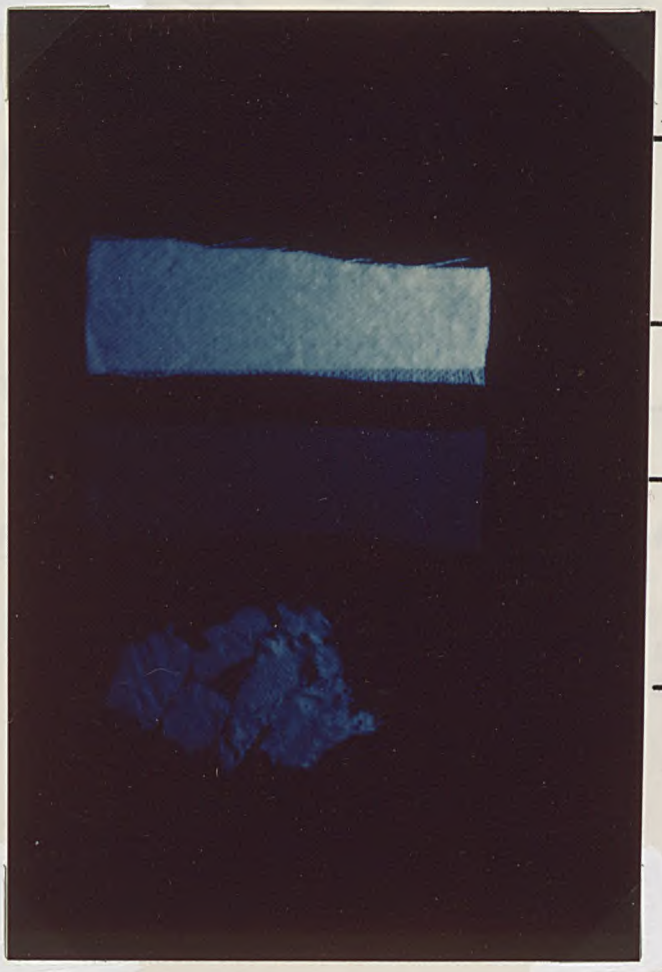
Illustration of the initial non-fluorescence of FWA-1 applied to bleached wool by method 2 (c - middle band only) followed by enhanced fluorescence (middle bands only) on further treatments with morpholine (d), boiling water (e) and aqueous sodium carbonate (f).

The side bands do not fluoresce as these portions of the fabrics were exposed to light during a light fastness measurement experiment.



PHOTOGRAPH 4.3

Fluorescence of FWA 2 applied to wool serge fabric by methods (g) 2, (h) 3 and (i) 4.



PHOTOGRAPH 4.4

Fluorescence of:-

(j) brominated wool,

(k) Photine-HV applied to wool serge fabric by method 2,

(l) Photine-HV applied to brominated wool by method 2,

and

(m) reduced brominated wool on which Photine-HV was present.

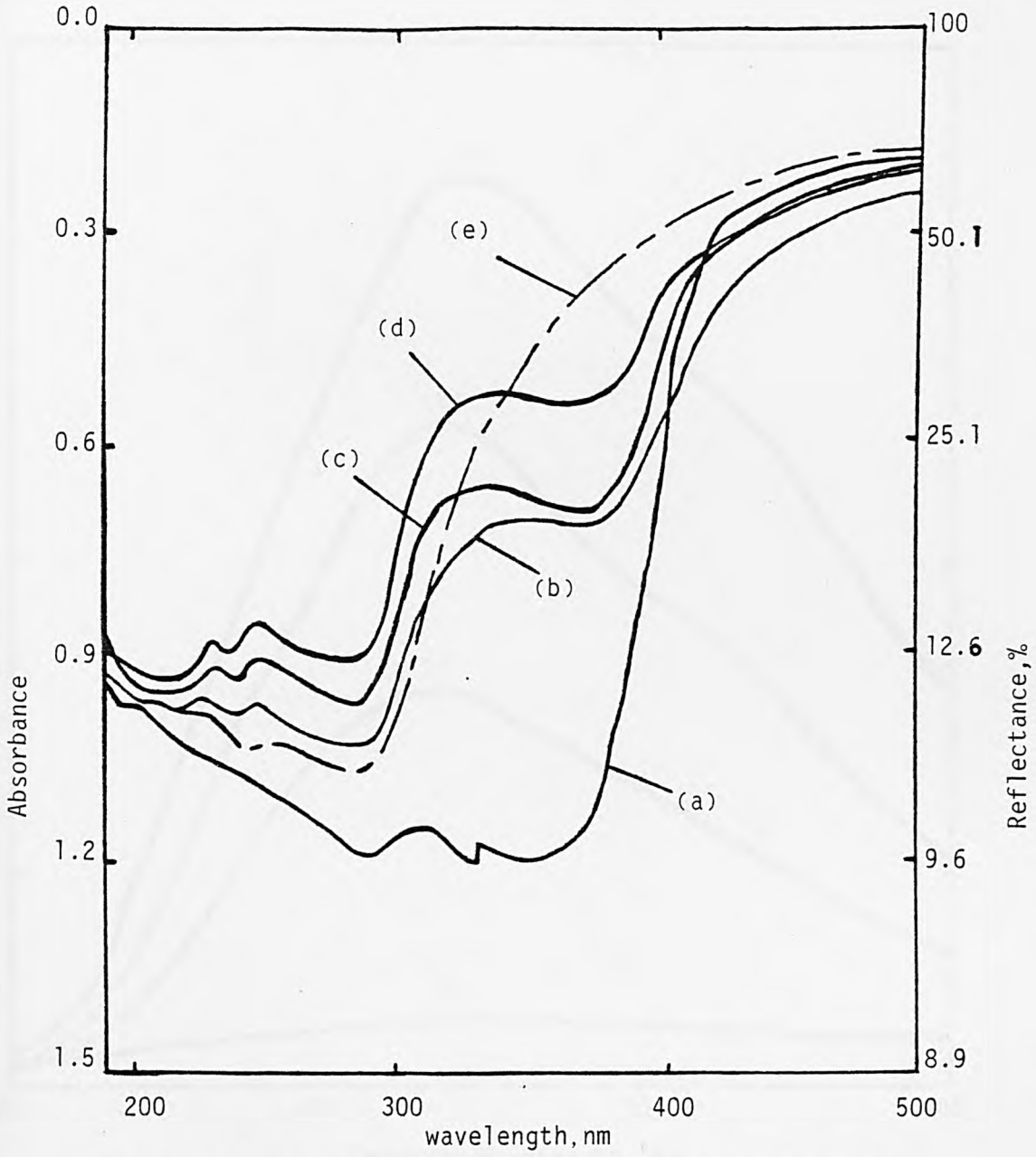


Figure 4.1 Diffuse reflectance spectra of FWA-1 applied to bleached wool by method 2 (a) and of its subsequent treatments with boiling water (b), aqueous sodium carbonate (c) and morpholine (d). The reflectance spectrum of bleached wool is also included (e).

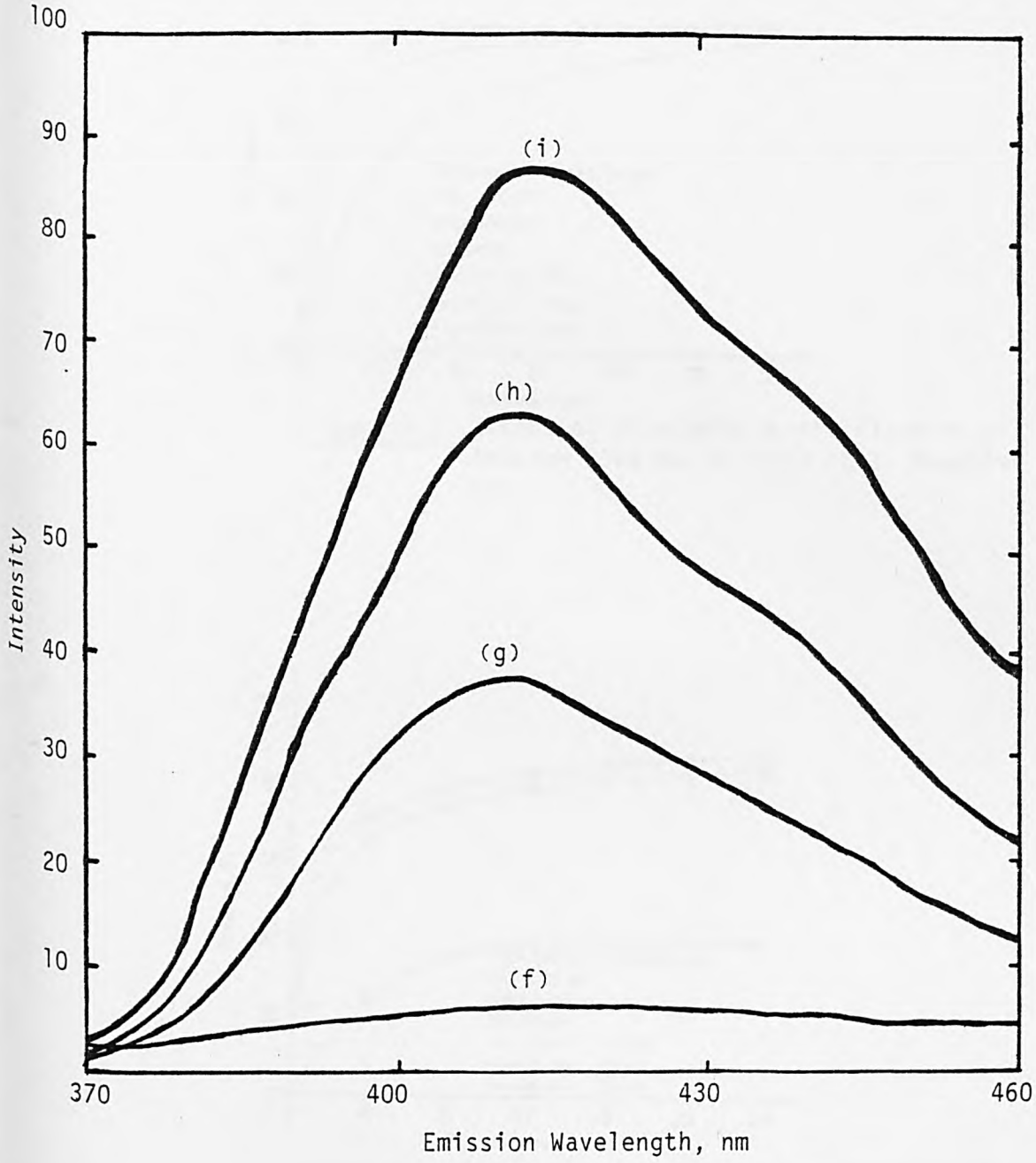


Figure 4.2 Fluorescence spectra (taken under similar conditions) of bleached wool (f) and of FWA 2 applied to bleached wool by method 2 (g). Subsequent treatments with aqueous sodium carbonate (h) and boiling water (i) of FWA 2 on bleached wool are also included.

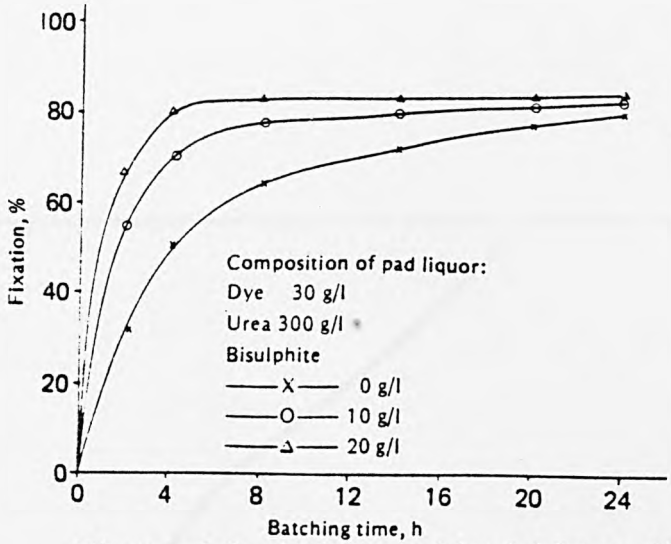


Figure 4.3 Effect of bisulphite on the fixation of Procion Blue MX-3G (ICI) (C.I. Reactive Blue 1) [2].

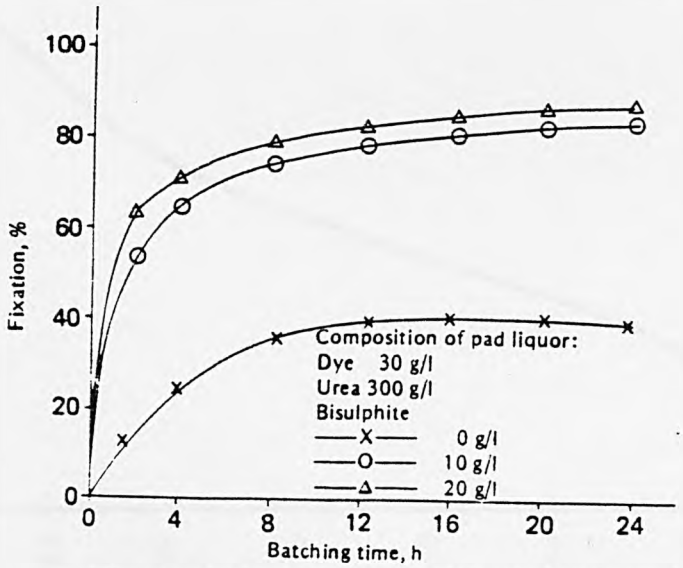


Figure 4.4 Effect of bisulphite on the fixation of Levafix Brilliant Red E-2G (BAY) (C.I. Reactive Red 89) [2].

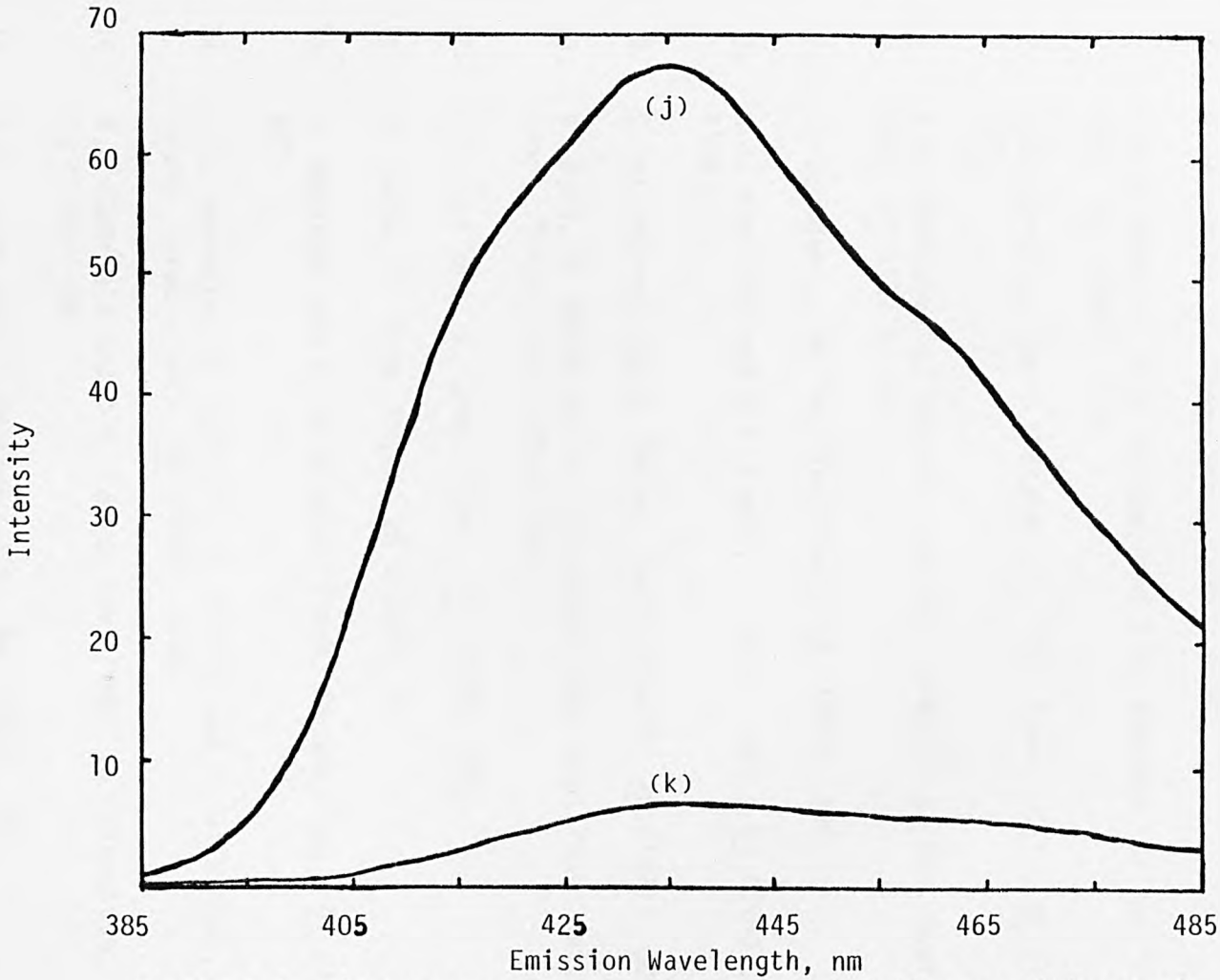


Figure 4.5 Fluorescence spectra (taken under similar conditions) of Photine-HV applied to wool serge fabric (j) and to brominated wool (k) by method 2.

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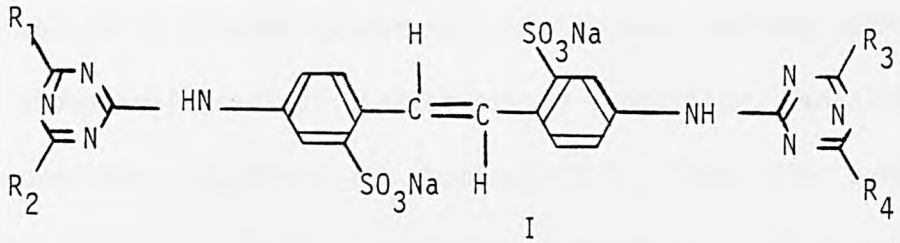
CHAPTER 5

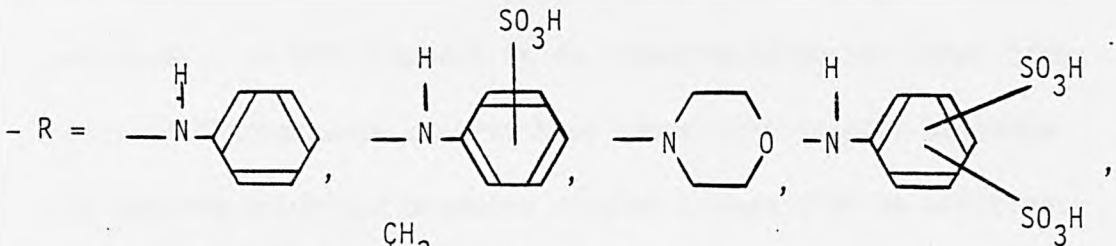
THE PHOTOSENSITISING PROPERTIES AND PHOTOSTABILITY OF STILBENE FLUORESCENCE WHITENING AGENTS

5.1. INTRODUCTION (also see Chap.2, pp.86-91).

Stilbenes having the general formula I are used widely in industry as fluorescent whitening agents (FWAs). They represent about 80% of all FWAs on the commercial market [1]. Such FWAs



I



$-N(CH_2CH_2OH)_2$, $-NCH_2CH_2OH$ (with CH_3), $-NH_2$, $-N(alkyl)_2$,

$-OCH_3$, $-Cl$, $-N(H)-CH_2CH_2SO_3H$, $-NHCH_2CH_2OH$.

give an appreciable whiteness when applied to most yellowed materials. It has been observed for the case of wool that the applied FWAs undergo photodegradation when the treated fabric is exposed to sunlight and also that they sensitise the photoyellowing process [2,3]. The photodegradation of stilbene FWAs on the surface of wool appears to involve a direct reaction between the stilbene and wool [4] and this may not be a photo-oxidative process. It has also been reported that stilbenes, on the surface of wool, can sensitise the formation of singlet oxygen. This may be produced by interaction of the triplet excited state of stilbene with ground state oxygen. The excited oxygen generated

by this manner can chemically interact with tryptophyl residues of keratin located some distance away from the stilbene molecule [5]. It is undoubtedly true that the amino acids most susceptible to photodegradation are those which are the most susceptible to attack by singlet oxygen [6]. Stilbenes, besides acting as photosensitisers of singlet oxygen production, can also behave as quenchers (physical and chemical) [7]. Thus, like pyrazolines [8], stilbenes can also undergo self destruction in photochemical processes. In this context it is interesting to note that time resolved fluorescence studies have shown that triplet stilbene (in benzene solution) produces singlet oxygen with an efficiency of 18± 5% [9]. In view of this result and the fact that most stilbene FWAs possess high fluorescence quantum yields and short fluorescence lifetimes, it is likely that such compounds are inefficient sensitisers of singlet oxygen.

This chapter presents experimental evidence to show that some stilbenes sensitise singlet oxygen formation and that others quench singlet oxygen. Furthermore, FWAs which are inefficient sensitisers of singlet oxygen, when applied to wool, photoyellow the wool and are photodegraded at similar rates to conventional sensitisers.

5.2. EXPERIMENTAL

5.2.1. Materials

The following chemicals were used as supplied: Trans-stilbene, thioanisole, 4,4′-diaminostilbene dihydrochloride, anhydrous sodium acetate, 3-methylindole and ethylenediaminetetraacetic acid (disodium salt, dihydrate), (all Aldrich), indole (Fisons), Methylene Blue, N-acetyl-DL-tryptophan and DL-tryptophan (all Sigma), Rose Bengal (Eastman, Kodak Co.), potassium chromate (BDH), 4,4′-diaminostilbene-2,2′-disulphonic acid (Vickers Laboratories Ltd.), Photine-HV (Hickson and Welch Ltd.), Lissapol N(ICI), hydrogen peroxide (GPR, 100 volumes, about 30% w/v H_2O_2, BDH), formic acid (95-97%, Aldrich), glacial acetic acid and pyridine (both Fisons SLR grade), methanol (Fisons AnalaR and HPLC grade), methanol-d_1 and methanol-d_4 (Aldrich, Gold Label). Spectroscopic solvents for fluorescence quenching experiments (acetonitrile and methanol) were used as supplied (Aldrich, Gold Label spectrophotometric grade).

The wool fabric employed was a botany serge (2/2 twill of weight 200 g/m^2) supplied by Salts of Saltaire.

5.2.2. Methods

UV spectra were recorded using a Perkin-Elmer 402 ultraviolet-visible spectrophotometer. Specific optical densities were made up using a Cecil CE 272 linear readout ultraviolet spectrophotometer. Melting points were determined using a Gallenkamp

melting point apparatus and are uncorrected. GLC analyses were carried out using a Perkin-Elmer Sigma 3 gas chromatograph fitted with a 1.5% OV225 (80-100 mesh; length 2m; outer dimension $\frac{1}{8}$", inner dimension 2mm; support material Chromosorb W acid washed dimethyldichlorosilane) column. Peak areas were calculated using a Pye-Unicam DP88 computing integrator, and compared with those of the internal standard. HPLC analyses were carried out using an ODS Hypersil (5μm) column (150 x 4 mm) and degassed (2 hrs. in an ultrasonic bath at room temperature) eluent (70% water, 20% methanol and 10% glacial acetic acid). UV spectroscopy (Cecil CE 2012 reference channel variable wavelength UV monitor) was used for detection of the peaks.

5.2.2.1. Fluorescence quenching

Fluorescence spectra were recorded on a Perkin-Elmer MPF-4 spectrofluorimeter and are uncorrected. Solutions of trans-stilbene were made up to an optical density of 0.1 at the excitation wavelength (320 nm in methanol and 326 nm in acetonitrile). Fluorescence spectra of the degassed (flushed with dry argon for five minutes) solutions were obtained in the presence and absence of indole (10^{-2} M), under similar conditions.

5.2.2.2. Photo-oxidation of indoles

Mixtures of indoles (10^{-2}M) and stilbenes (10^{-2}M) in pyrex photolysis tubes were irradiated within a circular array of

16 x 8 W (F8T5/BLB) Sylvania black light lamps giving a maximum output at 350 nm which is absorbed only by the stilbenes. Also included in the above was a photo-stable internal standard phenyl methyl sulphone - 1 mg being employed for subsequent post irradiation GLC analyses and 72 mg for other analyses - total volume of the irradiated solution solvent was 20 cm^3 (water or methanol). Dry oxygen was continuously bubbled through the reaction mixture. The kinetic stability of a particular indole was followed by monitoring the decrease in its concentration with time. This was achieved by measuring the area of its GLC peak or the height of its HPLC absorption peak (at 270 nm) relative to those of the internal standard.

5.2.2.3. Measurement of kinetic solvent isotope effects

Solutions containing indole (10^{-3} M or 10^{-4} M), trans-stilbene (10^{-2}M) and phenyl methyl sulphone (2.14 mM) were made up in CH_3OH, CH_3OD (methanol-d_1) and CD_3OD (methanol-d_4). The solutions were photolysed using the above procedure with the following modifications: the solutions were flushed with dry oxygen for 30 minutes and stoppered prior to being irradiated in a reactor fitted with a merry-go-round apparatus. The photochemical reactions of the 10^{-3} M indole solutions were monitored by GLC analysis whilst HPLC was used for the 10^{-4}M indole solutions.

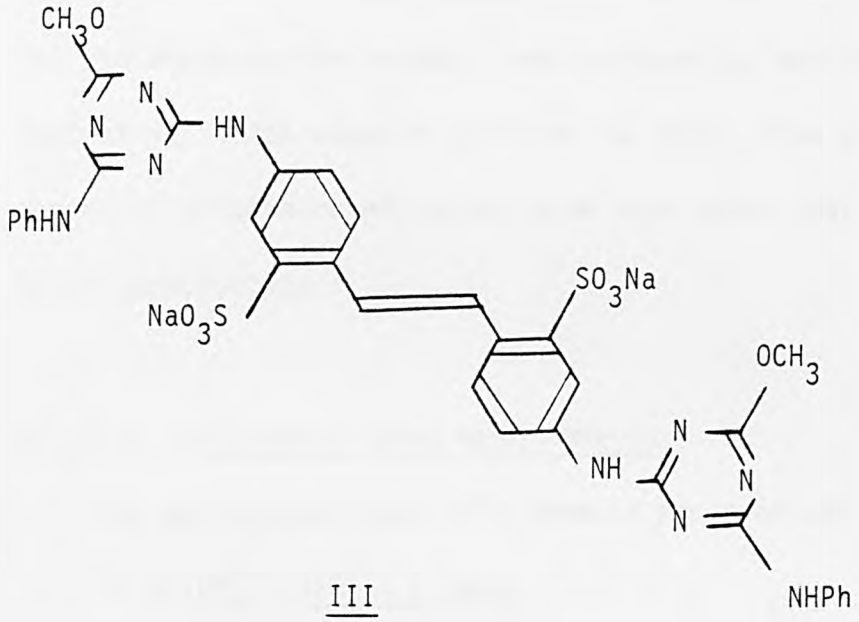
5.2.2.4. Stern-Volmer rate constant measurements

Solutions of indole (10^{-3}M) containing phenyl methyl sulphone (641 μM) and either Methylene Blue (sufficient to give an optical density of 1.0 at 661 nm) and disodium 4,4′-diacetamidostilbene-2, 2′-disulphonate (10^{-4}, 5×10^{-4} or 10^{-3}M) or Rose Bengal (sufficient to give an optical density of 1.0 at 548 nm) and disodium 4,4′-diaminostilbene-2, 2′-disulphonate (10^{-4}, 5×10^{-4} or 10^{-3}M) were made up in water. The dye-sensitised photo-oxidation of the indole in these solutions was carried out in a similar manner to the indolic oxidations using daylight lamps (8 x 20 W Chryselco) filtered through a 2% aqueous potassium chromate light filter solution. The reactions were followed by GLC analysis.

5.2.2.5. Wool bleaching

Wool serge fabric was bleached overnight by a pad-batch (room temperature) method, padding on (100% wet pick-up) 80 g/l hydrogen peroxide (100 volumes, about 30% w/v H_2O_2), 5 g/l formic acid (95-97%) and 10 g/l Lissapol N [10]. This was followed by rinsing in cold water and drying in air at room temperature.

5.2.2.6. Application of 4,4′-diacetamidostilbene-2,2′-disulphonic acid (II) and Photine-HV,III, to bleached wool and subsequent photoyellowing



III

Pre-bleached wool samples were treated in baths set at pH 4 (4% of weight fibre (o.w.f.) sodium acetate plus required acetic acid), at a liquor to material ratio of 30:1 in the presence of ethylenediaminetetraacetic acid (disodium salt, dihydrate - 1% o.w.f.) and Lissapol N (ICI - 1% o.w.f.) with either FWA II or III at 0.0 and 0.5% o.w.f. The temperature was raised to 80°C at 1°C/min. and maintained for one hour, followed by cold water rinsing and drying at room temperature. Both FWAs gave exhaustion values above 80% by this application method.

The whitened samples were photoyellowed for 24 hours as described previously [3].

5.2.2.7. Extractions [11]

Strips of equivalent weight from each of the whitened and photoyellowed fabrics (see above) were wetted with aqueous Lissapol N solution and rinsed in cold water. Extraction of the brighteners and photodegradation products was achieved by boiling (5 mins.) each strip in 25% aqueous pyridine (50 mls), five times. The amount of brightener extracted from each sample was determined by UV spectroscopy.

5.2.2.8. Yellowness Index measurements

The yellowness index (YI) formula employed was the following:

$$YI = \frac{100\ (1.316X - 1.164Z)}{Y}$$

where X, Y and Z are the CIE tristimulus values obtained from the Macbeth Micromatch reflectance spectrophotometer. The lower the yellowness index value the whiter is the wool colour.

5.2.2.9. Preparation of disodium 4,4′-diaminostilbene-2,2′-disulphonate

This was prepared from 4,4′-diaminostilbene-2,2′-disulphonic acid according to the procedure described by Zahradnik [1].

5.2.2.10. Synthesis of 4,4′-diacetamidostilbene-2,2′-disulphonic acid

This was prepared by the method described by Needles and Seiber [12] and its structure was elucidated by NMR and IR spectroscopy.

5.2.2.11. Synthesis of phenyl methyl sulphone

This was synthesised from thioanisole using the method described by Bell and Bennett for the oxidation of 1,4-dithian by hydrogen peroxide [13]. The product was identified by its melting point (m.p. 81°C, lit. m.p. 88°C [14]) and elemental analysis (found: C, 53.83; H, 5.10. $C_7H_8SO_2$ requires C, 53.82; H, 5.16%.)

5.3. RESULTS AND DISCUSSION

Tryptophan is one of the amino acids which is most prone to photodegradation in proteins such as wool [2]. Studies were therefore carried out on the degradation of this amino acid, in addition to some closely related indoles, using stilbenes as photosensitisers. UV spectra (Figures 5.1 - 5.3) of each component in the reaction mixtures implicated that only stilbenes absorbed the irradiation light used (see under photo-oxidation of indoles in the experimental section). The results are shown in Table 5.1.

It can be seen clearly from Table 5.1 that stilbene and 4,4'-diaminostilbene dihydrochloride successfully photosensitise the degradation of indolic compounds. The other two stilbenes proved to be very inefficient sensitisers.

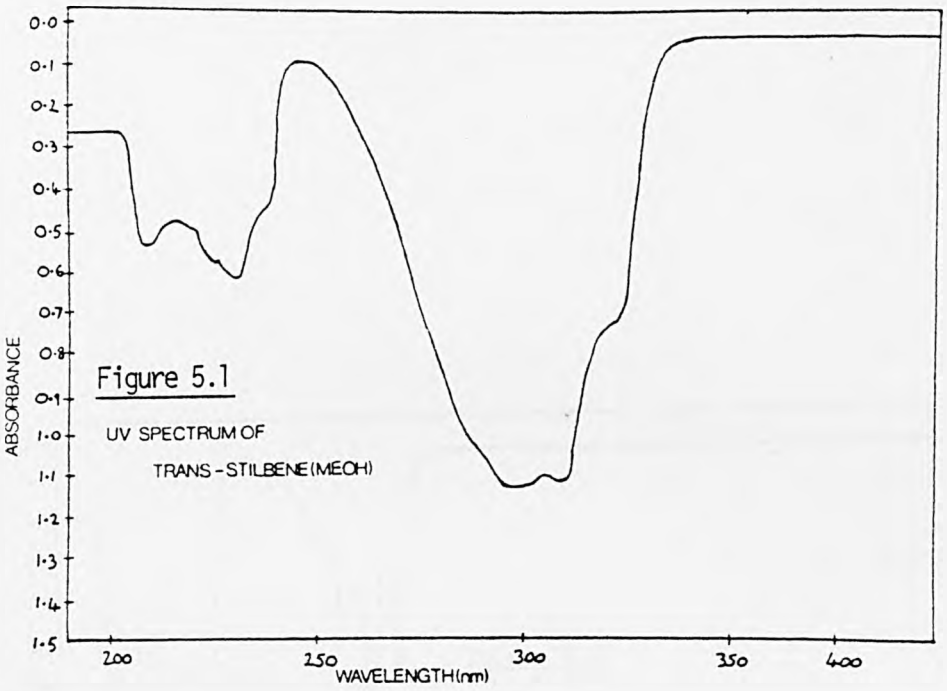


Figure 5.1

UV SPECTRUM OF
TRANS-STILBENE(MEOH)

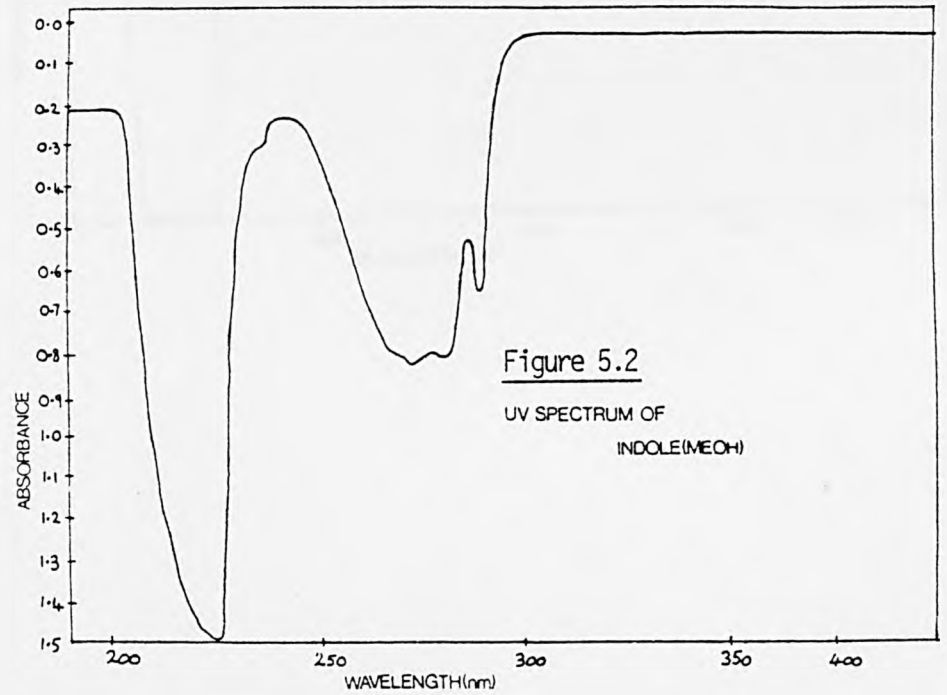


Figure 5.2

UV SPECTRUM OF
INDOLE(MEOH)

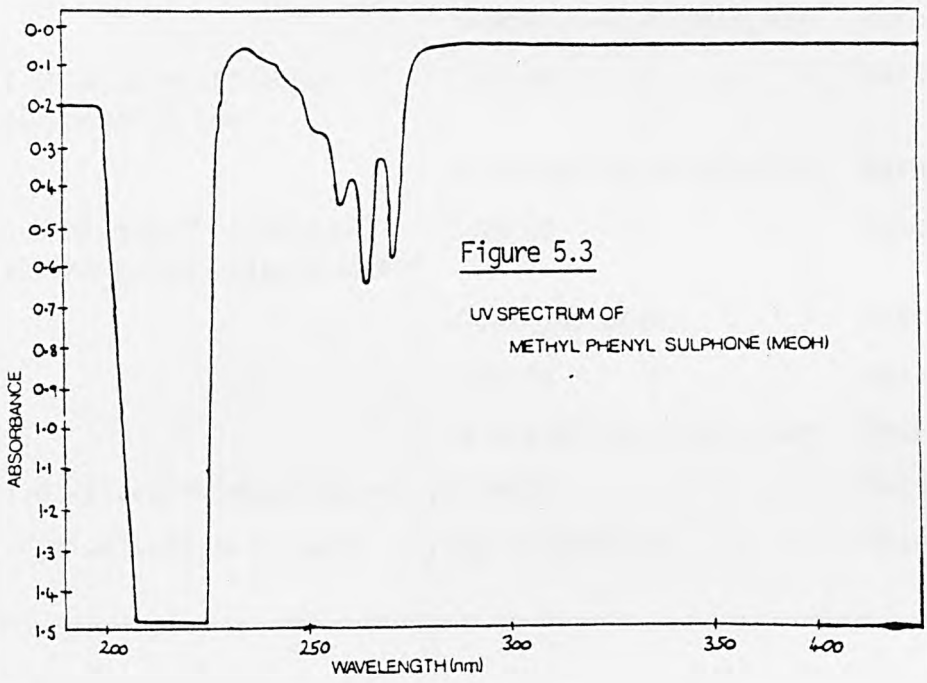


Figure 5.3

UV SPECTRUM OF
METHYL PHENYL SULPHONE (MEOH)

TABLE 5.1

The photooxidation[a] of indoles sensitised by stilbenes

Stilbene Sensitiser	Indole	Solvent	% Indole Oxidised
Trans-stilbene	Indole	Methanol	74[b]
"	3-Methylindole	Methanol	96[b]
"	N-Acetyl-DL-tryptophan	Methanol	74[c]
4,4′-Diaminostilbene dihydrochloride	Indole	Methanol	88[b]
"	N-Acetyl-DL-tryptophan	Methanol	100[c]
Disodium-4,4′-diamino-stilbene-2,2′-disulphonate	Indole	Water	40[b]
" "	DL-Tryptophan	Water	11[c]
" "	Indole	Methanol	0[b]
" "	N-Acetyl-DL-tryptophan	Methanol	10[c]
4,4′-Diacetamidostilbene -2,2′-Disulphonic acid	Indole	Water	45[b]
	DL-Tryptophan	Water	10[c]

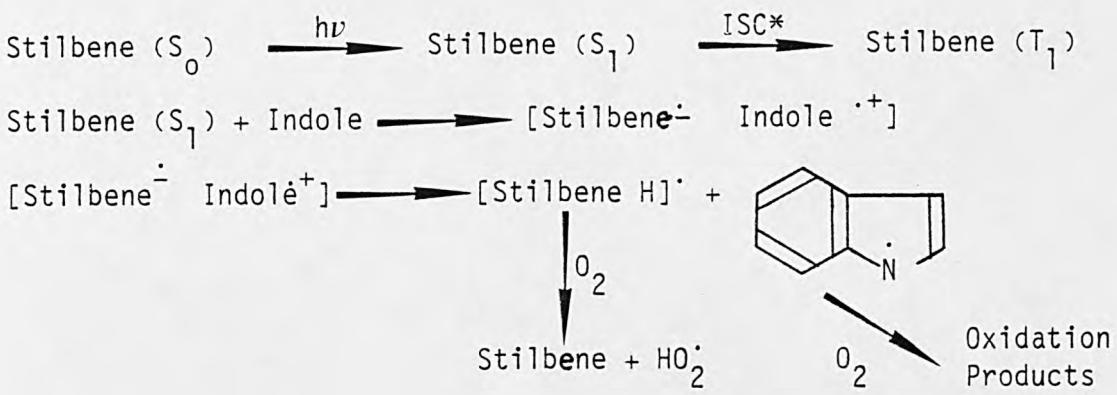
a. Irradiation time 24 hr.

b. Determined by GLC.

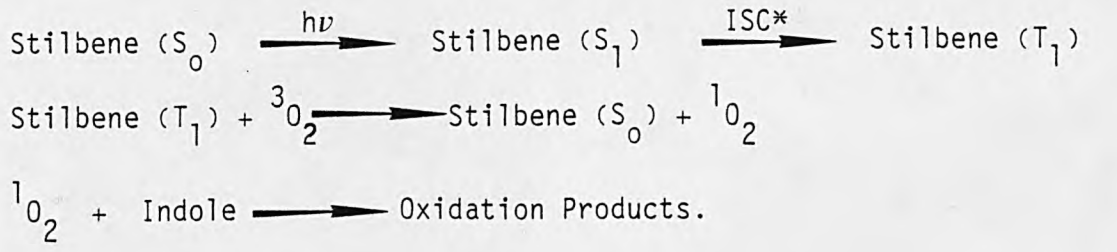
c. Determined by HPLC.

Two possible mechanisms can be suggested for the oxidation reactions:

MECHANISM A : CHARGE-TRANSFER TYPE



MECHANISM B : SENSITISATION TYPE



S_0 = Ground state.

S_1 = Excited singlet state.

T_1 = Excited triplet state.

*ISC = Intersystem crossing.

In Mechanism A, the excited state of the stilbene interacts primarily with the indole to give radicals which subsequently react with oxygen. To prove that this mechanism is operating, the effect of indole upon the fluorescence was studied. A drop

in the fluorescence output due to the excited state of stilbene was not observed even at a high concentration of 10^{-2}M indole (Figures 5.4 - 5.7). Similarly, with a very short lifetime of the triplet state of stilbene [15] makes it unlikely that it will undergo a bimolecular reaction with indole at a concentration of 10^{-2}M. Thus, although it is known that amines can react with stilbenes via a charge-transfer process [16,17], this process does not appear to be operating under the reaction conditions employed for the experiment.. A technique known as the kinetic solvent isotope effect was used in order to establish that singlet oxygen was involved in the oxidation reactions. This technique is a test [18] for the participation of singlet oxygen in photo-oxidation reactions and relies upon the fact that its lifetime is markedly dependent upon the nature and isotopic composition of the solvent. As the lifetime of singlet oxygen is generally longer in a deuterated solvent compared to its non-deuterated counterpart, the observation of a solvent isotope effect upon the rate of a photo-oxidation reaction is often used as evidence for the involvement of singlet oxygen. The rate of a singlet oxygen mediated photo-oxidation reaction is given by equation 5.1 [19]. Provided that a change from a non-deuterated to the

$$\frac{-d[A]}{dt} = \frac{Ia\emptyset_T Kr[A]}{Kd + (Kr + Kq)[A] + Kq\,[S]} \qquad (5.1)$$

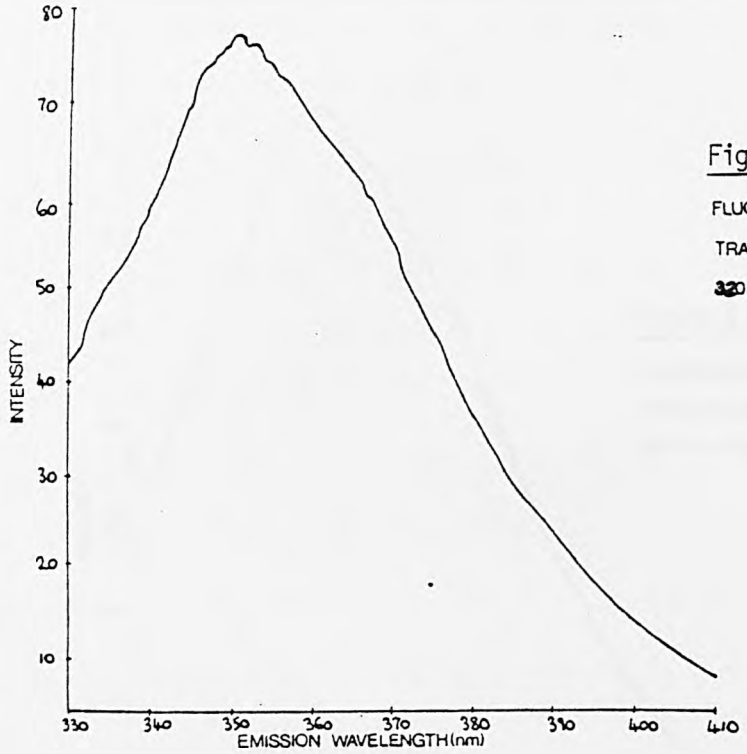


Figure 5.4

FLUORESCENT SPECTRUM OF TRANS - STILBENE (O.D. = 0·1 at 320nm in MeOH)

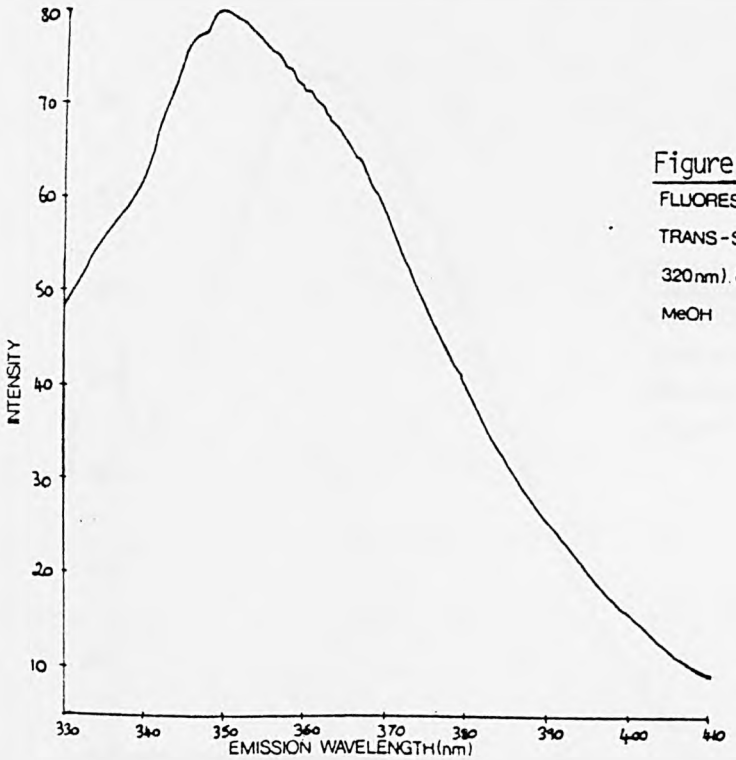


Figure 5.5

FLUORESCENT SPECTRUM OF TRANS - STILBENE (O.D. = 0·1 at 320nm) & 10^{-2} M INDOLE IN MeOH

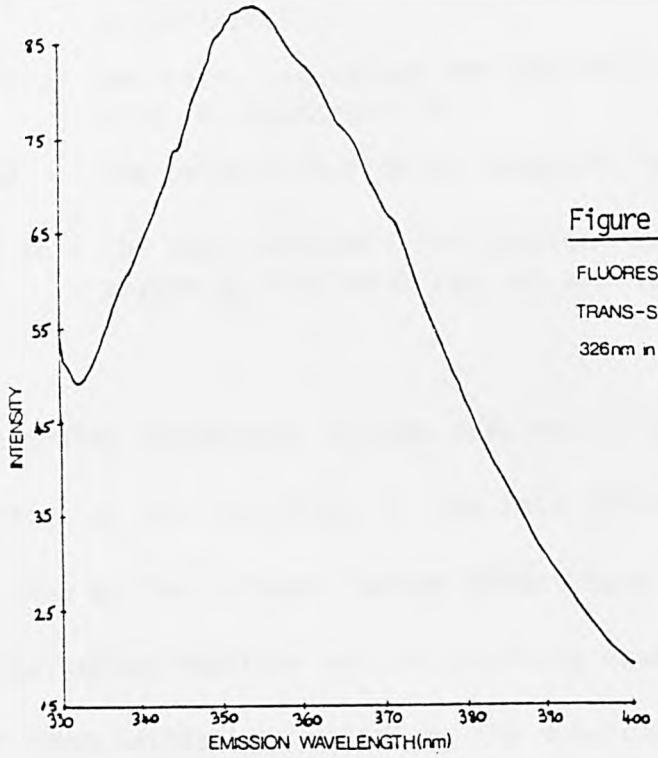


Figure 5.6

FLUORESCENT SPECTRUM OF TRANS-STILBENE(O.D.=0·1 at 326nm in CH_3CN)

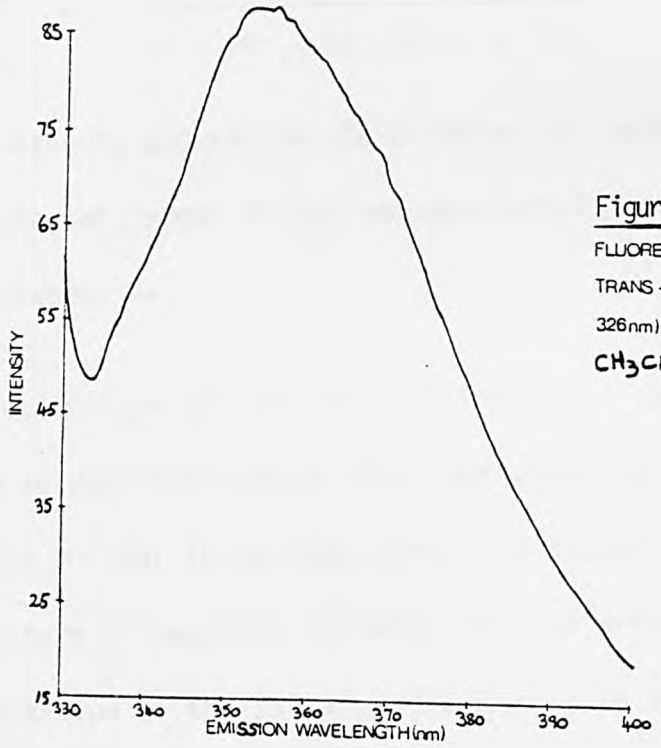


Figure 5.7

FLUORESCENT SPECTRUM OF TRANS-STILBENE(O.D.=0·1 at 326nm) & 10^{-2}M INDOLE IN CH_3CN

where

Ia = the intensity of light absorbed by the sensitiser(S),

$\emptyset_T$ = the quantum yield of triplet production from the sensitiser(S),

Kr = the rate constant for reaction of singlet oxygen with the substrate (A),

Kd = the unimolecular decay constant for singlet oxygen, and

Kq and Kq' = the rate constants for physical quenching of singlet oxygen by the substrate (A) and sensitiser (S) respectively.

corresponding deuterated solvent does not affect the photophysical properties of the sensitiser or the rate constants of the system other than Kd the solvent isotope effect upon the rate of the photo-oxidation reaction will be given by equation 5.2. For direct photo-oxidation reactions the substrate behaves as both

$$\frac{RATE_D}{RATE_H} = \frac{Kd + (Kr + Kq)\,[A] + Kq'\,[S]}{Kd' + (Kr + Kq)\,[A] + Kq'\,[S]} \qquad (5.2)$$

where Kd and Kd' are the unimolecular decay constants for singlet oxygen in the non-deuterated and deuterated solvents respectively.

the sensitiser (S) and the substrate (A). Because deuteration of a solvent only causes minor perturbations in the properties of the solvent it has been generally assumed that Kd is the only term in equation 5.2 which will be affected by the isotopic composition of the solvent. However, this assumption is one which has rarely been verified. For the observation of maximal

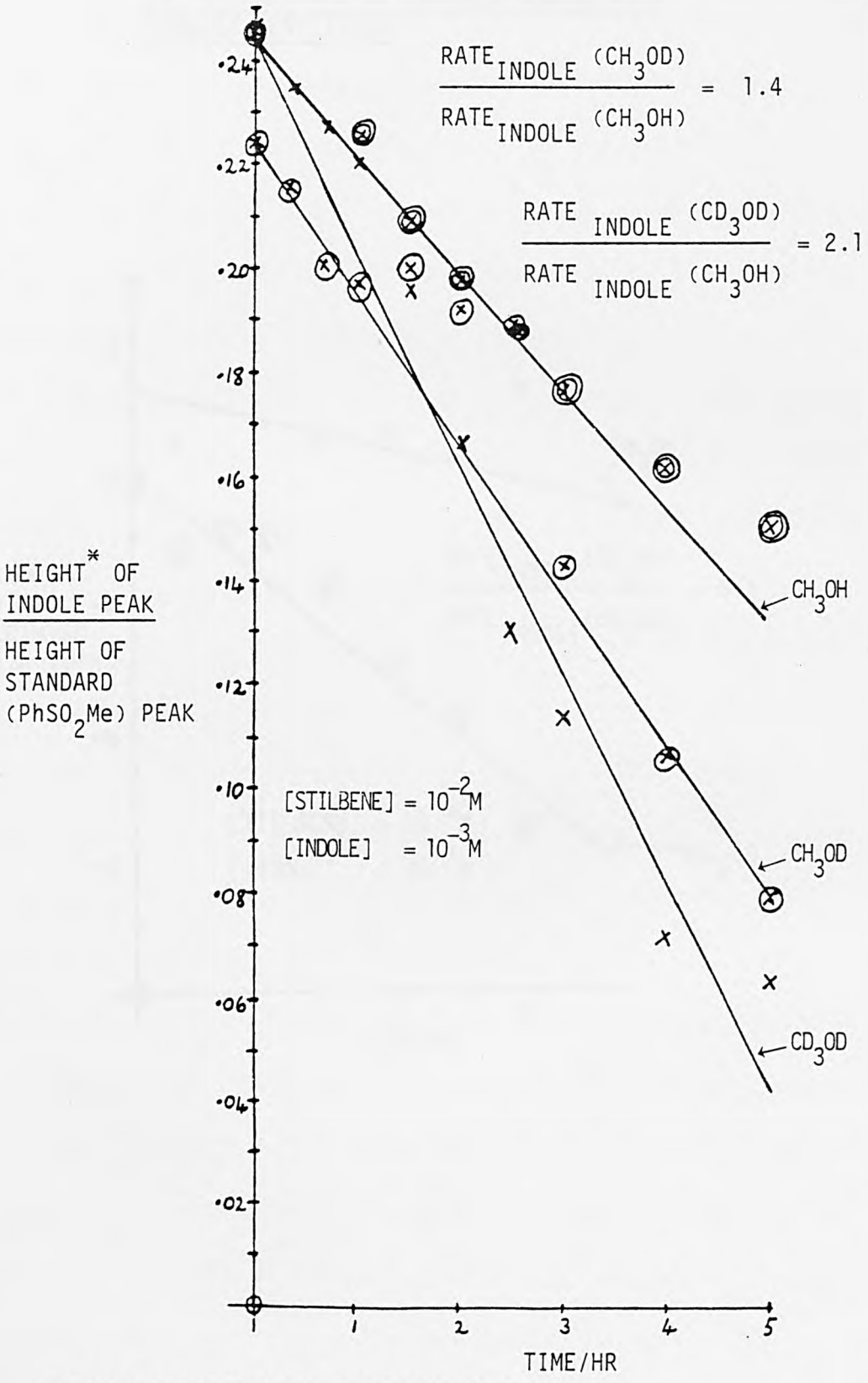
solvent isotope effects the reaction with singlet oxygen must involve a diffusional process and the concentration of the substrate and the sensitiser must be kept as low as possible in order that $Kd >> (Kr + Kq)[A] + Kq'[S]$. This is a far more reliable technique than using quenchers of singlet oxygen [20], but even so it is not without its pitfalls [19]. A comparison of the rates of oxidation of indole (10^{-3}M) in methanol, monodeuterated methanol (CH_3OD) and fully deuterated methanol (CD_3OD) sensitised by stilbene (10^{-2}M) yielded the following isotope effects (Figure 5.8):

$$\frac{RATE_{INDOLE\ (CH_3OD)}}{RATE_{INDOLE\ (CH_3OH)}} = 1.4$$

$$\frac{RATE_{INDOLE\ (CD_3OD)}}{RATE_{INDOLE\ (CH_3OH)}} = 2.1$$

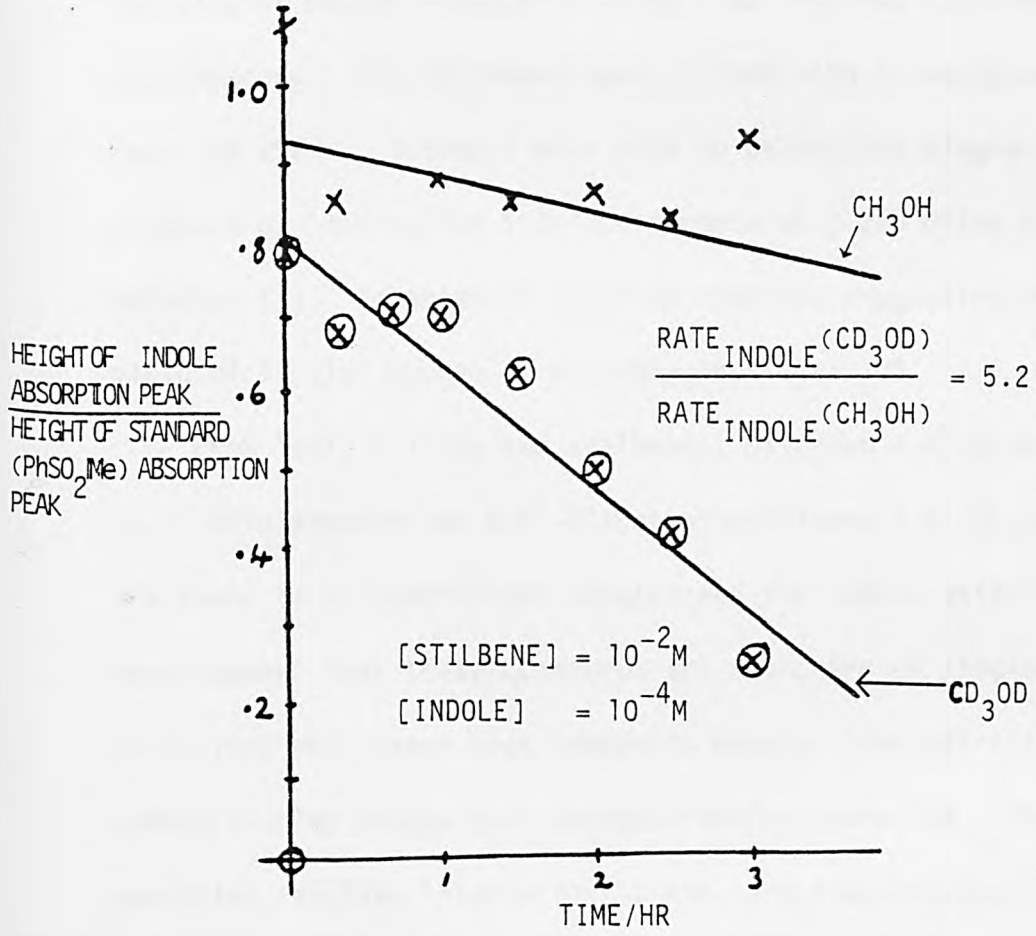
The values for the isotope effects are remarkably very low. Without any complicating factors the expected isotope effect for the CD_3OD/CH_3OH system is approximately 22 [21]. A possible reason for the low values is that the inequality $Kd >> (Kr + Kq)[A] + Kq'[S]$ (A = indole and S = stilbene for this case) does not hold with the high concentration of indole employed for the measurements [19]. The isotope effect value was increased to 5.2 for the CD_3OD/CH_3OH system by dropping the indole concentration to 10^{-4}M (Figure 5.9). This value is still lower than expected. A possible reason is that the stilbene is itself acting as a

Figure 5.8. SOLVENT ISOTOPE EFFECT OF INDOLE OXIDATION IN THE PRESENCE OF TRANS-STILBENE AS A SINGLET SENSITISER



* THIS IS PROPORTIONAL TO CONCENTRATION

FIGURE 5.9. SOLVENT ISOTOPE EFFECT OF INDOLE OXIDATION IN THE PRESENCE OF TRANS-STILBENE AS A SINGLET SENSITISER



quencher of singlet oxygen. Recently, many well-known sensitisers have been shown to act as quenchers of singlet oxygen [22] and this leads to the observed kinetic solvent isotope effects being lower than expected. Nevertheless, the observation of an isotope effect in this work demonstrates that singlet oxygen is involved.

Attempts were also made to see if the stilbenes are sensitisers for singlet oxygen production using time resolved fluorescence spectroscopy. The stilbenes were excited with a neodymium YAG laser at 355nm. Attempts were made to detect the singlet oxygen produced by looking for its fluorescence at 1.27μ using a germanium detector [9]. No emission could be detected suggesting that the yield of singlet oxygen is probably less than 10%.

From Table 5.1 the two stilbenes, disodium 4,4′-diaminostilbene -2,2′-disulphonate and 4,4′-diacetamidostilbene-2,2′-disulphonic acid are found to be inefficient sensitisers for indole oxidation. It would appear that these compounds are quenchers of singlet oxygen. It is very well known that compounds bearing lone pair(s) of electrons quench singlet oxygen by a charge-transfer mechanism. Charge-transfer quenching involves interaction of the very electron-poor singlet oxygen molecule with electron donors to give a charge-transfer complex (or perhaps even complete electron-transfer in some cases); intersystem crossing restrictions are relaxed in the complex, which can then dissociate to donor and ground-state oxygen [23 - 27], (equation 5.3). The types of compound which probably quench

$$D + {}^1O_2 \rightleftharpoons [D^+ \ldots O_2^-]^1 \rightleftharpoons [D^+ \ldots O_2^-]^3 \rightleftharpoons D + {}^3O_2 \quad (5.3)$$

Donor

or react by the charge-transfer mechanism include amines, phenols, some metal complexes, and perhaps such compounds as sulphides, iodide, azide, superoxide ion, and similar electron-rich compounds. Some compounds (such as amines, sulphides, and phenols) which react by this mechanism not only quench but also react. The proportion of reaction versus quenching is dependent on structure and probably on solvent. It is likely that the charge-transfer complex which results can either transfer the electron back, giving ground state oxygen, or combine to give product, DO_2, (equation 5.4). To test the hypothesis that the two stilbenes

$$D + {}^1O_2 \longrightarrow (D^+ \ldots O_2^-) \begin{cases} \nearrow D + {}^3O_2 \\ \searrow DO_2 \end{cases} \quad (5.4)$$

mentioned above are likely quenchers of singlet oxygen the Methylene Blue and Rose Bengal (dyes which are capable of sensitising singlet oxygen formation) sensitised oxidation of indole in the presence and absence of 4,4′ - diacetamidostilbene-2,2′ - disulphonic acid (as disodium salt) and disodium 4,4′ -diaminostilbene -2,2′ -disulphonate, respectively, were studied. By measuring the rates of reaction in the presence of varying known amounts of the stilbenes Stern-Volmer plots were constructed (Figure 5.10). The slope of each line is given by equation 5.5 and was derived

$$\text{Slope (Ksv)} = \frac{Kq}{Kd + Kr\,[\text{indole}]} \quad (5.5)$$

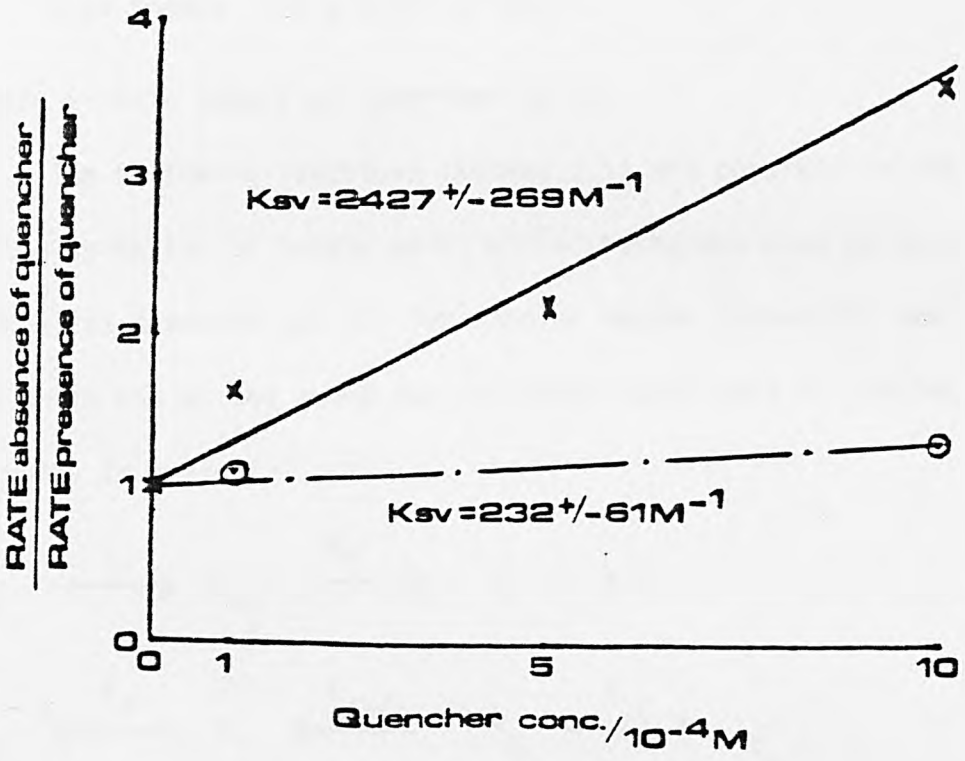


Figure 5.10

Stern-Volmer plots of the oxidation of indole using 4,4′-diacetamidostilbene-2,2′-disulphonic acid(-. θ) and 4,4′-diaminostilbene-2,2′-disulphonic acid (disodium salts) (—x—) as quenchers of singlet oxygen.

where

Kq = bimolecular quenching rate constant for quenching singlet oxygen,

Kd = unimolecular decay constant for singlet oxygen in solvent employed, and

Kr = bimolecular rate constant for reaction of singlet oxygen with indole ($7.7 \times 10^{5} M^{-1} s^{-1}$ [28]).

using kinetic theory as described below.

The following reactions (Scheme 5.1) are possible in the photo-oxidation of indole using either Methylene Blue or Rose Bengal as a sensitiser (S) for singlet oxygen production and in which one of the above two stilbenes (quenchers of singlet oxygen) is present:

$$S_{S_o} \xrightarrow{Ia} S_{S_1} \xrightarrow{K_F} S_{S_o} + h\nu$$

$$S_{S_o} \xleftarrow{K_T} S_{T_1} \xleftarrow{K_{ISC}} S_{S_1} \xrightarrow{K_{IC}} S_{S_o}$$

$$S_{T_1} + {}^3O_2 \xrightarrow{K_{O_2}} S_{S_o} + {}^1O_2$$

$${}^1O_2 \xrightarrow{Kd} {}^3O_2$$

$${}^1O_2 + \text{indole} \xrightarrow{Kr} \text{oxidised products}$$

$${}^1O_2 + \text{stilbene} \xrightarrow{Kq} {}^3O_2 + \text{stilbene}$$

Scheme 5.1

The rate of disappearance of indole is given by equation 5.6.

$$\frac{-d[\text{indole}]}{dt} = Kr[\text{indole}][^1O_2] \qquad (5.6)$$

Using the steady state approximation for the singlet and triplet excited states of the sensitiser equation 5.7 is obtained.

$$[S_{T_1}] = \frac{I\, a\emptyset_T}{K_T + [^3O_2]K_{O_2}} \qquad (5.7)$$

where the quantum yield of triplets $\emptyset_T = \frac{K_{ISC}}{K_F + K_{IC} + K_{ISC}}$.

Using the steady state approximation for singlet oxygen:-

$$[S_{T_1}][^3O_2]K_{O_2} = [^1O_2]\{Kd + Kr[\text{indole}] + Kq[\text{stilbene}]\} \qquad (5.8)$$

Substituting equation 5.7 into equation 5.8 gives equation 5.9.

$$[^1O_2] = \frac{Ia\emptyset_T[^3O_2]\, K_{O_2}}{\{Kd + Kr[\text{indole}] + Kq[\text{stilbene}]\}\{K_T + [^3O_2]\, Ko_2\}} \qquad (5.9)$$

Provided $K_T << [^3O_2]\, K_{O_2}$ the rate of disappearance of indole, obtained by substituting equation 5.9 into equation 5.6, is given by equation 5.10.

$$\frac{-d[\text{indole}]}{dt} = \frac{Kr[\text{indole}]\, Ia\emptyset_T}{Kd + Kr[\text{indole}] + Kq[\text{stilbene}]} \qquad (5.10)$$

The rate of disappearance of indole in the absence of stilbene is therefore given by equation 5.11.

$$\frac{-d[\text{indole}]}{dt} = \frac{Kr[\text{indole}]\, Ia\emptyset_T}{Kd + Kr[\text{indole}]} \qquad (5.11)$$

Equation 5.5 for the slope of each line was thus obtained as follows:

$$\frac{\text{Equation 5.11}}{\text{Equation 5.10}} = \frac{\text{RATE}_{\text{absence of stilbene}}}{\text{RATE}_{\text{presence of stilbene}}} = \frac{Kd + Kr[\text{indole}]+Kq[\text{stilbene}]}{Kd + Kr[\text{indole}]}$$

$$= 1 + \frac{Kq[\text{stilbene}]}{Kd + Kr[\text{indole}]}$$

$$= 1 + Ksv[\text{stilbene}] \quad (5.12)$$

Equation 5.12 is the Stern-Volmer equation for the system under study where $Ksv = \frac{Kq}{Kd + Kr[\text{indole}]}$ (equation 5.5).

Using the data in Figure 5.10 and a value of $7.7 \times 10^{5} M^{-1} s^{-1}$ for Kr quenching rate constants of $(1.8 \pm 0.5) \times 10^{5}$ and $(1.9 \pm 0.2) \times 10^{6} M^{-1} s^{-1}$ are obtained for 4,4′-diacetamidostilbene - 2,2′-disulphonic acid (as disodium salt) and disodium 4,4′-diaminostilbene - 2,2′-disulphonate quenchers, respectively. Clearly the stilbenes are not particularly efficient quenchers of singlet oxygen.

Since the 4,4′-diacetamidostilbene-2,2′-disulphonic acid is a quencher of singlet oxygen it might be expected to be a stable FWA when applied to wool. The stilbene was applied to wool and the treated wool exposed in a damp state to light. Both photoyellowing of the wool and degradation of the stilbene was observed. The rate of degradation of a commercial stilbene FWA (Photine-HV) applied to wool was also assessed and it was found that the diacetamido derivative was no more stable than Photine-HV (Table 5.2).

TABLE 5.2

Photoyellowing of stilbenes applied to wool

Stilbene	YI^a	YI^b	ΔYI
4,4′-diacetamidostilbene-2,2′-disulphonic acid	23.55	42.44	18.89
Photine-HV	15.34	37.09	21.75
Control (i.e. 0.0% FWA)	23.71	34.39	10.68

a = before irradiation; b = after irradiation.

The amount of stilbenes degraded during yellowing were determined by extracting undegraded FWAs with aqueous pyridine [11] where it was found that 37% of Photine-HV and almost 100% of 4,4′-diacetamido-stilbene-2,2′-disulphonic acid was degraded. These results confirm earlier work [3] which showed that the photodegradation of FWAs on wool does not solely involve singlet oxygen. It is remarkable that 4,4′-diacetamidostilbene-2,2′-disulphonic acid which is a good quencher of singlet oxygen possesses so little photostability when applied to wool.

5.4. CONCLUSION

The conclusions drawn from these studies are that stilbene FWAs will photosensitise the yellowing of wool and will degrade upon irradiation when applied to wool irrespective of whether they are efficient sensitisers of singlet oxygen production or whether they are efficient quenchers of singlet oxygen.

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CHAPTER 6

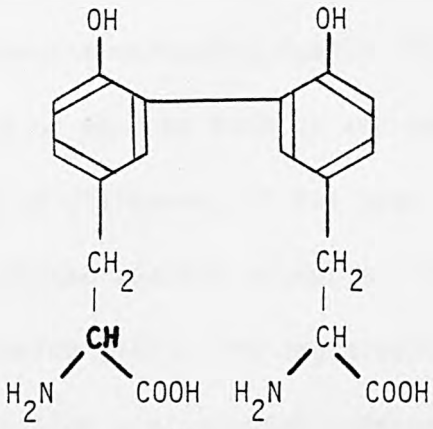
RETARDATION OF THE PHOTOYELLOWING OF WOOL AND WOOL TREATED WITH FLUORESCENT WHITENING AGENTS BY THE ACTION OF REDUCING AGENTS

6.1. INTRODUCTION (also see Chap. 2, pp.91-97).

The use of thiourea dioxide (TDO) as a reducing agent in the textile industry is well known [1,2]. Its mode of action is thought to depend on its degradation to sulphinic acid (H_2SO_2). Such reducing agents can be used to bleach wool but the whiteness attained is usually slightly inferior to that obtained with hydrogen peroxide. Furthermore, there have been several reports [3,4] that the inclusion of reducing agents such as thioglycolic acid and triarylphosphines within the wool structure leads to some stabilisation of wool towards photoyellowing. This effect is most likely due to an increased level of photobleaching.

In order to design effective photobleachers it is necessary to have some understanding of the mechanism of photoyellowing [5-7]. It is clear that tryptophyl residues play a key role [8,9]. These are photodegraded to N-formylkynurenine which can act as photosensitisers in further photodegradation reactions by either producing singlet oxygen [10] or by participating in hydrogen abstraction reactions giving radicals which in turn produce hydroperoxides by auto-oxidative processes. Furthermore, the generation of tryptophyl radicals can lead to degradation of tyrosyl residues by hydrogen abstraction of the phenoxyl hydrogen [11]. The aryloxy radical generated by this process can trigger off the formation of di (I)[12], tri (II) and other polytyrosines. Such materials are suspected as being particularly sensitive to photooxidative processes [13], leading to quinones, and highly

coloured condensation products such as melanin [14].



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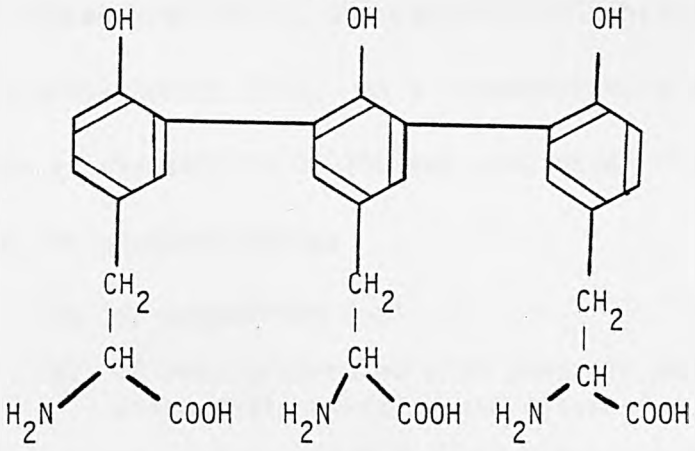


II

The strategy used for minimising photooxidative degradation was to irradiate the wools in the presence of oxygen and a reducing agent. In this way it was hoped that carbonyl compounds produced by degradation of tryptophyl residues would be reduced thereby reducing their propensity to act as a photosensitiser. Furthermore, the presence of a reducing agent will transform any hydroperoxides into innocuous alcohols thereby minimising damage. The reducing agent should also protect tyrosyl residues and related species such as dityrosine by preventing radical induced phenolic oxidations

which lead to the highly coloured and reactive quinones.

A pronounced disadvantage of fluorescently whitened wool is that all fluorescent whitening agents (FWAs) photosensitise the photoyellowing of wool by both UV and near visible blue light [5]. In the case of stilbenes, it has been demonstrated that one of the FWA photodegradation products also photosensitises wool to photoyellowing [15]. The degradation products most likely to cause discolouration are carbonyl compounds [7] and these have, as stated previously, the capacity to sensitise protein and lipid peroxidation [10]. As a consequence, a study has been undertaken of the ability of TDO and some other reducing agents to reduce the photoyellowing:

(a) of unmodified wool,

(b) of wool pretreated with phenolic materials which mimic tyrosine and dityrosine,

and (c) of wool to which FWAs have been applied.

6.2. EXPERIMENTAL

6.2.1. Materials

The following chemicals were used as supplied:

Lissapol N (Vickers Laboratories Ltd), o,o′-Biphenol,p,p′-Biphenol, 1,1′-bi-2-naphthol and 2,4-dimethylphenol (all Aldrich), a non-ionic surfactant G.1285 (Atlas), Photine-HV (Hickson and Welch Ltd), thiourea dioxide (formamidine sulphinic acid), thiourea and sodium benzene sulphinate (all Aldrich), Rongalit C (BASF) and aqueous formaldehyde solution (38% w/v, Fisons).

The wool fibre employed was a botany serge (2/2 twill of weight 200 g/m^2) supplied by Salts of Saltaire.

6.2.2. Methods

6.2.2.1. Wool Bleaching

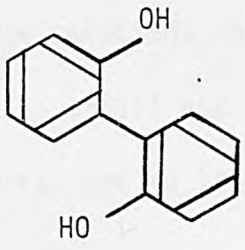
Wool serge fabric was bleached overnight by a pad-batch (room temperature) method, padding on (100% wet pick-up) 80 g/l hydrogen peroxide (100 volumes, about 30% w/v H_2O_2), 5 g/l formic acid (95-97%) and 10 g/l Lissapol N [8]. This was followed by rinsing in cold water and drying in air at room temperature.

6.2.2.2. Wool Chlorination

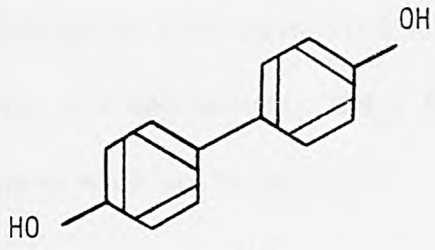
Wool was chlorinated in the continuous KROY fabric chlorinator at R. Armitage Ltd (Bradford) using chlorine gas to give approximately 2% active chlorine of weight fibre (o.w.f.). Normal 'antichlor' conditions using 10 g/l sodium sulphite were employed.

6.2.2.3. Application of o,o'-Biphenol (III), p,p'-Biphenol (IV), 1,1'-Bi-2-naphthol (V) and 2,2'-Dihydroxy-3,3',5,5'-tetramethyl-diphenyl (VI) to wool serge fabric by a pad-dry method

METHOD A



III



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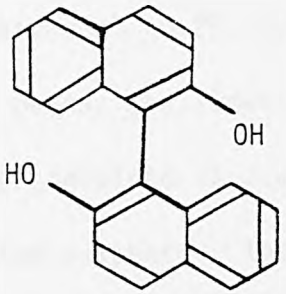

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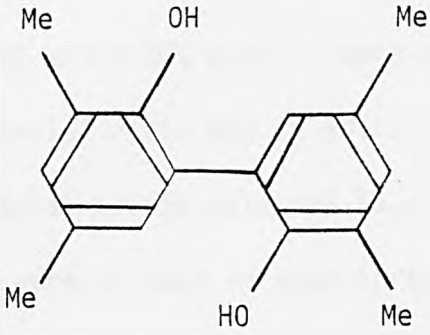


VI

Solutions of the above-named phenols (~ 0.054 mol l^{-1}) were made up in aqueous solution (specific gravity = 0.91; the saturated solutions of compounds IV and V were used after agitation of the corresponding suspensions in an ultrasonic bath for 2 hours at room temperature and filtration). To these, Lissapol N (1g per 100 ml ammonia solution) was added (the suspensions formed on adding this to solutions of compounds V and VI were utilised for subsequent work). Each liquor was padded onto wool serge fabric, achieving approximately 100% wet pick-up, and the treated fabrics were allowed to dry in air overnight at room temperature.

6.2.2.4. Applications of o,o′-Biphenol (III) and 2,2′-Dihydroxy-3,3′,5,5′-tetramethyldiphenyl (VI) to wool serge fabric from an emulsifiable concentrate

METHOD B

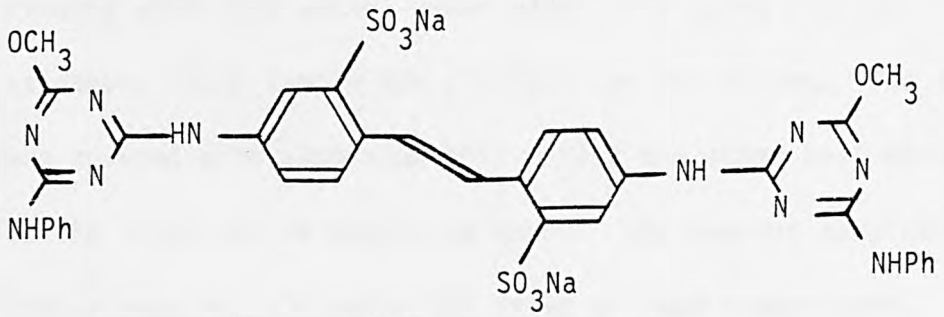
Stock solutions composing of the above biphenols (10% w/v in the case of III and 5% w/v for VI, v = net volume) and a non-ionic surfactant (G.1285; 10% w/v) were made up in methanol.

The appropriate volumes to correspond with the required amount of biphenol (0.25, 0.50, 1.00 and 2.00% o.w.f.) were added to the baths; the liquor to material ratio was of 50:1; the baths also contained 1% o.w.f. glacial acetic acid and 5% o.w.f. anhydrous sodium sulphate. The baths were allowed to equilibrate in the presence of the wool at room temperature in a Jeffreys Dyemaster (John Jeffreys Ltd, Rochdale, Lancashire), before (10 minutes) and after (15 minutes) the addition of the appropriate volumes from the stock solutions. The temperature was then raised at 1°C/min to bring the baths to the boil and maintained there for an hour, followed by rinsing in cold water and drying at room temperature.

6.2.2.5. Diffuse Reflectance Spectra

Reflectance spectra of fabric samples treated with the above aromatic alcohols were obtained as described previously [16] except that barium sulphate was used as a reference in this case.

6.2.2.6. Application of Photine-HV, (VII), to bleached wool



VII

A pre-bleached wool sample was treated in a bath set at pH 5 (4% o.w.f. sodium acetate plus required acetic acid), at a liquor to material ratio of 30:1 in the presence of ethylenediamine-tetraacetic acid (disodium salt, dihydrate - 1% o.w.f.) and Lissapol N (1% o.w.f.) with Photine-HV (a commercial fluorescent brightener) at 0.5% o.w.f. The temperature was raised to 80°C at 1°C/min and maintained for one hour, followed by cold water rinsing and drying at room temperature.

6.2.2.7. Irradiation of wool samples

The wool samples (20 cm x 5 cm, supported on rectangular glass plates of similar dimensions) were exposed to 3 x 20 W long wavelength u.v. fluorescent tubes with a maximum output at approximately 350 nm. The samples (after wetting with aqueous Lissapol N and rinsing in cold water) were immersed under different 2% aqueous reductive bleaching agent solutions and/or water only (net volume of 2 litres was used, which formed a depth of 20 mm). These were irradiated at room temperature in a flat, open, stainless steel tray (55 cm x 21.5 cm x 4.5 cm). The samples after exposure with the bleaching agent solutions were also irradiated (after rinsing with cold water) under water only using similar conditions as above. Each sample was divided into two halves. One half was covered with aluminium foil whilst the other half was exposed to the light for 24 and/or 48 hours. The exposed samples were then rinsed in cold water and dried at room temperature.

6.2.2.8. Extractions [17]

A strip of the Photine-HV whitened fabric and a strip (same weight) of the same sample which had been exposed under 2% aqueous thiourea dioxide solution (a reductive bleaching agent; see above) were made wet with aqueous Lissapol N solution and rinsed in cold water. Extraction of the brighteners and any photodegradation products was achieved by boiling (5 mins) each strip in 25% aqueous pyridine (50 mls) three times. The amount of brightener extracted from each sample was determined by u.v. spectroscopy.

6.2.2.9. Yellowness Index (Y.I.) Measurements

The yellowness index formula employed was the following:

$$YI = \frac{100\ (1.316X - 1.164Z)}{Y}$$

where X, Y and Z are the CIE tristimulus values obtained from the Macbeth Micromatch reflectance spectrophotometer. The lower the yellowness index value the whiter is the wool colour.

6.2.2.10. Light Fastness Measurements

Dry wool samples were exposed to Grey Scale 4 using the Xenotest (original quartz Hanau) according to the ISO standard procedure (ISO 105-B02: 1978).

6.2.2.11. Synthesis of 2,2′-Dihydroxy-3,3′,5,5′-tetramethyldiphenyl(VI)

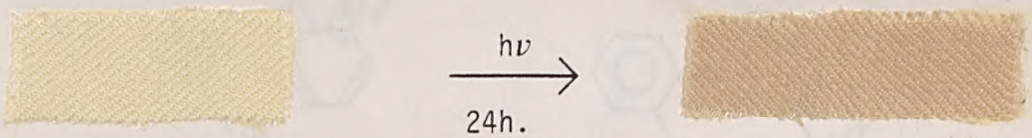
This was prepared from 2,4-dimethylphenol according to the procedure described by Haynes, Turner and Waters [18]. The product was identified by its elemental analysis (Found: C,79.07; H,7.48. $C_{16}H_{18}O_2$ requires C,79.30; H,7.48) and mass spectrum ($M^{+\cdot}$ = 242).

6.3. RESULTS

Irradiation of wool serge, bleached wool and chlorinated wool in 2% aqueous TDO solution led to photobleaching as shown by a decrease in yellowness index values (Table 6.1/Column A). Further irradiation of these treated wools in water led to photoyellowing (Table 6.1/Column B). In the case of wool serge this was not as marked as when untreated wool serge was irradiated in the absence of TDO. The light fastness values (Table 6.1/Columns C and D) show that for wool serge the TDO treatment does produce some photostability in agreement with the results presented in Column A of Table 6.1. Dityrosine, unlike tyrosine [19,20], has never been reported to be associated with the yellowing of wool, and has probably not been studied in this context. Sufficient evidence has been obtained during the course of this work to suggest that one of the sources of photoyellowing in wool is dityrosine. In order to mimic the effect of dityrosine, o,o′-biphenol was padded onto wool serge. Exposure of the treated wool under water to irradiation led to a marked yellowing (see fabrics below) whereas exposure to irradiation in the presence

of TDO produced bleaching (Table 6.1/Column A). However, as with the untreated wools, the TDO treated sample underwent yellowing upon further irradiation in the presence of water (Table 6.1/Column B). Also, exposure under TDO solution of the irradiated (24h. under water only) sample of o,o′-biphenol led to some bleaching (Table 6.1/Column A) and this TDO treated sample underwent yellowing upon further irradiation under water (Table 6.1/Column B). Similar experiments were carried out with bleached wool treated with a commercial FWA (Photine-HV). Irradiation of the sample under water led to yellowing whereas irradiation in the presence of TDO produced some bleaching (Table 6.1/Column A). The treatment, however, does not provide a lasting effect since further irradiation of the TDO treated sample under water produced yellowing (Table 6.1/Column B). As can be seen from the light fastness values (Table 6.1/Columns C and D), application of the FWA decreases light fastness on both the TDO treated and untreated samples.

FABRICS



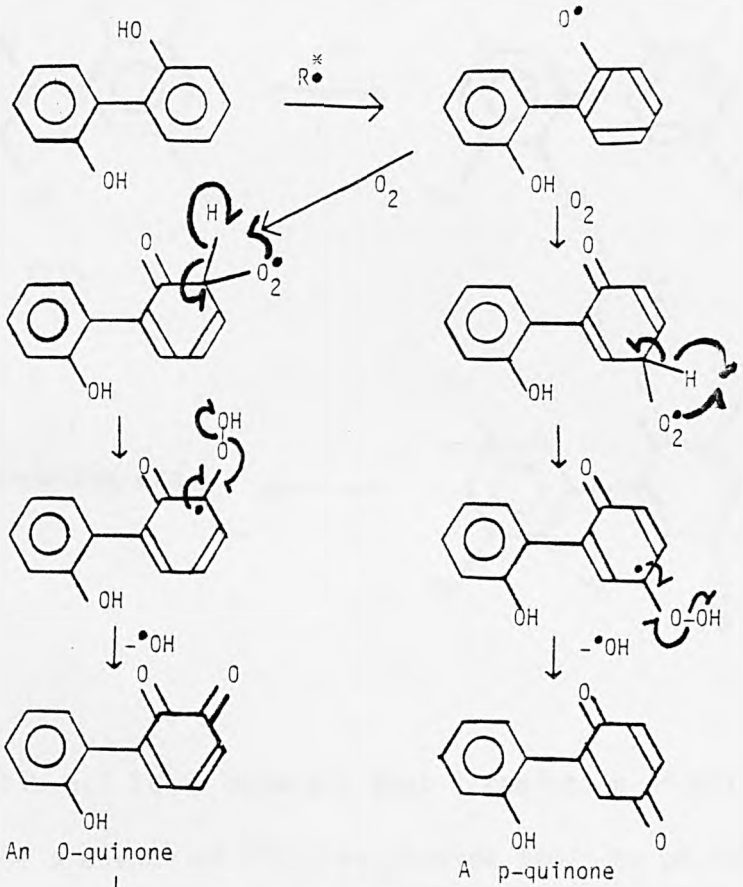
BEFORE EXPOSURE

AFTER EXPOSURE

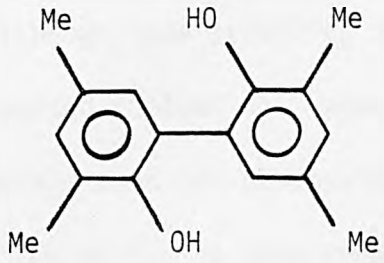
o,o′-Biphenol on wool serge.

In order to further substantiate our claim that models for dityrosine enhance photoyellowing and thus by extrapolation conclude

that dityrosine and related compounds play an important part in the photoyellowing of wool, various biphenols were applied to wool (Table 6.2). That the applications were successful was substantiated by diffuse reflectance measurements outlined in the experimental section. In all cases exposure of the samples under water to light led to severe photoyellowing. Furthermore, the results show that as the loading of the biphenol on the fibre is increased (using Method B), so an increase in photoyellowing is observed. A possible mechanistic route for the formation of the highly coloured products on wool surface is shown below using o,o′-biphenol as an example:

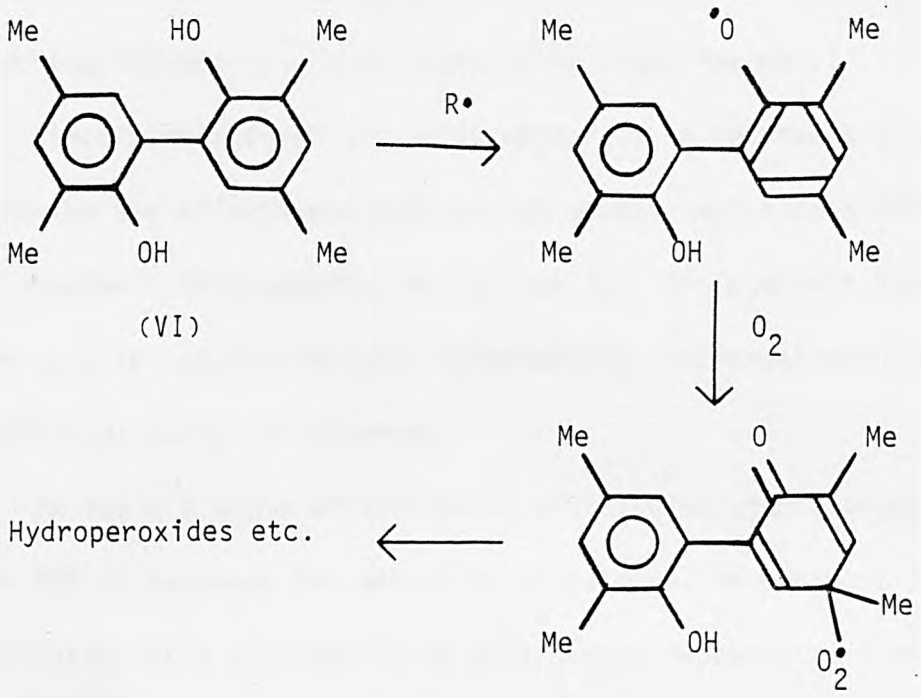


The behaviour of phenol VI is interesting to note since the positions o- and p- to the phenolic hydroxyl groups are blocked thereby



(VI)

preventing coupling in these positions. Even so, yellowing is observed although it is not as marked as with the less hindered o,o′-biphenol. The phenol VI can still give rise to peroxy compounds:



From Table 6.1 it is apparent that irradiation of different samples of wool in the presence of thiourea dioxide leads to photobleaching which can be reversed upon further irradiation under water.

An investigation was therefore carried out on the ability of a variety of compounds related to TDO to bring about photobleaching (Table 6.3). Thiourea retarded photoyellowing but did not arrest it (Column A) although some stability to further irradiation in water was imparted (Column B). When a mixture of thiourea and formaldehyde was used the photoyellowing was retarded even more (Column A) but as can be seen from Column B the effect was not permanent. Formaldehyde alone had a small effect on retarding photoyellowing but not like the strong effect observed when it was used in conjunction with thiourea. Rongalit C (sodium formaldehyde sulphoxylate - $HOCH_2SO_2Na$) could be used to achieve photobleaching (Column A) and an increase in light fastness.

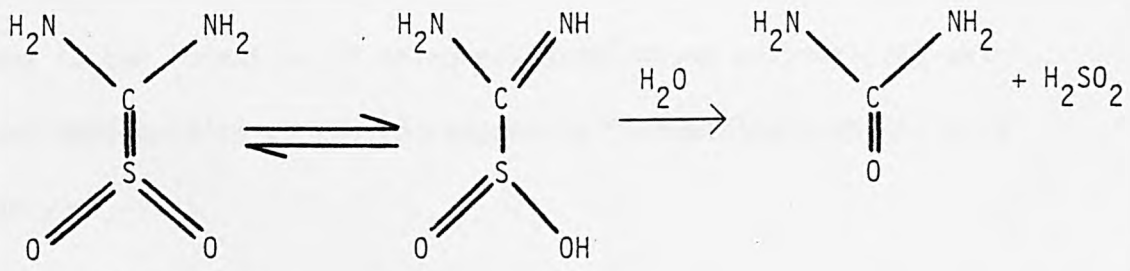
Since simple sulphinic acids appear to be retarders of photoyellowing the effectiveness of sodium benzene sulphinate ($PhSO_2Na$) was studied. This material on its own had little effect but when used in conjunction with formaldehyde, increased retardation of photoyellowing was observed.

In Table 6.4 the effectiveness of reducing agents other than TDO to decrease the amount of discolouration produced by degradation of o,o′-biphenol on wool during exposure to light in the presence of the reducing agents is assessed. None behaved as effectively as TDO.

6.4 DISCUSSION

The proposal that the photoyellowing of wool can be alleviated by having a reducing agent such as TDO present in the wool appears to be correct from the results presented in Table 6.1. Furthermore, TDO prevents the yellowing of Photine-HV treated fabric although it does not prevent destruction of the FWA - as determined by extraction of undegraded FWA after irradiation [33% of Photine-HV (applied to bleached wool at pH 5) was observed to degrade whilst irradiating under 2% aqueous TDO solution whereas 37% of Photine-HV (applied to bleached wool at pH 4) degraded during irradiation under water only; similar extraction conditions were used for each case]. Thus, it is clear that TDO retards yellowing by reducing the coloured products formed on photodegradation of amino acids and the FWA. Furthermore, o,o′-biphenol, which mimics the action of dityrosine, undergoes extensive photoyellowing when applied to wool but these yellow products are then reduced by TDO.

The TDO is thought to be a source of sulphinic acid (H_2SO_2):

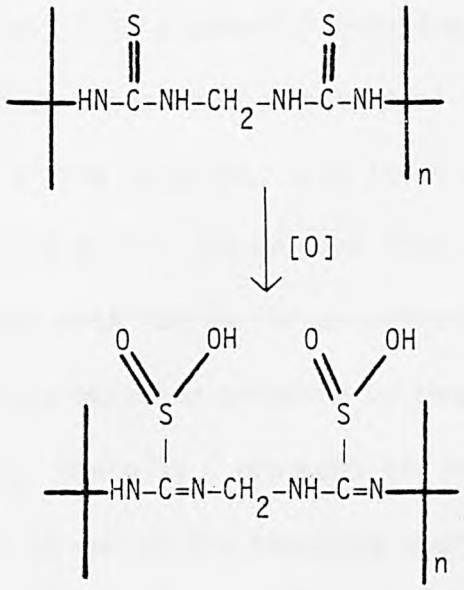


TDO

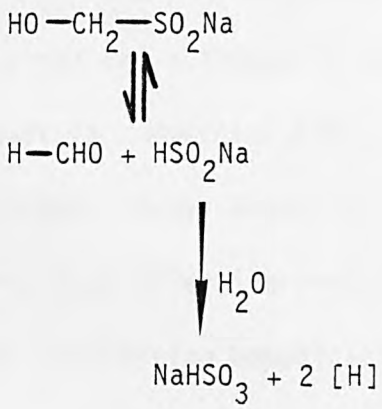
This material is a powerful reducing agent producing sulphur dioxide on oxidation which in solution also acts as a reducing agent. Sulphinic and sulphurous acids should be able to act as excellent reducing agents for excited states since they provide a source of readily abstractable hydrogen atoms.

The finding that many biphenols (Table 6.2) undergo rapid photodegradation on the surface of wool shows that much of the observed wool photoyellowing may be due to dityrosine which is present to a substantial extent in the cuticle of wool [21]. Indeed, dityrosine may be nature's "light protector" since, on oxidation, the products form a highly effective light screen.

Thiourea can, under certain conditions, be photooxidised to thiourea dioxide [22,23]. Whether this reaction occurs upon photooxidation of thiourea applied to wool is not known. The fact that thiourea retards photoyellowing but does not photobleach is probably due to it either being an effective source of TDO and hence H_2SO_2 or its transformation to TDO is slow compared with the rate of photoyellowing. The improved performance of thiourea when used in conjunction with formaldehyde is probably due to the formation of thiourea-formaldehyde polymer [24] which can photooxidise to the corresponding formamidinesulphinic acid polymer, viz:



Formaldehyde itself may also have a beneficial effect by crosslinking disulphide linkages [25] but from the result obtained following irradiation of wool in the presence of this compound, this effect is not marked. Rongalit C, a good source of sodium sulphinate and formaldehyde behaves like TDO by promoting photo-bleaching:



Sodium benzenesulphinate cannot act as a source of sodium

sulphinate but it is a powerful reducing agent. The results in Table 6.3 show that a combination of formaldehyde and the sodium salt of the sulphinic acid leads to a synergistic effect.

From Table 6.4 it can be seen that none of the reducing agents compare with TDO as far as efficiency in decreasing the amount of discolouration produced by degradation of o,o′-biphenol is concerned. Rongalit C produces the next best performance; whether this is due to the reducing agent reducing the yellowing of both wool and biphenol has not been ascertained. As shown in the retarding of the photoyellowing of wool, there is an obvious beneficial synergistic effect obtained by using the reducing agents in the presence of formaldehyde.

6.5. CONCLUSION

The application of TDO to wool promotes photobleaching of the fibre but unfortunately the white colouration produced is not permanent to further irradiation in water. TDO also reduces the yellow photodegradation products of o,o′-biphenol (a model for dityrosine) and although it does not prevent the photodestruction of Photine-HV (a commercial FWA), it again reduces any coloured products formed. Other potential reducing agents were tried and all were less effective photobleaching agents than TDO. However, an interesting beneficial synergistic effect was uncovered when these reagents were used in the presence of formaldehyde.

TABLE 6.1

Yellowness indices and light fastnesses of wool samples after irradiation under 2% aqueous thiourea dioxide (TDO) solution and/or water only at 350 nm

SAMPLE	Y.I. before exposure (1)	Y.I. after exposure for 24 h. under water only (2)	Y.I. after exposure for 48 h. under water only (3)	ΔY.I. (2-1)	ΔY.I. (3-2)	Y.I. after exposure for 24 h. under TDO solution (4)	Y.I. after exposure for 24 h. of TDO treated sample under water only (5)	COLUMN A ΔY.I. (4-1)	COLUMN B ΔY.I. (5-4)	COLUMN C Light fastness before any treatment	COLUMN D Light fastness after exposure for 24 h. under TDO solution
Wool serge fabric	21.88	26.79	29.82	4.91	3.03	15.13	21.79	-6.75	6.66	4	4+
Bleached wool	19.55	28.52	31.36	8.97	2.84	16.08	26.81	-3.47	10.73	4	4
Chlorinated wool	20.74	26.70	30.06	5.96	3.36	20.14	29.92	-0.60	9.78	4	4
o,o'-Biphenol padded onto wool serge fabric	22.87	48.12	—	25.25	—	17.27	24.66	-5.60	7.39	—	—
Irradiated (24 h. under water only) sample of o,o'-biphenol on wool serge fabric	48.12	—	—	—	—	37.84	41.05	-10.28	3.21	—	—
0.5% o.w.f. Photine-HV on bleached wool	9.34	30.23	36.68	20.89	6.45	7.87	27.03	-1.47	19.16	3-4	3-4

TABLE 6.2

Photoyellowing[a] of phenols applied to wool

PHENOLS	Y.I. (before irradiation)	Y.I. (after irradiation)	ΔY.I.
o,o'-Biphenol[b]	22.87	48.12	25.25
p,p'-Biphenol[b]	23.46	39.51	16.05
1,1'-Bi-2-naphthol[b]	26.69	45.17	18.48
2,2'-Dihydroxy-3,3',5,5'-tetramethyldiphenyl[b]	31.01	49.75	18.74
1.00% o.w.f. o,o'-Biphenol[c]	28.01	48.57	20.56
2.00% o.w.f. o,o'-Biphenol[c]	25.94	51.28	25.34
0.25% o.w.f. 2,2'-Dihydroxy-3,3',5,5'-tetramethyldiphenyl[c]	25.48	35.91	10.43
0.50% o.w.f. 2,2'-Dihydroxy-3,3',5,5'-tetramethyldiphenyl[c]	27.06	40.74	13.68
1.00% o.w.f. 2,2'-Dihydroxy-3,3',5,5'-tetramethyldiphenyl[c]	27.09	43.97	16.88
2.00% o.w.f. 2,2'-Dihydroxy-3,3',5,5'-tetramethyldiphenyl[c]	26.97	47.43	20.46

a. Irradiated for 24 h under water.
b. Applied to wool using Method A.
c. Applied to wool using Method B.

TABLE 6.3

Yellowness indices and light fastnesses of wool serge fabrics after irradiation under 2% aqueous reducing agents and water only at 350 nm

Reducing agent (2% aqueous solution)	Y.I. before exposure (1)	Y.I. after exposure for 24 h under water only (2)	Y.I. after exposure for 48 h under water only (3)	ΔY.I. (2-1)	ΔY.I. (3-2)	Y.I. after exposure for 24 h under reducing agent solution (4)	Y.I. after exposure for 24 h under water only of wool serge fabric treated with 2% aqueous reducing agent solution (5)	COLUMN A ΔY.I. (4-1)	COLUMN B ΔY.I. (5-4)	Light fastness after exposure for 24 h under reducing agent solution
None	21.88	26.79	29.82	4.91	3.03	—	—	—	—	4
$CS(NH_2)_2$	"	"	"	"	"	25.12	25.93	3.24	0.81	4
$CS(NH_2)_2$ and equivalent concentration of $CH_2{=}O$ (0.26 M)	"	"	"	"	"	22.24	23.55	0.36	1.31	4
0.26 M $CH_2{=}O$	"	"	"	"	"	24.38	26.89	2.5	2.51	4
Rongalit C	"	"	"	"	"	16.74	18.19	-5.14	1.45	4+
$PhSO_2Na$	"	"	"	"	"	28.14	31.42	6.26	3.28	—
$PhSO_2Na$ and equivalent concentration of $CH_2{=}O$ (0.12 M)	"	"	"	"	"	24.44	28.42	2.56	3.98	3-4

TABLE 6.4

Yellowness indices of wool serge fabrics treated with o,o'-biphenol after irradiation under 2% aqueous reducing agents and water only at 350 nm

Reducing agent (2% aqueous solution)	Y.I. before exposure (1)	Y.I. after exposure for 24 h. under water only (2)	ΔY.I. (2-1)	Y.I. after exposure for 24 h. under reducing agent solution (3)	Y.I. after exposure for 24 h. under water only of the wool sample treated with 2% aqueous reducing agent solution (4)	ΔY.I. (3-1)	ΔY.I. (4-3)
None	22.87	48.12	25.25	—	—	—	—
$CS(NH_2)_2$	"	"	"	39.95	42.72	17.08	2.77
$CS(NH_2)_2$ and equivalent concentration of $CH_2{=}O$ (0.26 M)	"	"	"	27.94	31.41	5.07	3.47
0.26 M CH_2O	"	"	"	41.16	44.17	18.29	3.01
Rongalit C	"	"	"	23.42	27.54	0.55	4.12
$PhSO_2Na$	"	"	"	37.92	41.72	15.05	3.80
$PhSO_2Na$ and equivalent concentration of $CH_2{=}O$ (0.12 M)	"	"	"	34.30	39.59	11.43	5.29

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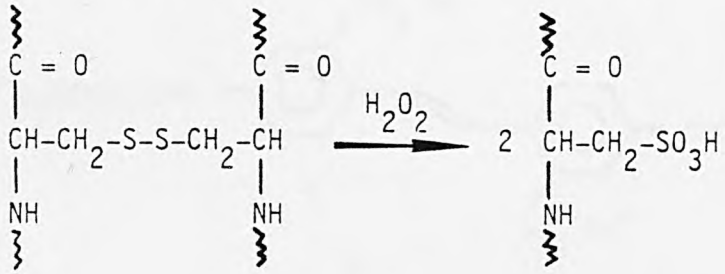
CHAPTER 7

SOLUTION AND SOLID STATE STUDIES OF FLUORESCENCE QUENCHING BY DISULPHIDE BONDS

7.1. INTRODUCTION

It is well known that the tryptophan residue present in wool is the main component responsible for the fluorescence of wool [1]. This fluorescence intensity can be doubled by reducing the disulphide bond of cystine component present in wool with tributylphosphine [2,3]. This observation clearly indicates that the presence of the disulphides is responsible for at least 50% quenching of wool's fluorescence. Furthermore, the original fluorescence of wool can also be enhanced by treating it with an alkaline solution of hydrogen peroxide. Hydrogen peroxide, an oxidative bleaching agent, is believed to generate two cysteic acid residues by attack on one cystine residue [4]:



Clearly, the loss of disulphide bonds accounts for the increment in fluorescence.

The literature does not contain much evidence on fluorescence quenching by disulphide bonds. This chapter therefore presents further experimental evidence on this aspect by studying in solution and in the solid state (i.e. wool fabric).

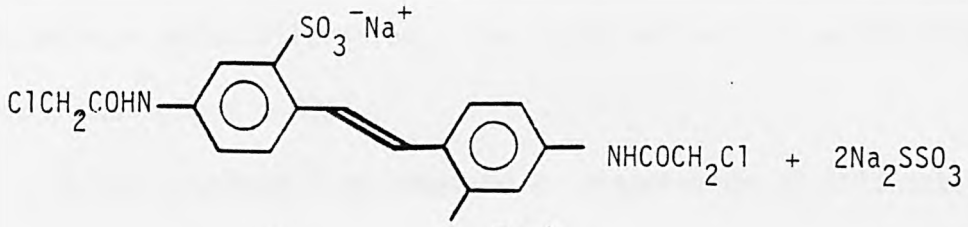
7.2. EXPERIMENTAL

7.2.1. Preparation of Bunte salts, 1 and 3 [5].

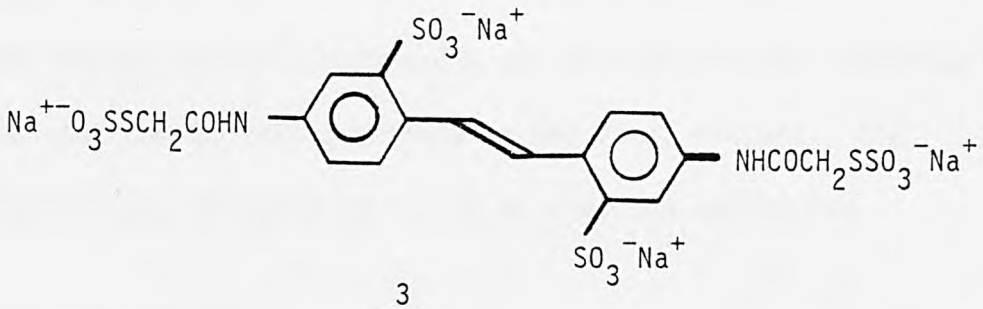
These were prepared as depicted by the following equations:

$$PhCH_2 - Cl + Na_2SSO_3 \xrightarrow{-NaCl} PhCH_2 - SSO_3^-Na^+$$

1



2



3

1 was prepared by refluxing equimolar quantities of benzyl chloride and sodium thiosulphate (this starting material contained 5 moles of water of crystallization; 31.6 mmol of each starting material was used) in 50% aqueous ethanol (20cm^3 of each used) for 2 hours. The cooled reacted mixture was then evaporated under reduced pressure. To the residue obtained was added 100cm^3 water and unreacted benzyl chloride was extracted from the resulting

suspension with 100cm^3 ether. The required compound was precipitated by adding sodium chloride to the aqueous solution. The precipitate was filtered and washed thoroughly with ether followed by drying over phosphorus pentoxide in a vacuum desiccator. The Bunte salt was purified by dissolving it in ethanol to remove any insoluble sodium chloride followed by evaporating the solution to dryness after filtration. The yield before the purification stage was 4.3g ($\sim$60%).

3 was obtained from compound 2 (preparation of this material has been previously described, see Chapter 4 p.143 for full details) by refluxing it with twice the number of moles of sodium thiosulphate in water for 2 hours. Adding a large quantity of sodium chloride to the cooled reaction mixture did not precipitate the compound; it was obtained by adding acetone to the final mixture. The precipitate was filtered and dried in a vacuum desiccator.

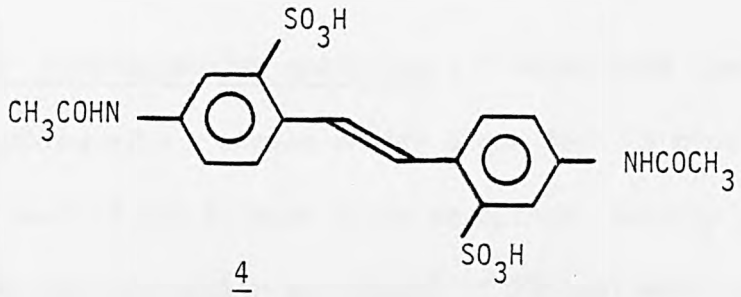
Analysis of compound 1

Proton nmr (in D_2O using DSS* as reference) : 4.33δ(2H, due to $-CH_2-$ protons) and 7.36δ (5H, due to the aromatic protons). IR (KBr disc) : The spectrum included bands at $\sim$ 700cm^{-1} and 770cm^{-1} which strongly imply for a monosubstituted benzene compound.

*DSS = Sodium 2,2-Dimethyl-2-Silapentane-5-Sulphonate.

7.2.2. Preparation of 4,4′-diacetamidostilbene-2,2′-disulphonic acid,4.

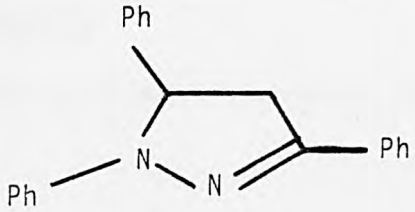
Compound 4 has the following structure:



4

The above compound has been synthesised previously, see Chapter 5 p.184 for details.

7.2.3. Preparation of a Pyrazoline, 5.



5

This compound was synthesised according to the procedure of Leaver and Rivett [6].

7.2.4. Fluorescence quenching experiments in solutions

7.2.4.1. Intermolecular quenching : Fluorescence spectra of degassed (by flushing with a stream of dry argon for ~5 mins.) solutions of the fluorescers (made up to an optical density of 0.1 in the appropriate solvent at the excitation wavelength)

only and with added disulphide bond containing quenchers (10^{-2} and/or > 10^{-2}M) were recorded under similar conditions using a Perkin-Elmer MPF-4 spectrofluorimeter.

7.2.4.2 Intramolecular quenching : Fluorescence spectra of degassed (by flushing with a stream of dry argon for ~5 mins.) solutions of compounds 3 and 4 (made up to an optical density of 0.1 in water at the excitation wavelength of 370 nm) were recorded under similar conditions employing the spectrofluorimeter used above. The spectrum of 3 (containing -S-S- linkages) was compared to that of 4 (lacking the -S-S- linkage) to observe any intramolecular quenching of fluorescence in the former compound.

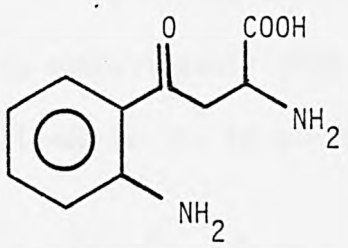
7.2.5. Wool bleaching

This has been described previously, see Chapter 6 p.210 for full details.

7.2.6. Application of Photine-HV to wool serge fabric and bleached wool

0.5% of weight fibre (o.w.f.) Photine-HV was applied to each type of fabric using the exhaustion method described previously, see Chapter 6 p.212 for details. Approximately 85% of Photine-HV was exhausted onto each type of fabric using this application method.

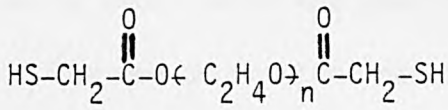
7.2.7. Application of kynurenine (Sigma Chemical Company Limited), 6, to wool serge fabric and bleached wool by a pad-dry method



6

A 3% (w/w) solution of kynurenine was made up in aqueous ammonia solution (specific gravity = 0.88). To this, Lissapol N (1% w/w; ICI) was added. The liquor was then padded onto wool serge fabric and bleached wool (achieving approximately 100% wet pick-up for each case) followed by drying of the treated fabrics in air overnight at room temperature.

7.2.8. Formation of more -S-S- bonds on Photine-HV treated wool serge fabric by treatment with polyethylene glycol dimercaptoacetate, 7



7

Molecular weight = 748

(Purchased from Evans Chemetrics, Darien, USA)

A 10% (w/w) solution of 7 was made up in water (used a net weight of 50g) to which was then added sodium lauryl sulphate as a wetting agent (0.05% o.w.f., mass of fibre used was 2.35g). To this

mixture was added solid sodium bicarbonate until the pH was 8. The liquor was then padded onto Photine-HV treated wool serge fabric (0.5% o.w.f. Photine-HV was applied to wool serge fabric, see above), achieving approximately 100% wet pick-up, and the padded fabric was allowed to dry in air overnight at room temperature.

7.2.9. Fluorescence spectra of fabric samples

Fluorescence spectra of fabric samples were obtained as described in Chapter 8 p. 257.

7.3. RESULTS AND DISCUSSION

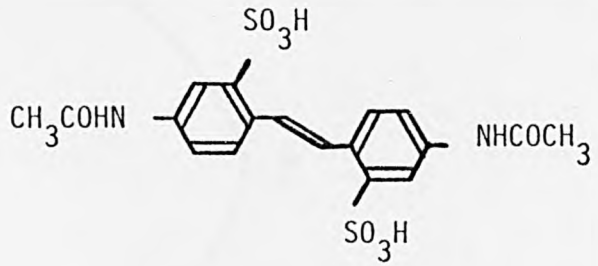
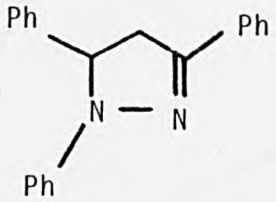
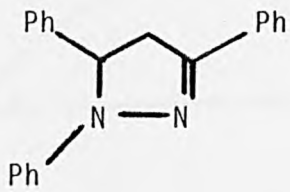
Table 7.1 shows the results obtained on studying fluorescence quenching of the intermolecular type by disulphide bond containing compounds in solution. This table shows that quenching was only observed in one of the four cases studied. This observed quenching was quite small under the conditions of the experiment (Figure 7.1). The reason for the lack of or small fluorescence quenching being observed can be explained using the Stern-Volmer equation [7]:

$$\frac{\Phi_o}{\Phi_q} = 1 + K_q . \tau . [Q]$$

where Φ_o = the quantum yield of fluorescence in the absence of quencher,

Φ_q = the quantum yield of fluorescence in the presence of quencher at a concentration [Q],

Table 7.1. Intermolecular Fluorescence Quenching by Disulphide Bond Containing Quenchers (Studies Performed at Room Temperature)

Disulphide bond containing quencher (conc.)	Fluorescer	Excitation wavelength (nm)	Solvent	Quenching (Yes/No)
$PhCH_2SSO_3^-Na^+$ (10^{-2} and > 10^{-2}M)		370	Water	No
$PhCH_2SSO_3^-Na^+$ (10^{-2} and 10^{-1}M)		370	Methanol	No
$PhCH_2SSCH_2Ph$ (10^{-2}M)		370	Methanol	No
$[HO_2CCH(NH_2)CH_2CH_2CONHCHCH_2S]_2.xH_2O$ (Glutathione, oxidised hydrate (Aldrich) F.W. = 612.63 (10^{-2}M))	Photine-HV	370	Water	Yes

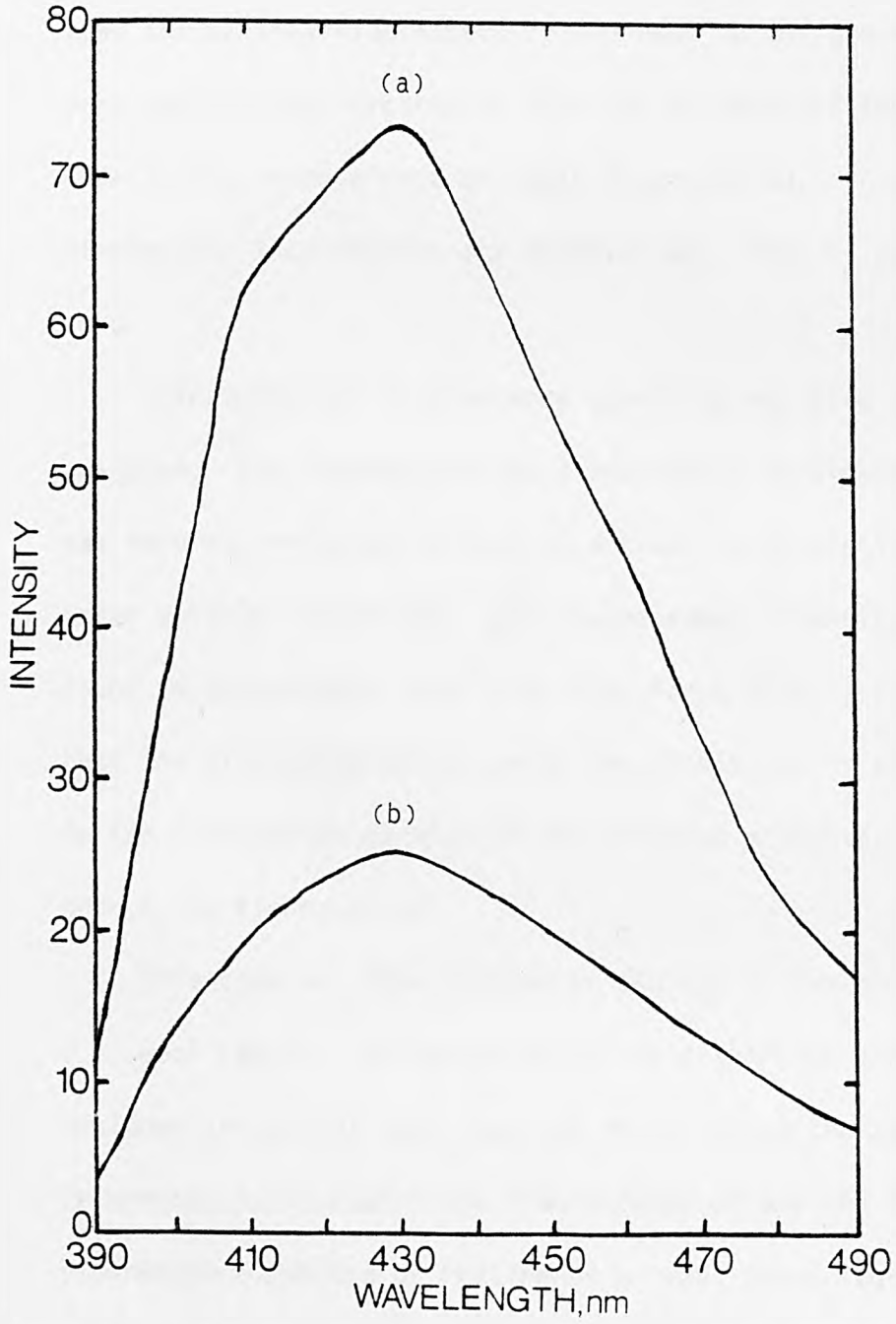


Figure 7.1

Fluorescence spectra (recorded under similar conditions) of:
(a) Photine-HV solution (optical density = 0.1 at 370nm in water)
and
(b) of the above solution (same concentration) in which 10^{-2}M oxidised glutathione was present.

K_q = rate constant for quenching

and τ = lifetime of the excited state in the absence of quencher.

τ for the systems considered must be very short indeed implicating that the excited fluorescers return back to the ground state very rapidly once excited so that the presence of the added quenchers have little or no effect on their fluorescence. In other words, bimolecular interactions are disfavoured. Thus K_q approximates to 0.

Intramolecular fluorescence quenching was also studied in solution. The fluorescence of 3 possessing disulphide bonds was recorded relative to that of 4 (lacking disulphide bonds) under similar conditions. The fluorescence intensity of 3 was found to be markedly lower than that for 4 (Fig. 7.2) suggesting that the disulphide bonds, which are within and in close proximity to the fluorescing portion of the stilbene molecule, 3, strongly quench the fluorescence.

Attention was then centred on studies in the solid state, i.e. wool fabric. To determine if the disulphide bonds of cystine residues in natural wool, most of which are on the surface, can intermolecularly quench the fluorescence of applied fluorescers, fluorescence spectra of Photine-HV on wool serge fabric and peroxide-bleached wool (in which most of the disulphide bonds of cystine residues have been cleaved to cysteic acid residues) were recorded under similar conditions (exhaustion method was used in applying

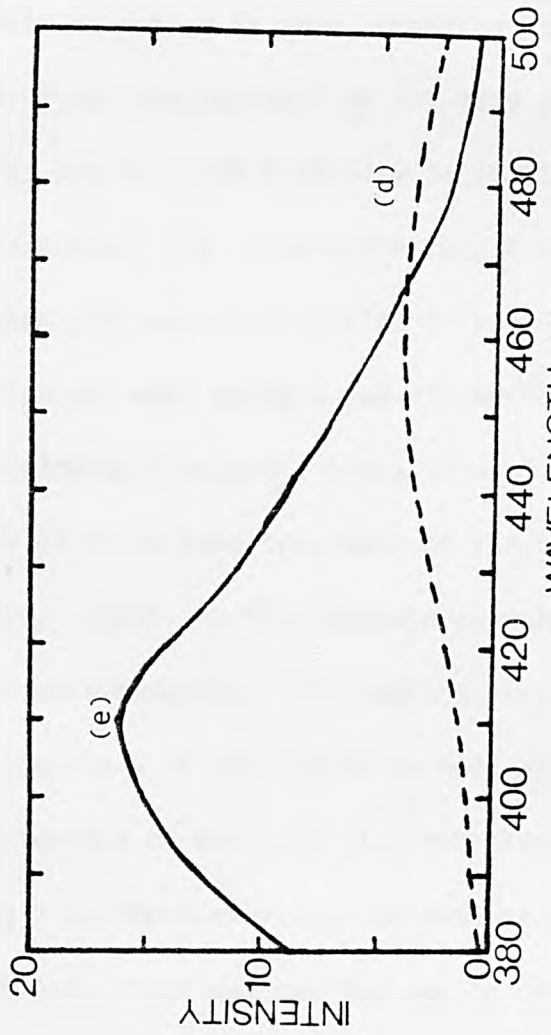
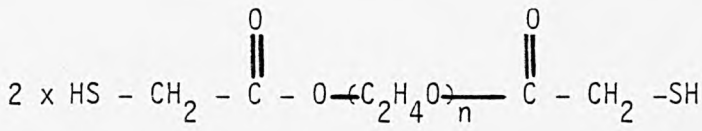


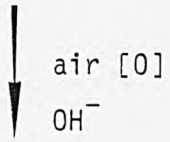
Figure 7.2

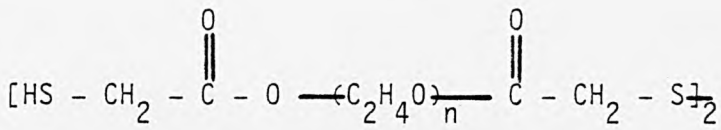
Fluorescence spectra (recorded under similar conditions) of compounds (d) 3 and (e) 4.

Photine-HV to each type of fabric - this method of application gives an even distribution of Photine-HV throughout each fabric). By comparison of the two spectra no fluorescence quenching could be found. Possible explanations for this result are that Photine-HV is a very strong fluorescer and if any disulphide bonds are quenching the fluorescence of Photine-HV on wool serge then it is impossible to notice it when comparing its spectrum with that of Photine-HV on bleached wool or not many of the applied Photine-HV molecules are in close proximity to cystine residues of wool. Another fluorescer, viz, kynurenine (6), a photo-oxidation product of tryptophan [8], was also applied to wool serge fabric and peroxide-bleached wool using a pad-dry method to determine quenching of fluorescence by disulphide bonds in wool. This method of application gives an even treatment of the compound on the surface of wool only. Again, no fluorescence quenching was found when the spectra were compared. The explanations given above for the quenching study of Photine-HV on wool apply equally well here. The surface of wool was also enriched in disulphide bonds by applying 7 to Photine-HV treated wool serge fabric (see experimental section). This was carried out to observe if the extra number of disulphide bonds deposited on wool surface can aid in quenching the fluorescence intermolecularly. 7 on wool undergoes oxidation to the disulphide:



7





The free thiol ends of this disulphide can also possibly oxidise further to form other disulphide bonds which ultimately may lead to a new polymeric compound containing many disulphide bonds. Fluorescent spectra were obtained of Photine-HV on wool serge fabric to which 7 was applied and of Photine-HV on bleached wool. Strong fluorescence quenching of the former fabric was observed implying that the extra disulphide bonds deposited on the surface of wool serge aid in quenching the fluorescence of Photine-HV by intermolecular means (Figure 7.3).

7.4. FUTURE WORK

The following experiments need to be performed for completion of this work:-

(i) To try intermolecular fluorescence quenching studies in solution with fluorescers having excited singlet states of longer lifetimes - the disulphide bond containing compounds will then probably quench the fluorescence very strongly

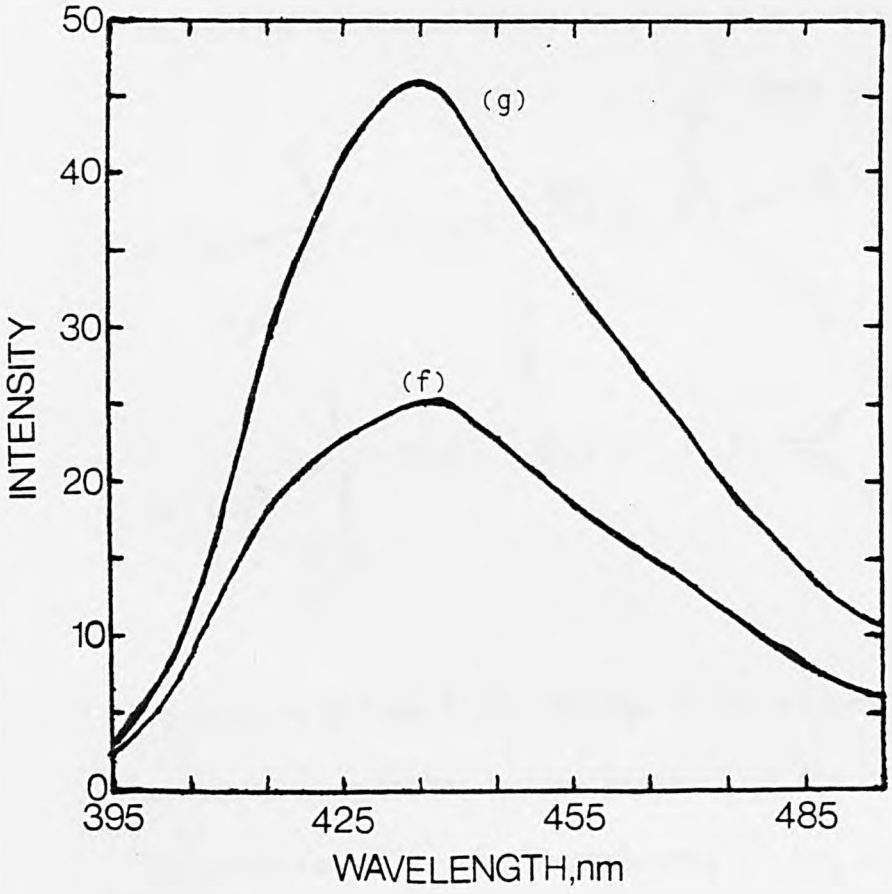


Figure 7.3

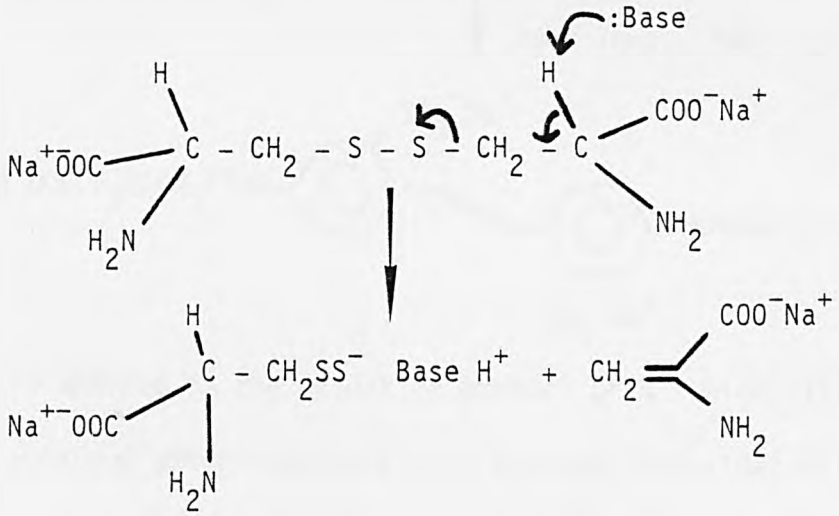
Fluorescence spectra (recorded under similar conditions) of:
(f) Photine-HV on wool serge fabric to which compound 7 was applied

and

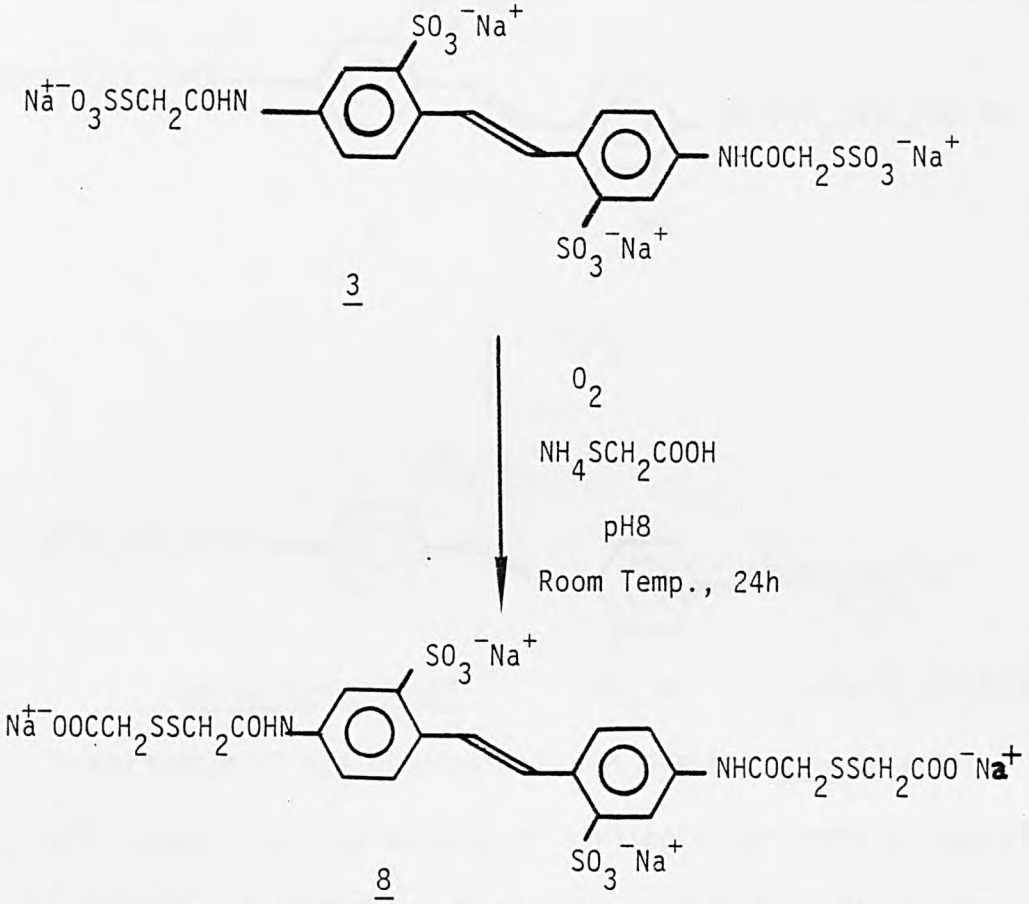
(g) of Photine-HV on bleached wool.

by intermolecular means.

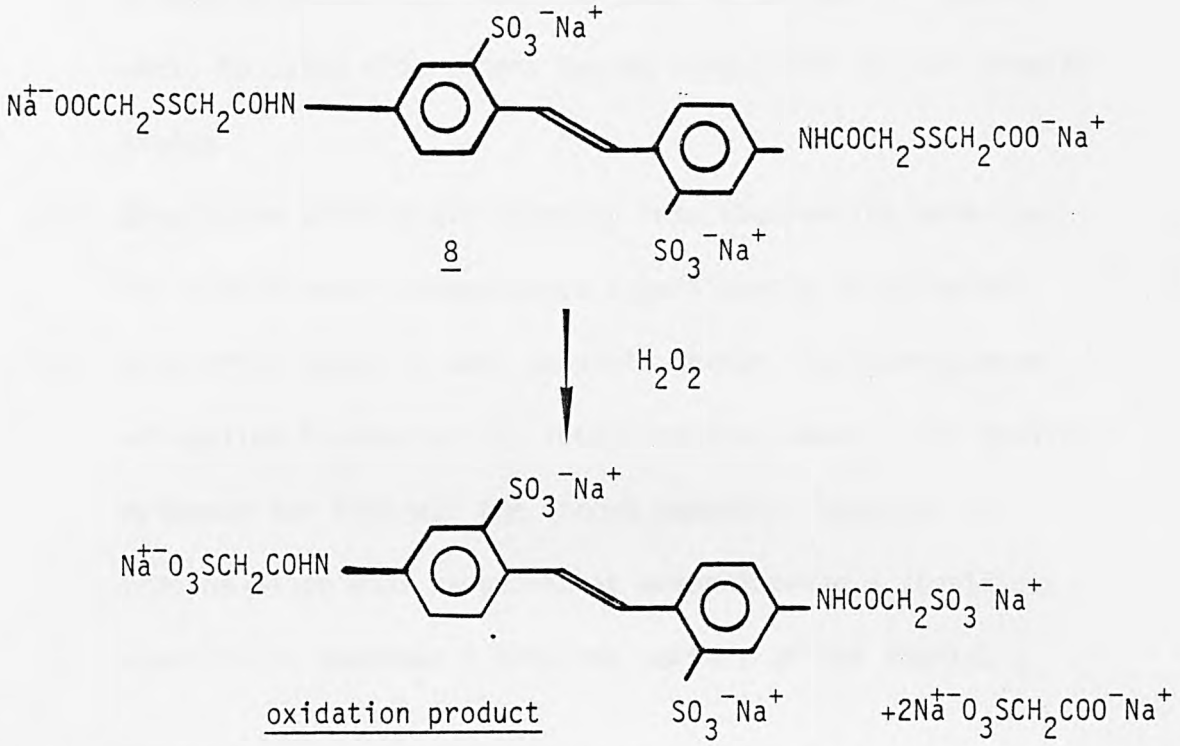
(ii) To use cystine as a disulphide quencher. This should be an ideal example as cystine residues in wool are responsible for quenching of at least 50% of wool's fluorescence. Studies should be carried out in water in which two molar equivalents of base, to a molar equivalent of cystine (to solubilise cystine) should be present. Excess base should be avoided as the elimination shown below will take place:



(iii) To prepare **8** from 3 and observe if the fluorescence of this compound is quenched by the presence of the disulphide bonds in comparison with the fluorescence of compound 4 (fluorescence of each compound to be recorded under similar conditions). This would be a further example on quenching by intramolecular means.



(iv) To observe if the oxidation product of 8 (which will be obtained after treatment with hydrogen peroxide) will have a higher fluorescence yield than 8 itself. This should be observed as the disulphide bonds of 8 will be cleaved according to the equation below. This is analogous to treating wool with hydrogen peroxide during which the disulphide bonds of cystine residues are cleaved and there is then an increment in the fluorescence of the fabric.



(v) To determine if the disulphide bonds present only in wool will quench the fluorescence of applied fluorescers by applying a lower level of Photine-HV (i.e. less than 0.5% of weight fibre) to wool serge fabric and peroxide bleached wool.

A lower level of Photine-HV is to be applied as it was stated in the results and discussion section that the strong fluorescence of Photine-HV on wool serge fabric (at a level of 0.5% of weight fibre) may be responsible for the non-quenching observed.

7.5. CONCLUSIONS

The following conclusions can be made from the work carried out so far on this particular topic:

(i) The small fluorescence quenching observed in solution between oxidised glutathione and Photine-HV suggest that disulphide bonds do quench fluorescence intermolecularly. For strong

effective quenching, attention must be turned, in future work, to using fluorescers having long-lived excited singlet states.

(ii) Disulphide bonds built directly into fluorescing molecules can quench their fluorescence significantly in solution.

(iii) Disulphide bonds in wool possibly quench the fluorescence of applied fluorescers by intermolecular means. The indirect evidence for this was the strong quenching observed of Photine-HV on wool serge fabric after loading a significant quantity of compound 7 onto the surface of the fabric.

7.6. REFERENCES

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CHAPTER 8

GENERAL EXPERIMENTAL AND INSTRUMENTATION

8.1. Application of Fluorescent Whitening Agents (FWAs) to Wool Substrate.

Two methods were generally used:

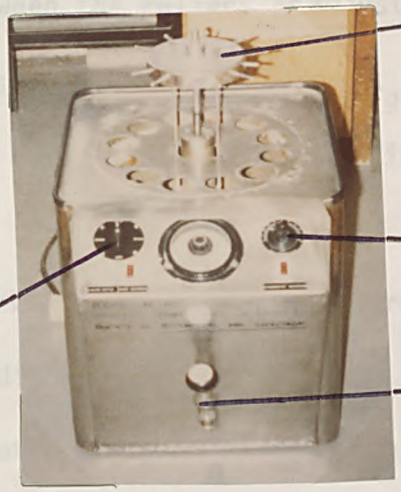
(i) Exhaustion at elevated temperatures onto wool serge and/or peroxide bleached wool at pH $\leqslant$ 7, and

(ii) Cold pad-batch methods onto wool serge and/or peroxide bleached wool.

The liquor formulations and the procedures used for the FWAs applied are outlined in the experimental sections from Chapter 3.

(i) Exhaustion Method

This was carried out in a Jeffreys Dyemaster (John Jeffreys Ltd., Rochdale, Lancashire). This consists of a cylindrical tank which serves as a water bath, and is thermostatically controlled. A top-plate is fitted, with a circular array of round holes to allow tubes of liquors to be placed vertically in the water bath. Wool samples are fixed to metal holders, each suspended from an overhead plate into the respective tubes. This plate is raised and lowered at a rate of 30 or 60 cycles per minute, thus agitating the fabric and the liquor very effectively. The rate of increase in temperature can also be pre-set, allowing a temperature gradient up to the final temperature to be controlled automatically. This method of treating wool is known as the Long Liquor treatment. The dyemaster described above is shown in Photograph 8.1.



Photograph 8.1.

(ii) Cold Pad-Batch Method

This is a three stage process. It involves drawing the wool into the pad liquor, and immediately afterwards passing it through a mangle to remove excess liquor. See Fig. 8.1(a).

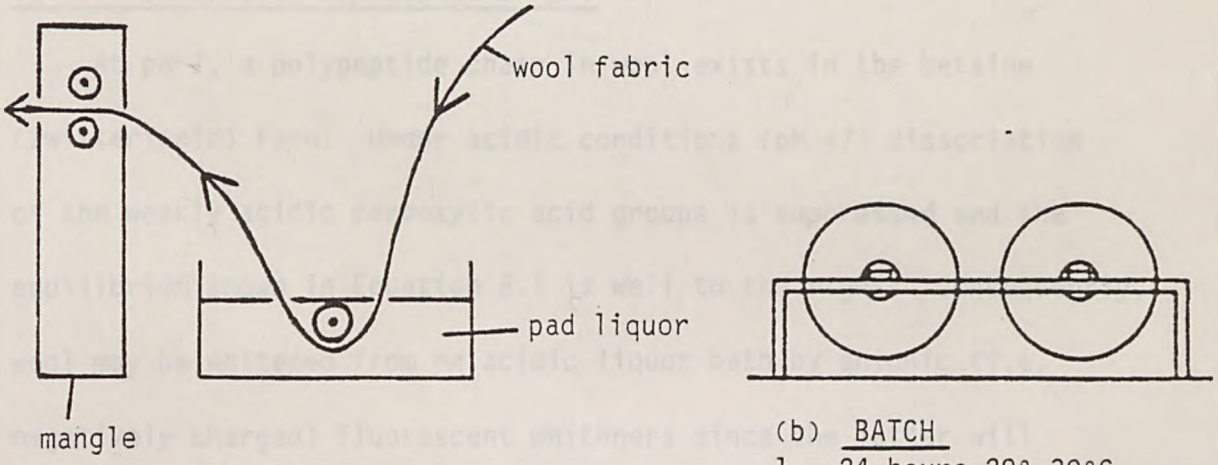


Figure 8.1.

The apparatus above [Fig. 8.1(a)] was designed and built at The City University. It is more normal to arrange the rollers horizontally, with end-plates fitted, and to place the pad liquor between the rollers.

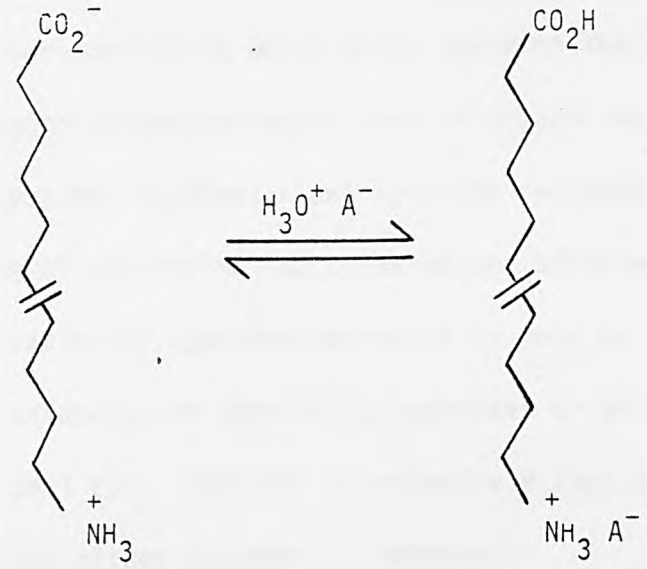
The pressure between the rollers is adjusted to allow 100% pick-up of the liquor; i.e. for x_g of wool, x_g of liquor is absorbed and is usually evenly distributed among the fibres. This is the "padding" process.

The wool is then batched by rolling it around a plastic tube approximately 2cm in diameter, and sealing it rolled up in a polythene bag to prevent evaporation. It is left suspended in this airtight state for the required time [see Fig. 8.1(b)]. When the fabric is removed, it is rinsed in cold water to remove excess liquor contents, and dried in air at room temperature. The combined process is known as the "pad-batch" treatment.

8.1.1. Exhaustion Dependence on pH.

At pH~7, a polypeptide chain in wool exists in the betaine (zwitterionic) form. Under acidic conditions (pH <7) dissociation of the weakly acidic carboxylic acid groups is suppressed and the equilibrium shown in Equation 8.1 is well to the right. Consequently, wool may be whitened from an acidic liquor bath by anionic (i.e. negatively charged) fluorescent whiteners since the latter will simply displace the colourless counter-ions within the fibre. In practice, weakly acidic conditions are employed since hydrolysis of the peptide linkages occur under strongly acidic conditions.

It is generally found that the amount of brightener fixed (= amount (%) of total fluorescent whitener applied which becomes bound to the fabric by physical forces or chemical bonds) is higher



Equation 8.1.

pH ~ 7 pH < 7

in acidic than under neutral conditions. Figure 8.2 illustrates this fact.

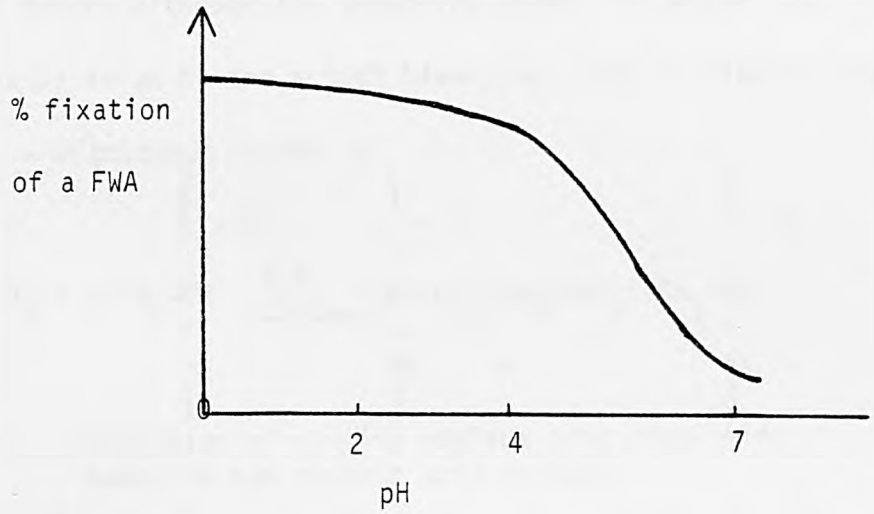


Figure 8.2. Exhaustion dependence on pH.

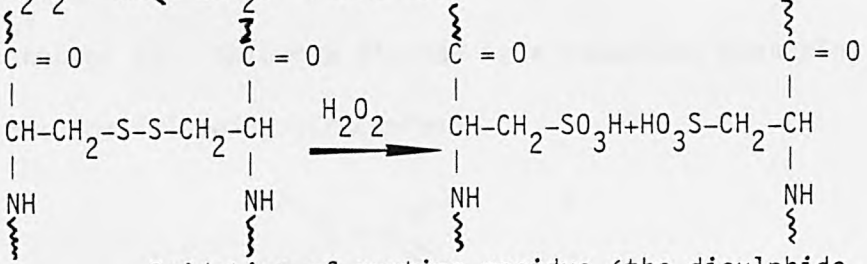
8.1.2. Peroxide Bleached Wool

This was obtained by treatment with hydrogen peroxide solution. Hydrogen peroxide (an oxidative bleaching agent) is a potent oxidant of many organic compounds. In proteins, it attacks principally

the sulphur-containing amino acids cysteine and methionine. In the presence of certain metal ions or organic acids, it may also attack cystine, tryptophan and tyrosine residues. The rates of oxidation of methionine and cysteine are affected differently by pH. Oxidation of cysteine decreases in rate as the pH is reduced, whereas oxidation of methionine increases slightly in going from pH 5 to pH 1 [1]. Cystine is relatively less susceptible to oxidation than either cysteine or methionine.

Improvements in the properties of wool obtained by bleaching with hydrogen peroxide in acid media have been reported [2]. In alkaline media, a higher degree of oxidation of the disulphide bond occurs although the bleaching effect is better [3]. [The peroxide anion is the actual bleaching agent in alkaline media,

$H_2O_2 + OH^- \rightleftharpoons H_2O + HOO^-$.]



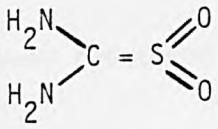
Oxidation of cystine residue (the disulphide bond) to two cysteic acid residues.

The reaction of wool keratin with hydrogen peroxide is relatively slow, except under alkaline conditions. When wool is immersed in hydrogen peroxide solutions (pH 2.5 - 9), some of the reagent is initially on the amino and imino groups [4]. The absorbed peroxide seems to be remarkably stable and is removed by washing. Oxidation of all the cystine can occur [5 - 7]. Simultaneous attack

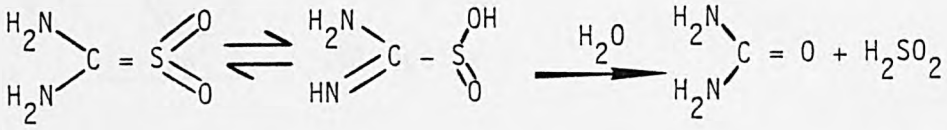
on the peptide bond may also occur, as Elöd et al.[8] showed that both silk (which contains no cystine) and wool were completely dissolved by 3% hydrogen peroxide solution in three days at 60°C; no high-molecular weight peptides were isolated. Hydrogen peroxide treated wool shows a decrease in acid combining capacity [7], but not in amino-nitrogen content [9]. This may be accounted for by the formation of cystine oxides, which are relatively strongly acidic [6]. Cysteic acid is also found in the acid hydrolysate [10]. The effects of peroxide oxidation on nineteen of the amino acid residues in wool have been described [11].

8.1.3. Thiourea dioxide Reductive Bleaching Agent

Some of the liquor formulations used had the presence of thiourea dioxide within them (see experimental sections from Chapter 3). Thiourea dioxide is a reductive bleaching agent and has the following structure:



This compound undergoes decomposition in aqueous medium:



It has been observed that the rate of decomposition of thiourea dioxide is greatly influenced by both temperature and pH (especially above pH 5.5). The decomposition reaction follows second order

kinetics, i.e.

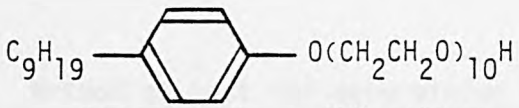
$$\frac{1}{C} - \frac{1}{C_o} = Kt$$

Thus a plot of 1/C v. time (t) gives a straight line. Activation energy (Ea) has been determined to be as 28.79 Kcal by plotting ln K v. 1/T (T = temp., values between 50 - 80°C employed). Sulphinic acid, H_2SO_2, is the active reducing species and is responsible for the bleaching effect itself decomposing to bisulphite according to the following scheme:

$$Na^+ {}^-O - S - OH \xrightarrow{H_2O} Na^+ HSO_3^- + H_2$$

8.1.4. Lissapol N

Most of the liquor formulations used had the presence of Lissapol N within them (see experimental sections from Chapter 3). Lissapol N is a wetting agent and has the structure shown below. It is necessary, because of the hydrophobic nature of wool, to enable aqueous solutions to penetrate the fibre. It also serves to achieve a level treatment across the surface of the wool sample.



Lissapol N - 28% active

8.2. Fluorescence Spectra of Fabrics

Fluorescence spectra of fabric samples were obtained using a Perkin-Elmer (MPF-4) fluorescence spectrophotometer. The fitment

for solids was designed and made at The City University and is similar to that described by McKellar and Allen [12]. The attachment is outlined in Fig. 8.3. The samples were placed at 50° to the incident beam to avoid scattering.

8.3. Diffuse Reflectance Spectra of Fabrics

Diffuse reflectance spectra of fabric samples were obtained using a Perkin-Elmer Lamda 5 UV/visible spectrophotometer. The Lamda 5 is a double-beam ratio recording instrument with a filter-grating monochromator in a Littrow configuration. It has a holographic grating of 1440 lines/mm and the light sources are deuterium and tungsten-halogen. Fig. 8.4 gives a diagram of the optical system for the general absorption mode. For measurements of turbid and solid samples an integrating sphere attachment is used, with barium sulphate as a reference. Solid samples are clamped into a special holder. The observed spectrum of a sample is the sum of the reflected light and the fluorescence. Therefore absorption appears weaker as fluorescence increases.

8.4. Method of test for determining light fastness of whitened fabrics

8.4.1. Purpose and Scope

This method is intended for determining the resistance of white colour (or any other one) of wool to the action of an artificial

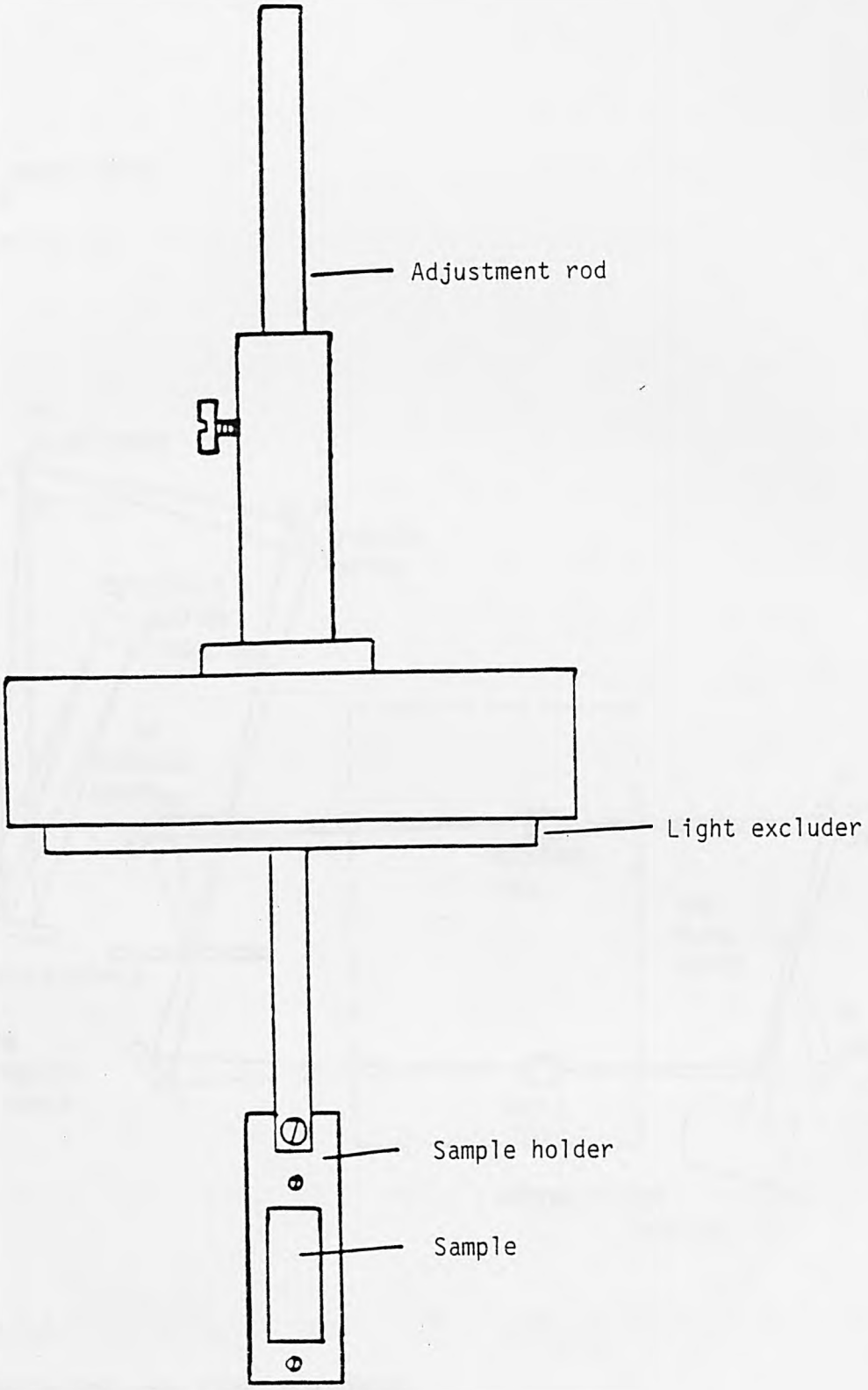


Figure 8.3.

Fluorimeter attachment for solid samples

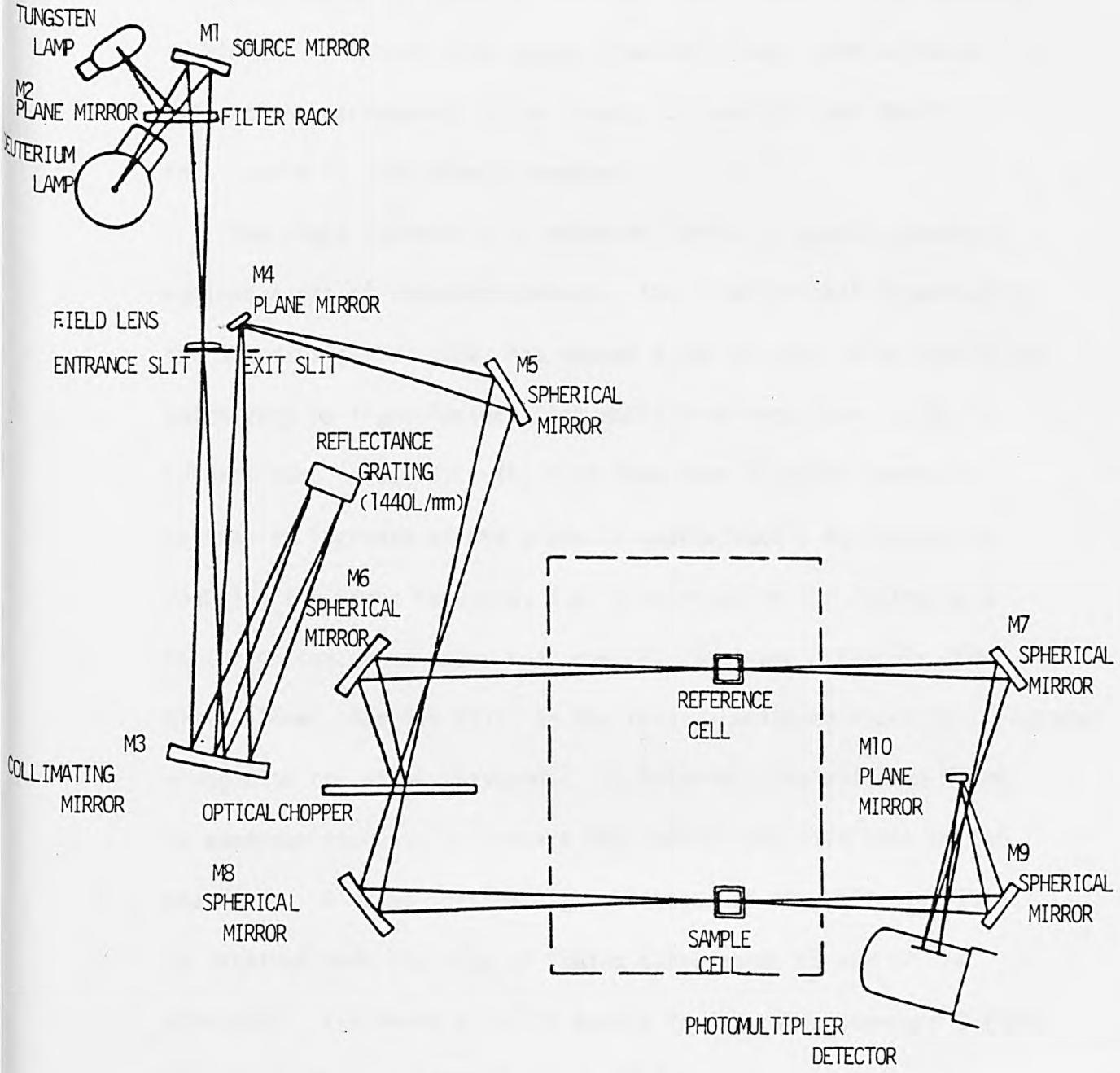


Figure 8.4.

THE PERKIN-ELMER LAMDA 5 SPECTROPHOTOMETER

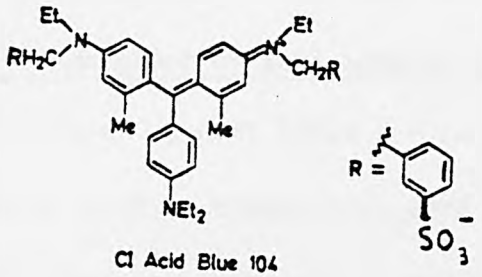
light source whose spectrum corresponds closely to that of daylight.

8.4.2. Test Method

This method is based on ISO 105 - B02 : 1978, Colour Fastness to Light : Xenon arc (the output from this lamp, with suitable filtration, corresponds quite closely to sunlight and therefore this source is used widely nowadays).

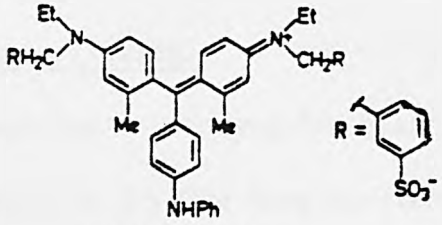
The light fastness of a whitened fabric is usually assessed against a set of standard dyeings. The International Organisation for Standardisation (ISO) has chosen a set of eight blue dyes (1-8) which vary in light fastness (on wool) from very poor (grade 1) to excellent (grade 8). The dyes have been selected carefully so that an increase of one grade is approximately equivalent to doubling the light fastness, i.e. a decrease in the fading by a factor of two; this means that dye (8) fades approximately 250 times slower than dye (1)! In the test, a whitened fibre is irradiated along with the eight standards. At intervals the whitened fibre is assessed visually to compare its fading rate with that of the standards. A value for the light fastness of the whitened fibre is obtained when its rate of fading corresponds to one of the standards. For example, if it begins to fade when standard 3 fades then it exhibits a light fastness of 3.

The standards which are dyed as follows can be obtained from the British Standards Institution:



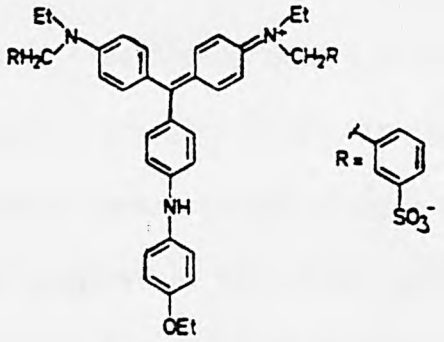
CI Acid Blue 104

(1)

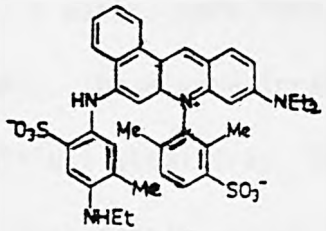


CI Acid Blue 109

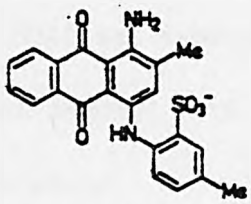
(2)



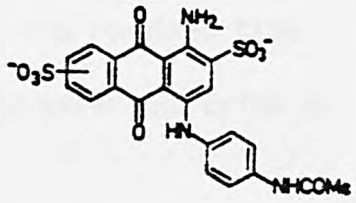
CI Acid Blue 83 (3)



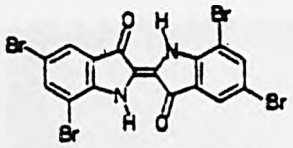
CI Acid Blue 129 (4)



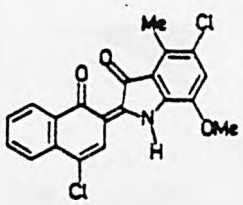
CI Acid Blue 47 (5)



CI Acid Blue 23 (6)



CI Vat Blue 5 (7)



CI Vat Blue 8 (8)

The fully detailed procedures for determining light fastness is described in IWS Test Method No.5 [13].

8.5. Irradiation of Wool Samples (:photoyellowing)

The wool samples (20cm x 5 cm, supported on rectangular glass plates of similar dimensions) were exposed to 3 x 20W long wavelength u.v. fluorescent tubes with a maximum output at approximately 350nm. These tubes were mounted on a metal piece which was within a wooden 'cupboard' (see Photographs 8.2 and 8.3). The samples (after wetting with aqueous Lissapol N and rinsing in cold water) were immersed under water (which formed a depth of 20mm). These were irradiated at room temperature in a flat, open, stainless steel tray (55cm x 21.5cm x 4.5cm) (see Photograph 8.3). Each sample was divided into two halves. One half was covered with aluminium foil whilst the other half was exposed to the light for the required time. The exposed samples were then rinsed in cold water and dried at room temperature.

Photograph 8.2

Photograph 8.3

(the lamps were directly above the tray during the irradiation period).

8.6. Yellowness Index Measurements

This is described in the experimental sections where such measurements were carried out.

8.7. I.R. spectra

These were recorded as Nujol mulls using sodium chloride cells or as potassium bromide discs using a Perkin-Elmer 157G grating spectrophotometer.

8.8. UV/visible absorption spectra

These spectra were recorded using a Perkin-Elmer 402 UV/visible spectrophotometer. If a solution was being made up to a known optical density a Cecil Instruments CE 272 Linear readout ultra-violet spectrophotometer was used.

8.9. ^{1}H NMR spectra

These were measured on a Jeol JNM-MH 100 NMR spectrometer.

8.10. Mass spectra

Measurements were carried out by Mr C. Whitehead, using a Kratos MS 30 Electron Impact mass spectrometer, linked to a Kratos DS 50 data system. The samples were introduced via a heatable probe and ran at 70eV.

8.11 Elemental analysis

CHN analyses were performed by Mr P. Hemming using a Carlo Erba Model 1106 Elemental Analyser.

8.12. Melting points

Melting points were recorded using a Kopfler block or a Gallenkamp melting point apparatus and are uncorrected.

8.13. REFERENCES

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12. J.F. McKellar and N.S. Allen, (1979), Photochemistry of man-made fibres, Applied Science Publishers, London.

13. IWS Test Method No.5, (July 1980), Method of test for determining colour fastness to light. This test method can be obtained from:
International Wool Secretariat,
Technical Centre,
Valley Drive,
Ilkley,
West Yorkshire,
LS29 8PB,
England.