"THE PHOTOYELLOWING OF WOOL"

by

Stuart Kenneth Russell Jones

Ph.D. Thesis

The City University
Dept. of Chemistry

August 1986

THE PHOTOYELLOWING OF WOOL

ABSTRACT

The principal aims of the work were to further the current understanding of the processes, physical and chemical, occurring in wool during irradiation with UV and visible light, and to develop a method of stabilizing the wool fibre to photodegradation.

Investigations of the changes occurring during the photoyellowing of wool were commenced by coating wool with a thin layer of the free-base form of amino acids existing in natural wool, and photoyellowing the samples under 350nm light. This highlighted tryptophan as the prime source of yellowing and rejected the possibility of a synergistic effect existing between two amino acids. It was shown that although all the amino acids in wool absorb light only below 300nm, the fibre was still yellowed by radiation of wavelengths greater than 330nm. Fluorescence work showed the existence of an unidentified species which absorbed at 360nm and fluoresced in the visible region. A comparison was made of the effects of a range of oxidizing agents on wool. Peroxide whitened the fabric and persulphate caused extensive yellowing. Permonosulphate and permanganate were also studied.

The search for a means of inhibiting photoyellowing was successful. Wool was irradiated in the presence of a reducing agent, with a view to reducing the photo oxidation products as soon as they were formed. A range of reagents were tested and it was found that sodium borohydride and zinc formaldehyde sulphoxylate not only prevented yellowing but caused a whitening of the fabric (not seen in the absence of irradiation) and imparted a measure of stability to subsequent irradiation. For several reasons, zinc formaldehyde sulphoxylate was chosen and extensive studies carried out to optimise the conditions. Significantly, this treatment is effective for bleached and optically brightened wool.

Other methods of retardation of wool photoyellowing which were investigated were the application of UV absorbers and radical inhibitors, such as hindered piperidines, diazo-bicyclo-octane and titanium dioxide, chemical modification of the fibre with cross-linking agents and the conversion of cystyl residues to their lanthionyl equivalents. Attempts were made to "exhaust" the yellowing capacity of the fibre by repeated alternate yellowing and bleaching. Diffuse

reflectance spectroscopy was used to study the effects of wool yellowing and fluorescent whitener de composition in situ on the fibre. This showed that in previous studies where residual FWA had been extracted after photo-yellowing, the results had not given a correct evaluation of the relative stabilities of the FWA's on the wool. Finally, the possibility of developing and applying a stable FWA to wool was studied. Dispersed whitening agents were tested, but with little success.

ACKNOWLEDGEMENTS

I express my sincere gratitude and appreciation to the following for their valuable support:-

- To my supervisors, Prof.R.S.Davidson & Dr.D.M.Lewis, for guidance and encouragement throughout the project;

- To [redacted] at the City University for help with spectrophotometric studies;

- To the I.W.S. technical staff for assistance during my visits to Ilkley;

- To [redacted] in the I.W.S. library, for endless assistance with the literature searches;

- To the Wool Foundation for funding the entire project;

- and to [redacted] for typing this thesis.

CHAPTER 1

Introduction to the Photoyellowing of Wool

CHAPTER 1

1.1 General Introduction to Wool

At the outset of this study on the Photoyellowing of Wool, it is instructive to note some of the characteristic features of the wool fibre. Wool is a member of the group of proteins called keratin. Other members include materials such as hair, nails, feathers, hooves and claws. The prime natural function of keratins is protection of animals from their environment. Keratins are composed mainly of protein, and are characterised by a high cystine content and the fact that they yield an α-type X-ray diffraction pattern which changes to a β-pattern upon stretching.

Wool fibres exhibit a great variation in length and diameter, dependent upon the breed and diet of the sheep, and the climate. For the purpose of this work, Merino wool was used, with an average fibre diameter of 20μm. This is one of the finest grades of wool, grown in Australia and New Zealand. In contrast are the British Corriedale and Lincoln wools, with fibre diameters up to 40μm.

A wool fibre consists of two major parts: the cortex, which is the bulk of the fibre, and the cuticle on the surface. Cuticle cells are flat and overlap each other with the exposed scale edges pointing towards the fibre tip, accounting for approximately 10% of the fibre. In contrast, cortical cells are rod-shaped and consist of bundles of microfibrils or filaments, which in turn comprise a helical arrangement of protein molecules. It is important to note that differences exist in the amino acid composition of these two types of cell. This is significant because wool photoyellowing is primarily a surface phenomenon. This is considered in more detail later.

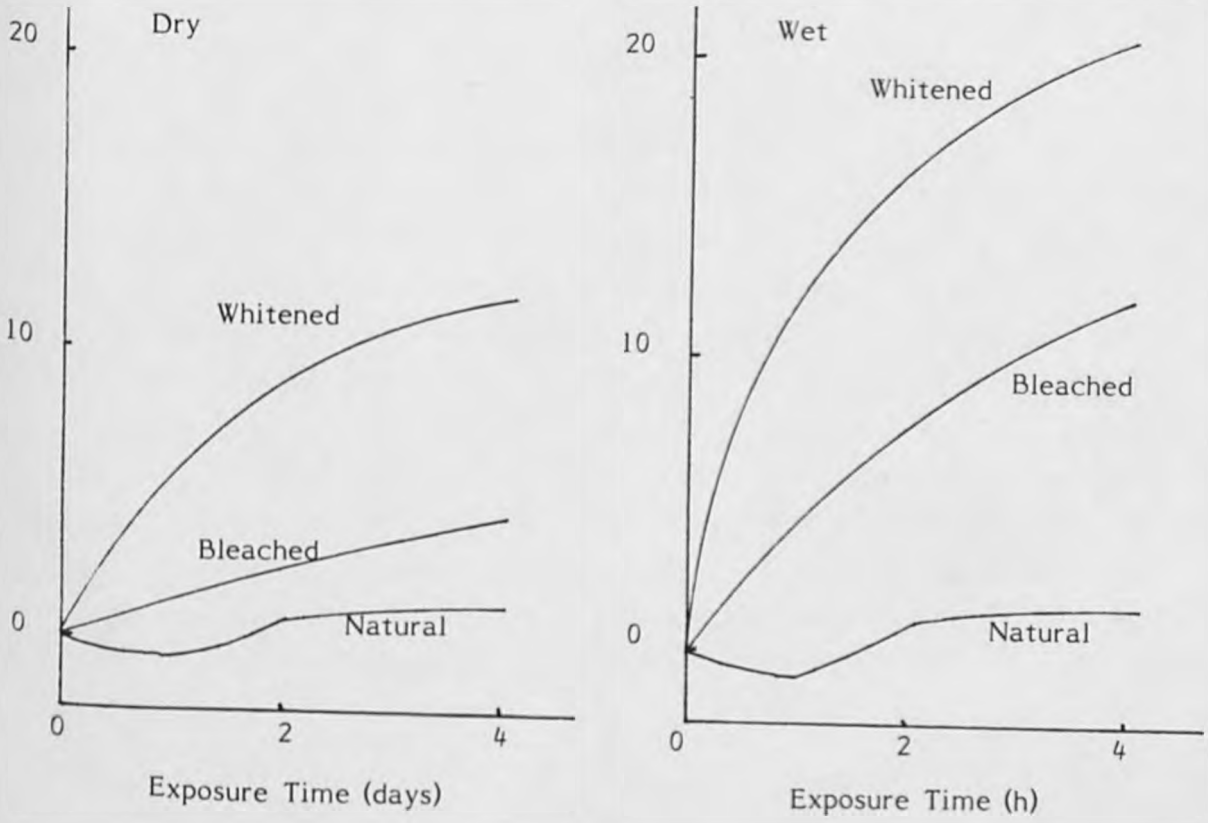
Wool consists of regularly coiled peptide chains, with inter-chain disulphide bonds and ionic interactions [1], which contribute to the elastic properties of the fibre. The pendant groups are on the outside of the helix, resulting in the natural hydrophobicity of the fibre.

1.2 The Photoyellowing Problem

For many years it has been known that wool discolours under irradiation by sunlight [2,3]. At the same time, wool also loses some of its tensile strength, and it has been suggested that these two processes may be related in some way. Photoyellowing occurs after relatively short irradiation periods, whereas phototendering only becomes apparent after more prolonged exposure to sunlight, and hence has been almost neglected by wool research laboratories until very recently. In the present work, only photoyellowing has been studied.

Two important parameters which affect yellowing are the presence of oxygen and water. A considerable quantity of oxygen has been shown to be consumed during ultra violet irradiation [4]. Irradiation of dry wool in the absence of oxygen produces a green discolouration, believed to be due to the formation of cystyl free radicals, which can be detected by esr [5-7]. On exposure to oxygen, these radicals decay and the green colour of the wool turns yellow [8]. The presence of water vapour also promotes yellowing: wet wool yellows more readily than dry wool [1,9,10], (see Fig.1.1).

Fig. 1.1 Rates of Photoyellowing of Natural, Peroxide-bleached, and Fluorescently Whitened Wool Fabrics in Simulated Sunlight

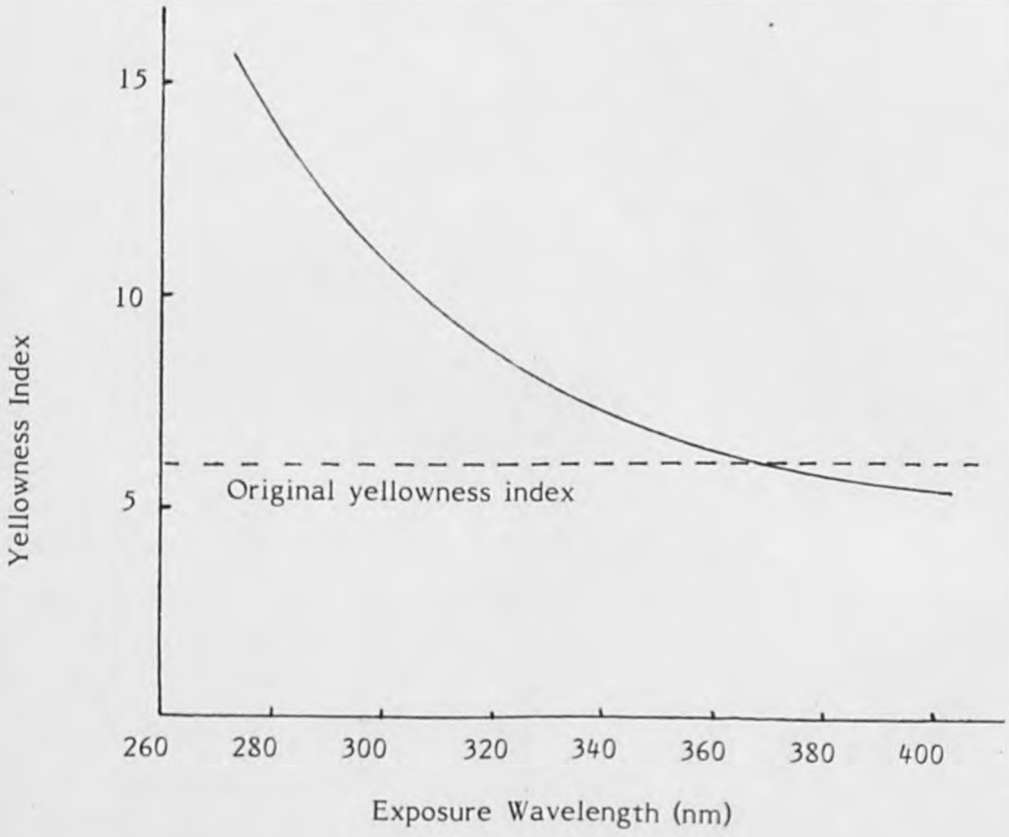


In its natural state, wool is not a white fibre but varies in colour from cream to canary yellow [11], colour differences depending upon breed and climatic conditions. The market for natural-coloured wool is extremely limited, and to match the whiteness of other textile fibres, bleaching is necessary [12-14], either oxidatively with hydrogen peroxide or reductively with hydrosulphite. Unfortunately, wool that has been chemically bleached, especially by peroxide, photoyellows much more rapidly than does natural wool [9]. This problem is further accentuated when a fluorescent whitening agent, (FWA), is applied [3]. This is necessary to achieve the whiteness obtainable with synthetic fibres. FWA's function by absorbing near UV, (at around 350nm), and fluorescing in the blue region of the visible spectrum. They thus brighten the wool by the addition of the fluorescence emission to the reflected visible light, and whiten the wool by the production of blue light, (blue being the complimentary colour of yellow). However, when wool thus treated is irradiated with sunlight, it yellows many times faster even than bleached wool, as shown in Fig.1.1. There are many types of FWA's in commercial use, 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. The effect of acceleration of photoyellowing appears to be independent of the nature or stability of the FWA used.

Probably the most important factor affecting the nature and extent of discolouration of wool is the wavelength distribution of the incident light. Two competing processes occur when untreated wool is exposed to sunlight, namely photoyellowing and photobleaching. Yellowing is caused by the ultra violet component of sunlight, ie. 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 [8]. In contrast, irradiation with wavelengths above 380nm into the visible region, causes bleaching to occur, the effect being maximal between 420-450nm [17,18]. 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 affect 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.

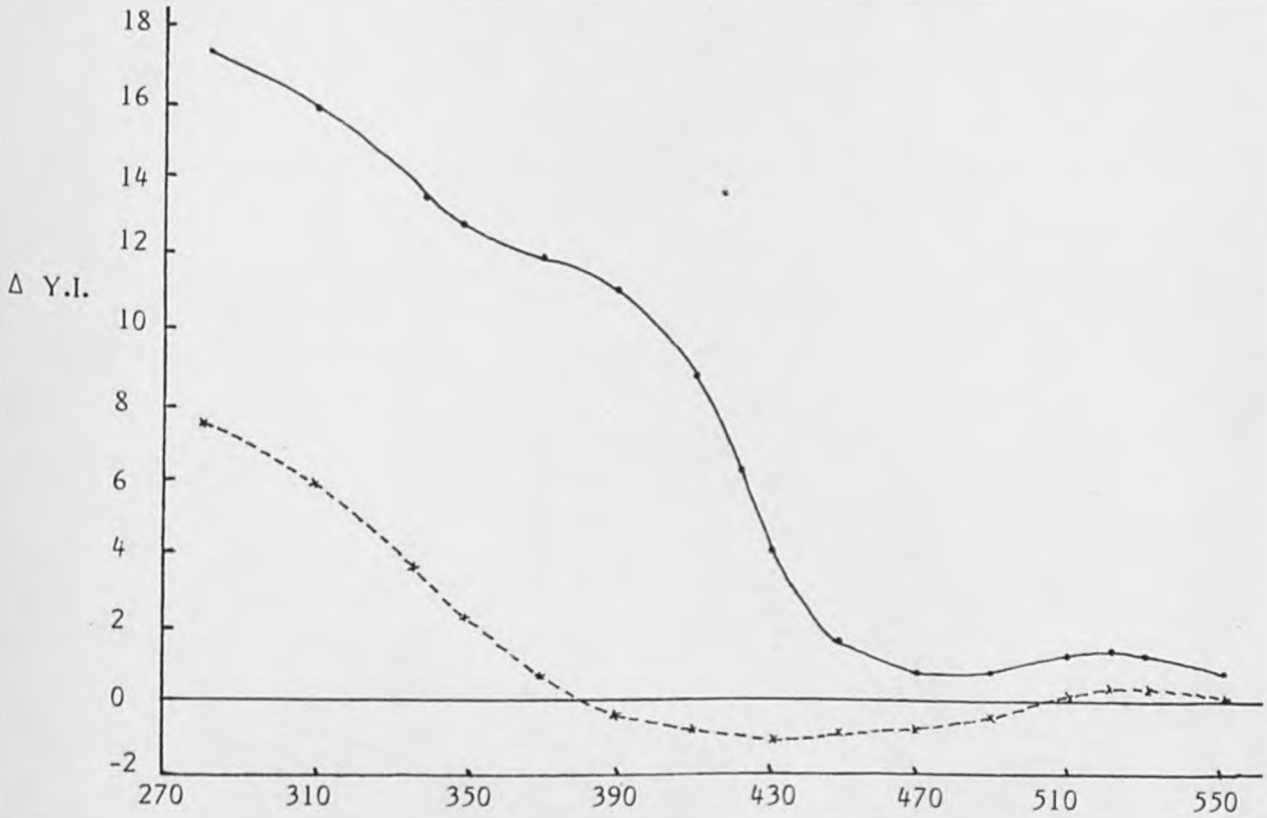
Fig. 1.2 Photoyellowing VS. Wavelength



Fluorescent whitening agents have a significant effect upon the degree of yellowing. It has been observed that in contrast to unbrightened fabrics which yellow at a maximum rate at 300nm, maximum yellowing of FWA-treated wool occurs in the region of 350-410nm, where brightener excitation occurs **[19]**.

The above effects are summarised in Figures 1.1 and 1.2

Fig. 1.3 Action Spectra for Yellowing of F.B.A. Treated [—] & Untreated Wool [- - -]



Many different light sources are used in the study of wool yellowing, and it should be noted that, due to their different spectral output, their effects on wool may not be the same [20,21].

1.3 Determination of Whiteness of Wool

At this stage it is necessary to define the methods used for quantifying the degree of yellowness or whiteness of a fabric. Colour is a sensation, and, as such, is not measureable. However, colour systems comprising three parameters have been developed whereby colours can be clearly determined and compared one with another. White is basically a colour like blue, green or red, and can be determined precisely by colorimetric methods. However, white samples are often more difficult to assess than coloured ones because white, besides being objectively quantifiable, is also a subjective connotation of quality which is greatly influenced by personal taste. Thus each whiteness formula has a specific "whiteness bias", ie. preferences, directly comparable with the varying whiteness preferences of different human observers.

In common with all other colours, white can be clearly defined by just three parameters of a colour system; in other words, in a "three-dimensional colour space". For practical purposes, the measured R_{650} (red), R_{450} (blue), and R_{550} (green) percentage reflectance values or the calculated X,Y and Z C.I.E. tristimulus values are used [23].

In the work presented in this thesis, the Jacquemart Whiteness Index and the Yellowness Index are used, as defined below:-

Yellowness Index:-

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

Y.I. increases with increasing yellowness.

Jacquemart:-

$$W = [\ (100-Y)^2 + K(X-Z)^2\ ]^{\frac{1}{2}};\quad K = 5.5$$

W decreases as wool becomes whiter.

The tristimulus values are according to C.I.E., (1976) system [24,25]. Under predetermined conditions of examination, the quoted formulae classify wool according to its degree of whiteness in the same order as a panel of experts [24].

1.4 Photoyellowing Theories

A number of theories have been advanced to explain the photoyellowing of wool. Much research has been carried out to substantiate the proposals made, but it still remains that no one theory is capable of explaining all the data. Three theories are to be discussed here.

1.4.1 Absorption by α-keto acids

Meybeck & Meybeck [26] proposed that the yellow colour produced on irradiation of wool is due to the visible light absorption by pyruvyl and glyoxyl residues, along with other α-keto acids formed by the photochemical

decomposition of the polypeptide chain. This theory has been refuted by Holt & Milligan [27] because in the case of wet wool, very few carbonyl groups were formed, even though extensive yellowing had occurred; and with dry wool, where an increase in carbonyl groups was observed, their rate of production was approximately the same for bleached, unbleached and fluorescently whitened wool, yet these fabrics yellow at markedly different rates. Thus it seems unlikely that light absorption by α-keto acyl groups in wool contributes directly to yellowing.

1.4.2. The Presence of Mobile Electrons

In 1974, Hoare [28] postulated that yellowing was caused by the presence of mobile electrons which could be photoexcited into conduction bands. Additional yellowing by irradiation, heat and alkali was caused by an increase in the number of unsaturated groups in the fibres, leading to an increase in the general level of mobile electrons capable of photoexcitation.

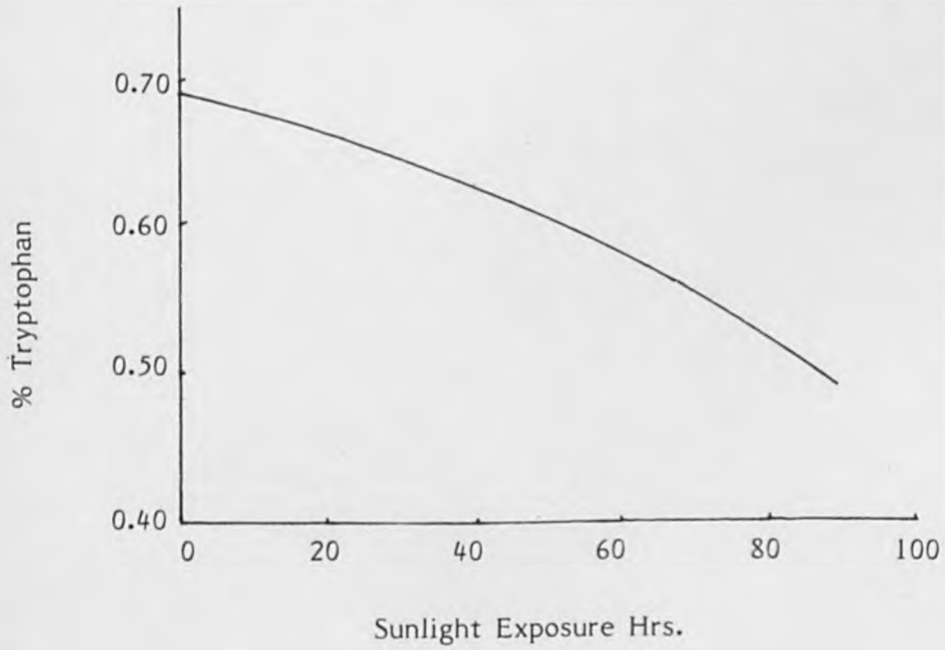
Doubt was again cast upon this theory by Holt and Milligan [29] when they showed that yellow compounds can be isolated from wool by enzymic digestion. Also, Nicholls and Pailthorpe [4] could find no evidence from esr studies for electrons in conduction bands in irradiated wool. Hence, this theory seems unlikely.

1.4.3. Photooxidation of Tryptophan Residues

By far the most widely accepted theory of wool yellowing proposes the photodecomposition of tryptophan residues to form yellow products. Substantial evidence is now available in support of this proposal.

(i) Tryptophan absorbs approximately a quarter of the radiation in the 290-310nm waveband. It is the amino acid most susceptible to sunlight yellowing, both in aqueous solutions, and in polymer films [31]. The rate of decomposition during sunlight irradiation is shown in Fig.1.4.

Fig. 1.4 Change in Tryptophan Content After Irradiation



(ii) The rates of photoyellowing of a series of 29 different keratin samples were proportional to their initial tryptophan contents, and to the destruction of tryptophan [32]. Correlation of yellowing with the destruction of other amino acids were less significant.

(iii) The incorporation of additional tryptophan residues into wool increases the rate of photoyellowing [33].

(iv) Yellow products were isolated from enzymic digests of photodegraded wool [29]. Only one of these could be identified; this was kynurenine, a known degradation product of tryptophan.

(v) When tryptophan residues were radiolabelled, radio active yellow products could be isolated [29].

(iv) Irradiation of tryptophan and trytophan-containing peptides are known to give complex yellow and brown photoproducts [34].

The main drawback with this theory is the observation that when wool is treated with hydrogen chloride and dimethyl sulphoxide to deplete the tryptophan content, the rate of photoyellowing is only a little slower than the untreated wool, suggesting that photooxidation of residues other than tryptophan must be responsible [35].

Oxindolyalanine

Nevertheless, in natural wool it seems very probable that the yellowing process observed is directly related to the photooxidation of tryptophan residues.

1.4.4. Photoyellowing of Fluorescently Whitened Wool

Wool treated with a fluorescent whitening agent is considerably whiter than untreated wool and yellows much more rapidly [3]. Practically all white wool marketed today is treated with a FWA, and it is therefore very important to understand why this acceleration in photoyellowing occurs under these conditions. Five possibilities are described in the literature:-

(i) Photodecomposition of the FWA leads to loss of whitening power.

(ii) FWA photoproducts quench the fluorescence of residual whitener, leading to further loss of whitening power.

(iii) The FWA decomposes to yellow photoproducts.

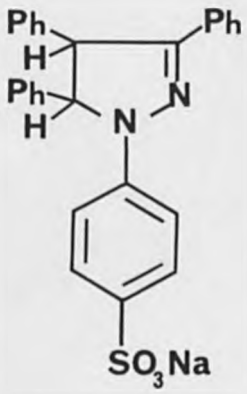
(iv) The FWA, or its degradation products, react photochemically with the wool to yield yellow products.

(v) The FWA promotes the yellowing processes that occur in untreated wool, and possibly initiates further yellowing reactions in the protein.

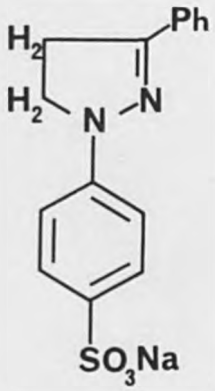
Extensive studies on the photodegradation of FWA's have been carried out at C.S.I.R.O. in Australia [35-38]. These have established that the FWA's are slowly photooxidised on the surface of the wool under sunlight irradiation. In some cases, the photoproducts do quench the fluorescence of the residual brightener. This does not, however, explain the rapid yellowing seen in fluorescently whitened wool.

The third possible cause of accelerated yellowing was the formation of yellow FWA photoproducts. Many types of FWA have been photooxidised and their products isolated. It has been found that on irradiation a stilbene FWA yields virtually no yellow material, even after extensive decomposition has occurred [42]. However, when irradiated in the wet state, wool whitened with the stilbene becomes very yellow, even though much of the whitener

remained unchanged. When brightened wool is irradiated, no yellow FWA decomposition products were formed [39]. The presence of oxygen has been found to have little effect on the rate of FWA photooxidation, whereas it has a marked effect on wool yellowing [38]. Furthermore, when a range of FWA's of varying photostabilities are compared, it is observed that the rate of photoyellowing of the treated wool samples is independent of the particular FWA used [37]. For example, tetraphenyl pyrazoline (I) is approximately five times more photostable than diphenyl pyrazoline (II), yet wool whitened with either I or II yellows at virtually the same rate.



(I)

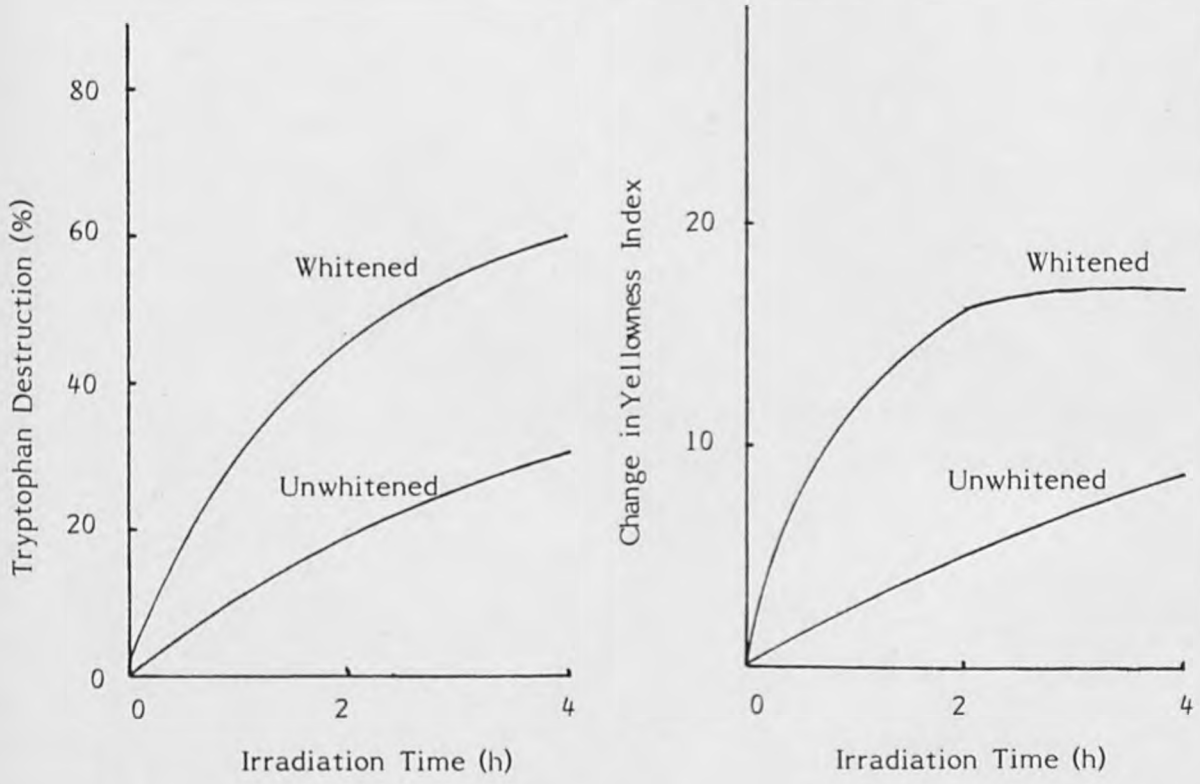


(II)

Use of radiolabelled stilbene and pyrazoline FWA's has shown that some brighteners, or their degradation products, do bind covalently to wool during irradiation [40]. However, no coloured degradation products have yet been found, so this is unlikely to be the cause of yellowing.

The evidence available suggests that FWA's promote yellowing by acting as sensitisers, probably to the processes occurring in natural wool [37,39,41]. Tryptophan and histidine are degraded much faster when a whitener is present, [41,43] and a good correlation exists between tryptophan decomposition and photoyellowing shown below, in Fig. 1.5 [41]:

Fig.1.5 Correlation Between the Destruction of Tryptophan Residues and Increase in Yellowness of Untreated & Fluorescently Whitened Silk During Exposure to Simulated Sunlight



Further, it has been shown that whitened wool, where 80% of tryptophan residues have been chemically modified, yellows at approximately half the rate of untreated whitened wool [35]. It therefore appears that the sensitisation of photoyellowing by FWA's is the controlling factor, rather than the stability of the FWA itself.

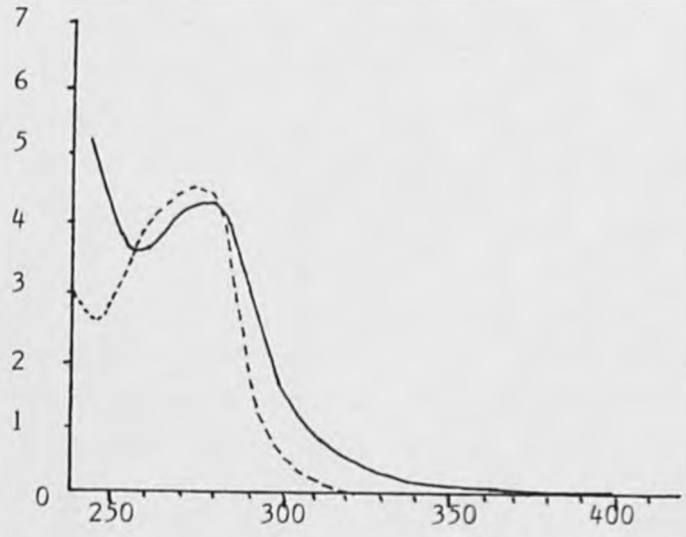
1.5. Effects of Irradiation on Wool

For over forty years a considerable amount of work has been done with a view to elucidating the chemical reaction involved in the photoyellowing of wool. It has emerged from these studies that the problem is very much more complex than was first imagined, and that no single reaction is responsible but rather, a combination of processes at several sites in the wool fibre.

1.5.1. Luminescence

An initial problem was that no amino acid has a significant U.V. absorption above 300nm, and that no light below 300nm reaches the earth, yet amino acids are photodegraded by sunlight. The absorbance curves of the individual amino acids in polymer films were known [4], as was the amino acid composition of wool, and hence it was possible to calculate the theoretical absorption curve due to the amino acid components of wool and compare it directly with that for wool itself. This is shown in Fig.1.6.

Fig.1.6 The Ultraviolet Absorption Spectrum of a 6μ Radial Section of Merino-Wool Keratin (---), and the Absorption Spectrum Calculated From the Amino-Acid Composition (- - -)



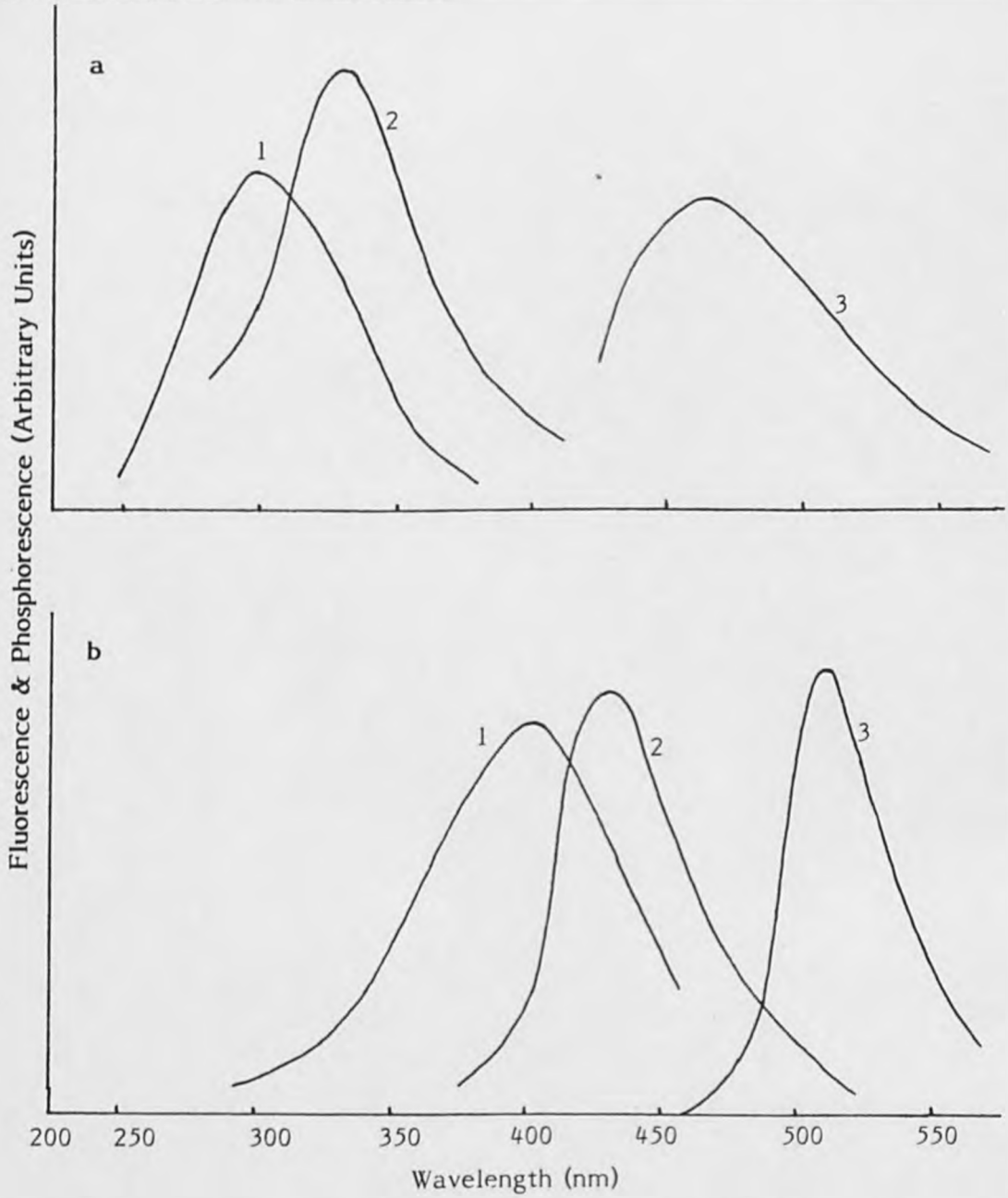
The absorption between 250nm and 300nm is due mainly to the presence of tryptophan and tyrosine, with minor contributions from cystine and phenylalanine. Whilst agreement between the observed and calculated absorbance curves are quite good in the region 250-290nm, at wavelengths above 290nm the wool fibre has a much higher absorbance than can be accounted for by amino acid absorptions, indicating the presence of other species which absorb strongly in this region. It has been postulated that this absorption could be due to the presence of photodecomposition products of wool as a result of sunlight exposure during growth [44], or to the presence of natural pigment precursors [45].

Attempts to determine which amino acid is responsible for the formation of yellow pigments in wool on irradiation have centred mainly upon tryptophan. One reason for this is that although both tyrosine and tryptophan absorb strongly in the 280-300nm 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 [45-48]. Whilst 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, considerable evidence is now available to support energy migration from tyrosine to tryptophan [49,50]. Seeking confirmation of energy migration in wool, Ghiggino et al. [51] determined the quantum yield of fluorescence of wool at room temperature and at 77K, with respect to the quanta absorbed by wool and quanta absorbed by the tryptophan. At both temperatures the quantum yield per quantum absorbed by tryptophan was found to be higher than for tryptophan in poly (vinyl alcohol) film, indicating that excitation energy transfer occurs from the tyrosine to the tryptophan.

Wool has also been found to exhibit phosphorescence, providing evidence of intersystem crossing by a radiationless process from the excited singlet state to the excited triplet state, and emissions of energy in the form of visible radiation in returning to the ground state. See Fig. 1.7.

Fig. 1.7

Excitation (1), Fluorescence (2), & Phosphorescence (3), Spectra of (a) Untreated & (b) F.B.A. Treated Wool

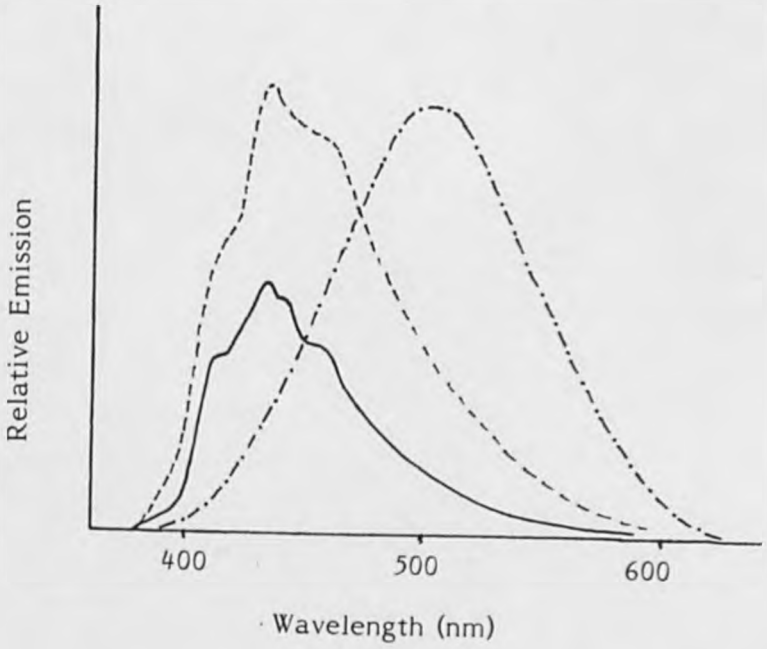


Ghiggino [51] examined the room temperature decay kinetics of wool phosphorescence, (excitation at 280nm), and found that under normal atmospheric conditions it decayed by first order kinetics with a lifetime of 0.08s, but under dry nitrogen its lifetime was 0.45s and decayed at an apparent second-order rate. They concluded that under dry nitrogen, the rate-determining step is 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.

Singlet oxygen, (0_2, $^1\Delta_g$), is the lowest of two singlet excited states of oxygen, lying only 22.54kCal. above the ground (triplet) state, and having a lifetime of 1 hour in the absence of collisions with other molecules.

Whilst studying the excitation wavelength dependence of the phosphorescence of wool keratin, Nicholls and Pailthorpe [4] found two quite distinct phosphorescent species, (Fig. 1.8).

Fig. 1.8 Triplet-state Emission Spectra at Room Temperature. - - - Tryptophan in a dry PVA Film for λex. = 280nm. ---- Wool Keratin for λex. = 290nm. -.-.-.- Wool Keratin for λex. = 350nm.



Exciting at 290nm they detected a phosphorescence emission at 435nm, which 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 at 350nm, a second phosphorescent species was detected, the source of which is not known but could be the same species which caused the enhanced absorption of wool in the 330-360nm range. Leaver [52] has subsequently proposed that the phosphorescent emission derived from excitation at 290nm is a combination of two exponential decay curves, with lifetimes 0.18 and 1.45s. The former he attributed to the species absorbing at 350nm and the latter to the triplet state of tryptophan.

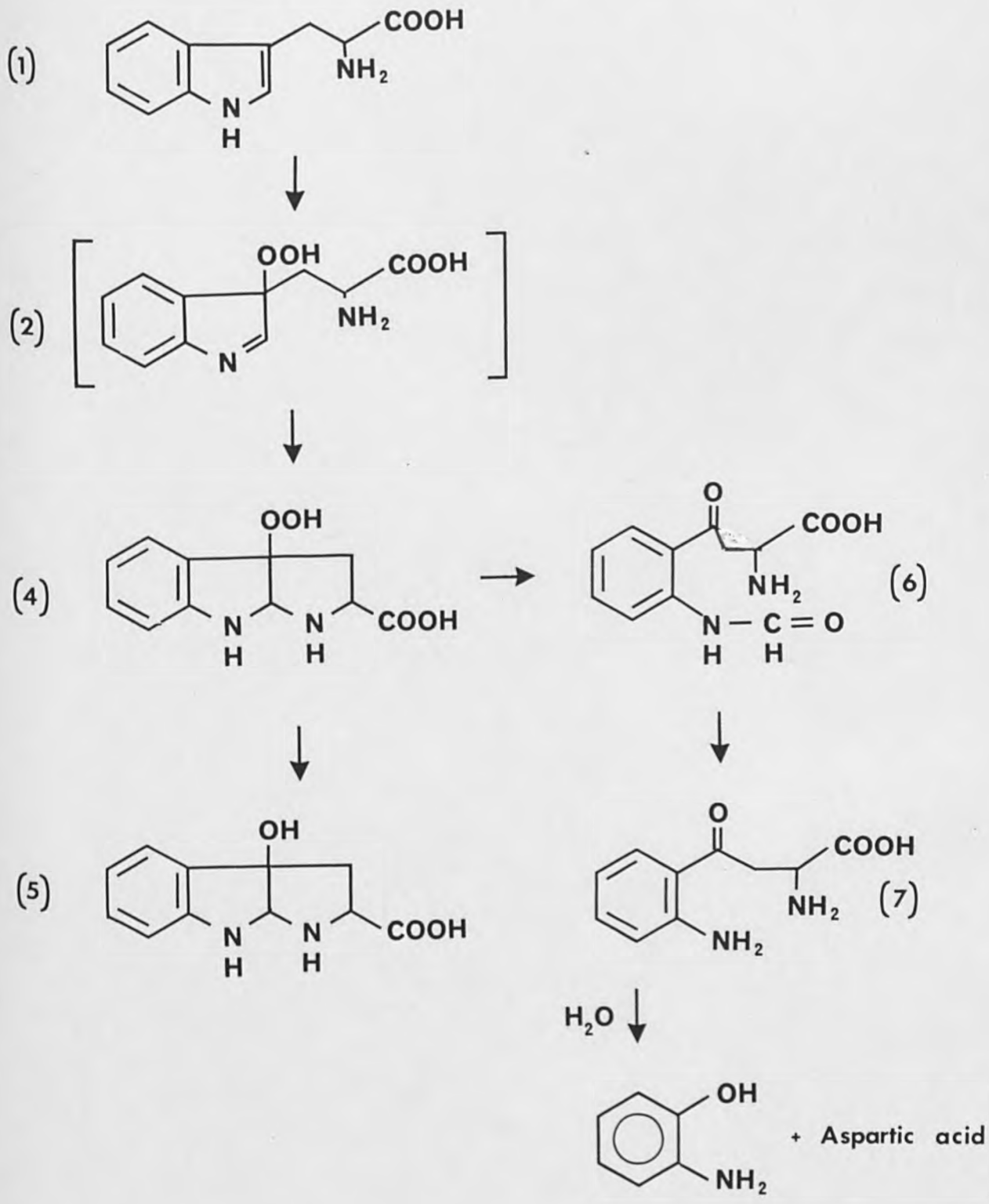
1.5.2. Tryptophan Degradation

Considerable evidence is now available [3,53-59] to support the theory that wool yellowing occurs as a result of photodecomposition of the tryptophan residue to form yellow products. If this is a correct interpretation of experimental results, then it is important to know what these photoproducts are, and the mechanisms by which they are formed.

To avoid the problems associated with the isolation of peptide-bound photoproducts from an insoluble substrate such as wool, much work has been done on the photodegradation of free tryptophan and its derivatives in aqueous solution. Two main approaches have been adopted, one involving direct photolysis, and the other by means of a photosensitising dye. In the latter case, most sensitising dyes used have been singlet oxygen generators and the photooxidation of tryptophan was the result of reactions with singlet oxygen [59].

Flash photolysis of an aqueous solution of tryptophan gave rise to at least nine primary degradation products [6], which have not been identified. After prolonged irradiation of tryptophan (1) two strongly yellow products were isolated, (N-formyl kynurenine [62,63] and kynurenine [64]), along with 3-hydroperoxypyrrolidinoindole, (4), and 3-hydroxypyrrolidinoindole (5). It has been proposed [65] that the short-lived intermediate, (2), (indolenine hydroperoxide), is involved as shown in Fig. 1.9.

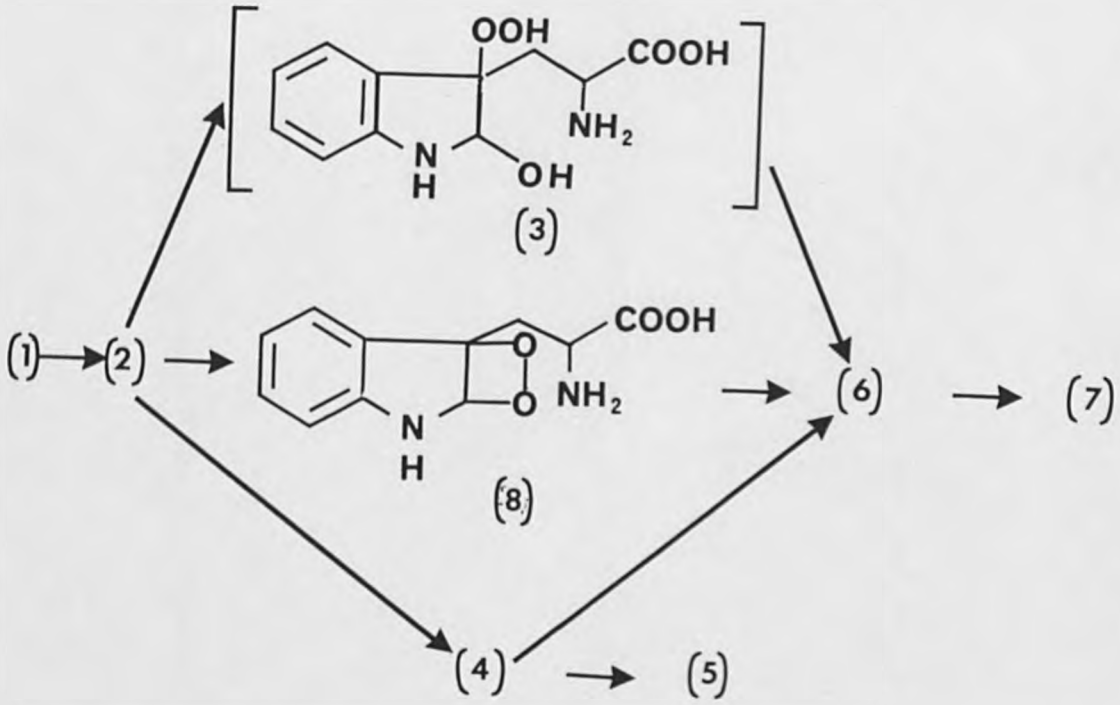
Fig. 1.9 Tryptophan Degradation Pathways



It should be noted that this mechanism, (2→4), where the lone pair on the α-amino group attacks the indole in position 2, is less likely to apply when tryptophan is incorporated into a protein chain. The ring closure would be sterically hindered, and the nitrogen deactivated by incorporation into the amide group. This may account for the reduced rate of yellowing observed for N-acetyl tryptophan [66].

It has been postulated that singlet oxygen is involved both in the direct photoyellowing of wool, and the FWA photosensitised yellowing of wool. If true, then the dye-sensitised photooxidation of tryptophan, (where singlet oxygen is the major reactant), becomes very relevant. The reaction scheme shown in Fig. 1.10 was proposed by Nakagawa et.al. [67].

Fig. 1.10 Further Tryptophan Degradation Scheme



N-formyl kynurenine (6) and (5) [68,69], were identified by Savige. It should be noted here that restriction regarding the ring closure formation of (4) which applied in the case of peptide-bound tryptophan would apply here too. However, N-formyl kynurenine would still be formed, via the dioxetane intermediate, (8). This is supported by the isolation of kynurenine, (7), from photoyellowed wool [29].

These results, whilst clearly implicating tryptophan residues in the photoyellowing of wool, still give little indication of the primary photochemical reactions involved.

Solution studies on the effect of irradiation on various amino acids and peptides [59] have shown that the photodegradation of tryptophan in aqueous solutions is strongly dependent upon the presence of molecular oxygen, and that the extent of yellowing and degradation pathway followed were controlled by the pH of the solution:

Fig. 1.11 Effect of pH on Photoyellowing

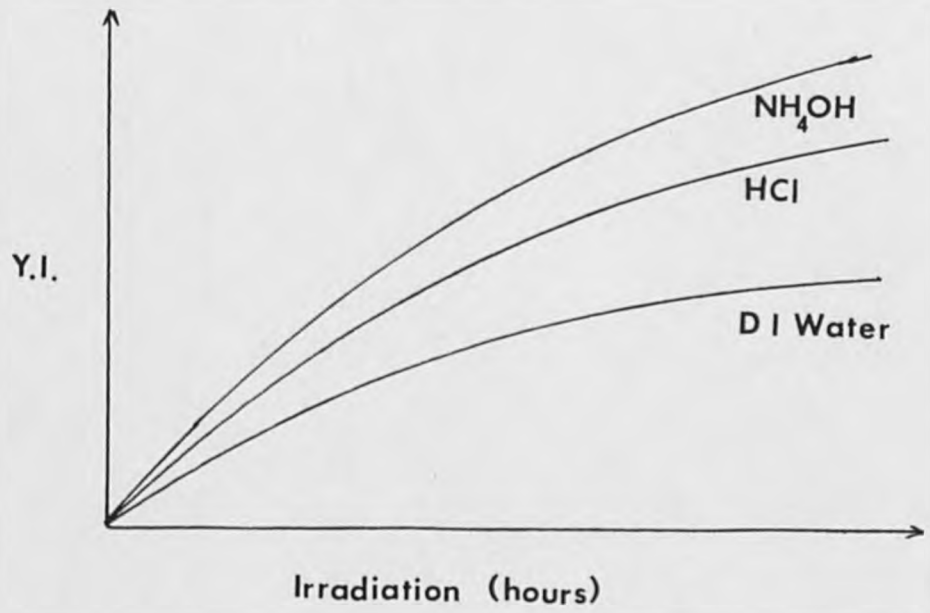
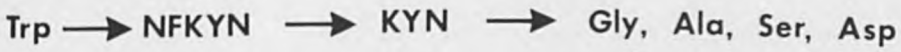
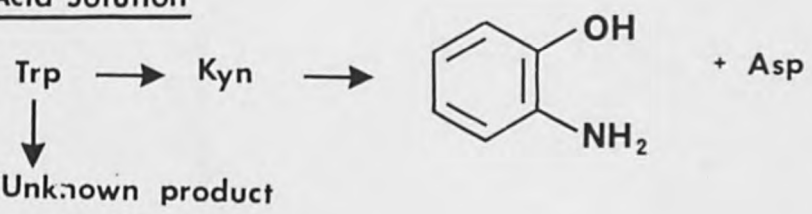


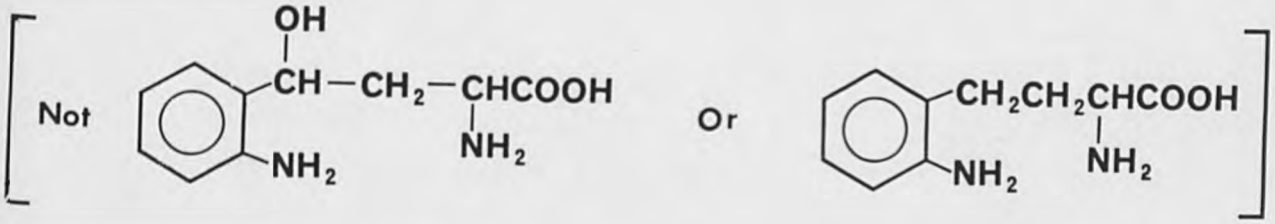
Fig. 1.12 The Effect of pH on Tryptophan Photodegradation

1. Deionised Water

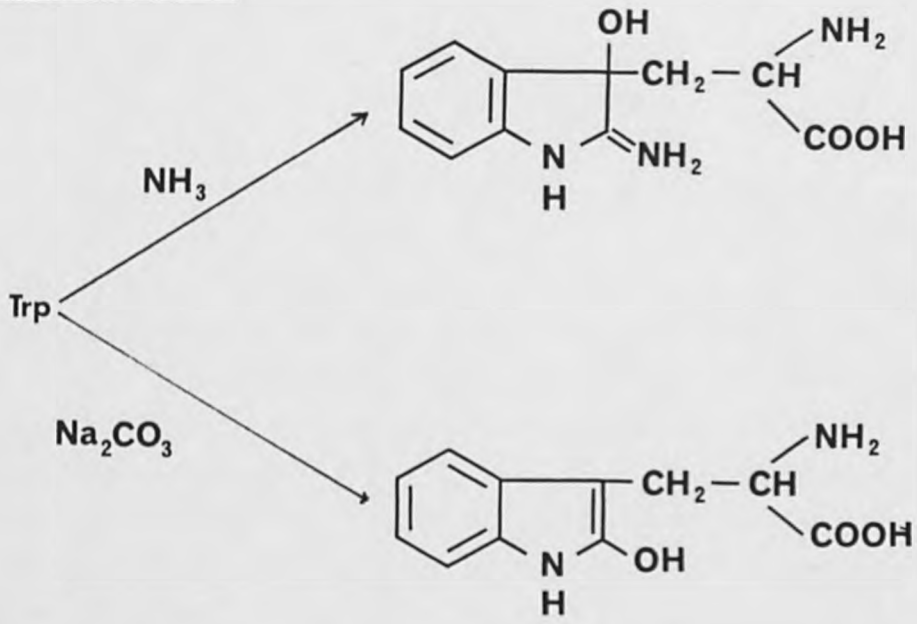


2. Acid Solution





3. Alkaline Solution



In their reviews on wool yellowing, both Milligan [70] and Nicholls [71] have concluded that a free radical pathway is not involved in wool yellowing, but is involved in peptide chain breakdown. Esr studies have shown an asymmetric low field signal due to the $RCH_2S^{\cdot}$ radical resulting from homolytic fission of the disulphide bond in cystyl residues [72], and a more intense line characteristic of photo-induced free-radicals in aromatic amino acids [73-75], possibly due to photolysis of tryptophyl or tyrosyl residues. Since disulphide and main chain cleavage would reduce the tensile strength of the fibre, it is likely that phototendering involves a radical mechanism. There is no evidence, however, to suggest radical involvement in the photoyellowing process. Other amino acids have been found to be degraded during photoyellowing, such as tyrosine, histamine, methionine and cystine [76,77], but in general coloured products are not formed to any appreciable extent.

1.5.3. Photochemical Reactions Involved in Wool Yellowing

It has already been established that wool absorbs energy below 320nm through tryptophan and tyrosine residues. However, energy is efficiently transferred from tyrosine to tryptophan, and hence all the absorbed energy initially lies in the tryptophan singlet excited state. About 8% of this is lost by fluorescence [51] the remainder is dissipated by internal conversion, quenching within the fibre, eg. by disulphides [78.79], 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 triplet state occurs [4].

It has also been established that wavelengths above 320nm are absorbed by an unidentified phosphorescent species. Its singlet excited state appears to follow similar deactivation pathways to that of the tryptophan singlet state, having a phosphorescence quantum yield of 0.06 [4] at 77K. Hence two distinct triplet state molecules are formed under sunlight irradiation. Both exhibit first order decay in the presence of oxygen, which is accelerated in the presence of water [4]. Further, it has been found that at least ten molecules of oxygen are consumed for every molecule of Trp destroyed, irrespective of whether the irradiation wavelength is below 320nm or above 340nm.[4].

As an explanation for these results, Nicholls and Pailthorpe proposed that these two triplet excited states react with ground state oxygen to produce singlet oxygen by energy transfer:-

$$^3T^* + {}^3O_2 \rightarrow S_0 + {}^1O_2 \ (^1\Delta_g)$$

Singlet oxygen then diffuses throughout the wool protein and reacts with histidine, tryptophan and methionine residues, (the amino acids known to react with singlet oxygen **[80]**). It is possible that singlet oxygen also reacts with the unidentified absorbing species.

Experimentally, these proposals are not easy to substantiate. It would be necessary to show both the formation of singlet oxygen in irradiated wool, and that a reaction occurs between the wool and the singlet oxygen to produce yellow products.

Treatment of wool with sodium azide, (1% owf, pad/dry), a known singlet oxygen quencher **[60]**, reduced the rate of both tryptophan degradation and oxygen consumption **[4]**, (see table 1.1)

Table 1.1 Tryptophan Loss & Oxygen Consumption Under Different Irradiation Conditions, 50% r.h.

$h\nu$ (nm)	ϕ^* TRP	$\phi^* O_2$
365	$1.3X10^{-4}$	$1.8X10^{-3}$
365+ NaN_3	$1.0X10^{-4}$	$1.2X10^{-3}$

Moreover, by passing oxygen over irradiated wool, 6 μmolg^{-1} h^{-1} of singlet oxygen was detected [4]. Hence, it has been shown directly that singlet oxygen is formed upon irradiation of wool. Indirect evidence supporting this comes from a study of peroxide bleached wool, which yellows more rapidly than unbleached wool [9], where it is shown to have a much greater phosphorescence [52]. This increase in phosphorescence, leading to a greater yield of singlet oxygen, could account for the increased yellowing. The cause of this increase in phosphorescence may be the oxidation of cystine residues, reducing the cystine-quenching of excited states in wool and thus enhancing the normal phosphorescence, or it could be due to the oxidation of certain amino acids to form more phosphorescent products.

It is likely that in the case of fluorescently whitened wool, the photosensitisation observed is due to a reaction between the triplet FWA molecule and oxygen to produce singlet oxygen, which then oxidises amino acids within the fibre. This is supported by the similarity which exists between the chemical changes occurring in untreated and brightened wool, (though more pronounced in the latter), and the dependence upon the presence of oxygen. Photosensitisation gives rise to a marked increase in the degradation of histidine, tryptophan, and methionine, the three amino acids susceptible to $^1O_2$ attack [80]; and the rate of photodegradation of fluorescently whitened wool was also significantly restricted by treatment with sodium azide [4], a singlet oxygen quencher.

Thus it is probable that a singlet oxygen mechanism is responsible for the photoyellowing of both untreated and fluorescently whitened wool.

From the "action spectra" for the photoyellowing of wool, (Fig.1.3) it can be seen that the most damaging wavelengths are those in the 290-310nm region of the spectrum. However, if this action spectrum is corrected for the energy distribution in sunlight, maximum yellowing is found to occur in the 340-350nm wavelength region, where the unidentified species has its absorption maximum. It therefore seems likely that when wool is irradiated by sunlight, yellowing is mainly dependent upon absorption by this 340nm absorbing species, rather than by any other amino acid [81]. However, the yellow photoproducts formed may be due to the subsequent oxidation of tryptophan by singlet oxygen formed from the triplet excited state of

this unidentified species. Thus, identification of this absorbing species, the behaviour of its excited states, and its reactivity with singlet oxygen would help greatly in understanding more fully the reactions leading to wool yellowing.

1.6. Photobleaching

Irradiation of wool with wavelengths above 380nm has been found to bleach wool **[82-87]**, a process occurring concurrently with yellowing in normal sunlight. The extent of photobleaching is controlled not only by the wavelength distribution of the incident light, but also by the degree of yellowness of the irradiated fabric. Hence the rate of bleaching is greatest in yellow wool, and virtually zero in fluorescently whitened wool **[85]**. Using blue light, it was found to be possible to bleach only half of the initial yellow colour of natural wool. From this, it was suggested that two chromophores may be present **[88]**, only one of which being susceptible to blue-light bleaching.

Pretreatment of wool with reagents such as sodium hydrosulphite, thiourea dioxide, thiourea formaldehyde resins or thioglycollic acid and its zinc salt have been found to accelerate the photobleaching effect **[89]**. As a result of this, King proposed that zinc complexed thioglycollic acid be used both as a photobleaching catalyst, and as an additive for detergents **[90]**.

Using high intensity visible light, (a 1000W water cooled mercury lamp, U.V. filtered), Launer achieved a bleaching effect equivalent to that achieved by the alkaline peroxide treatment in one minute. With this method, such problems as shrinking and felting are eliminated, as is the decrease in tensile strength. The process is not used commercially, the cost being prohibitive **[87]**.

Interest in photobleaching centres mainly in its potential for countering photoyellowing. It is known that in the wavelength distribution of normal sunlight, both processes occur simultaneously. It may be that a photobleaching catalyst can be found to so enhance the process that photoyellowing is virtually eliminated. However, this could only apply to untreated wool, because of the very low visible light absorption of fluorescently whitened wool. No mechanisms for the phenomenon have yet been established.

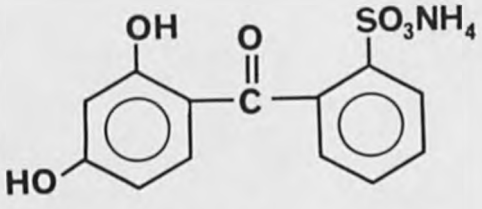
1.7. Prevention of Photoyellowing

The photoyellowing problem prohibits the use of whitened wool in the baby wear and fashion wear markets. Many attempts have therefore been made to develop a treatment to protect wool against photoyellowing, some with moderate success.

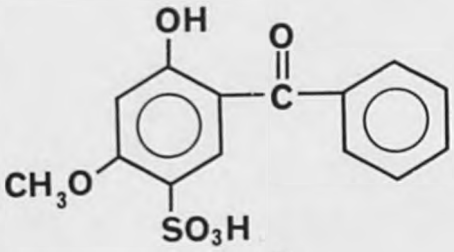
1.7.1. U.V. Absorbers

A U.V. screen functions by absorbing the U.V. wavelengths which cause yellowing, and dissipating the energy harmlessly as heat. Such compounds are already in commercial use with synthetic fibres with good effect [91,92].

Many U.V. absorbers have been tested on wool, usually as the sulphonic acid derivative [93-98]. Two of the most successful classes are the benzophenones and the benztriazoles. Using 17% owf loading, compounds I and II gave up to 50% protection against yellowing [93]:

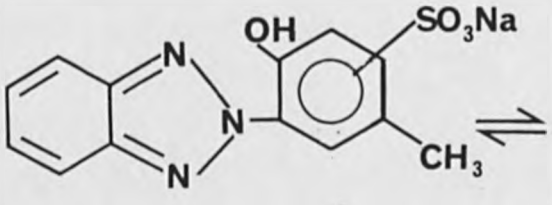


I

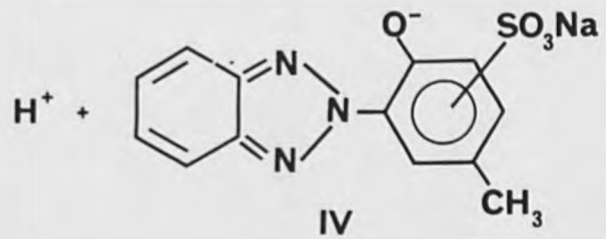


II

A protective effect was also obtained by Waters et.al. with 2- (2'-hydroxy-5'-methylphenyl) benzotriazole sulphonate, III [95,97].



III



IV

This compound is less effective for light sources having a visible as well as U.V. component, such as sunlight, because the phenolate anion, IV, exhibits the opposite effect and sensitises the wool to yellowing by visible radiation **[96]**. Generally, however, the protective effect of U.V. absorbers is disappointing. They retard yellowing less effectively than expected on the basis of their absorption properties, and require the use of high percentages on weight of fibre to achieve satisfactory results. This alters the handle of the fabric to an unacceptible extent, and hence the commercial use of U.V. screens is limited. A further limitation is their incompatibility with fluorescently whitened wool. Preferentially absorbing the same wavelengths as the FWA's, their use dramatically reduces the effect of the brightener.

More recently, a number of papers have been published on the use of U.V. screens for the prevention of phototendering **[97,99-103]**, with good effect. In all of these, it is the phenylbenzotriazoles and benzophenones which have been found to be effective.

1.7.2. Excited State Quenchers

Much evidence has been furnished for the theory that wool yellowing occurs as a result of singlet oxygen formation by reaction of triplet excited states with ground state oxygen. It is therefore likely that photoyellowing could be retarded by the effective quenching of these excited states. Most studies have considered only typtophan, since no other excited states have been identified in untreated wool.

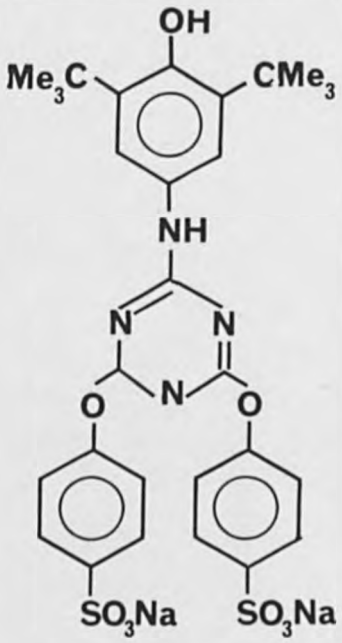
One of the most effective fluorescence quenchers for tryptophan in aqueous solution is acrylamide **[104]**, also effective in polymer films **[105]**. When applied to wool, however, the extent of tryptophan fluorescence quenching was only 25% of that expected from polymer film measurements. Furthermore, the reduction in photoyellowing of the wool in the presence of the quencher was minimal **[70]**. To account for this it was suggested a high percentage of tryptophan residues in wool are so located as to be inaccessible to the quenching molecules applied from aqueous solution. Nicholls & Pailthorpe **[4]** treated wool with sodium azide, 1% owf and observed a retardation in tryptophan decomposition, but did not report the effect on the photoyellowing of the fibre.

Abdullah et al. treated jute with singlet oxygen quenchers DABCO (DiAzoBiCycloOctane), and β-carotene, and observed no stabilising effect, as measured by photoyellowing [100].

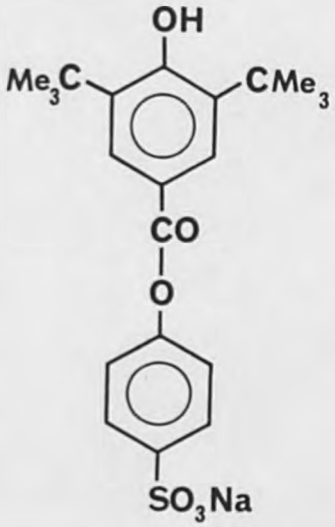
It therefore appears that this method of stabilisation is not successful with wool, using the quenchers and methods currently available.

1.7.3. Antioxidants

The function of an antioxidant is to destroy primary photoproducts before they can give rise to substrate degradation. Hindered phenols, eg. derivatives of 2,6-di-t-butyl phenol, are frequently used as antioxidants to suppress the autoxidation of olefins, which occur via hydroperoxide radicals. Two sulphonated derivatives, (V and VI) were applied, (5% owf), to wool fabrics previously whitened with Uvitex NFW [42]:



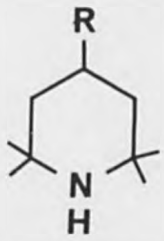
V



VI

Neither of the above were found to offer any protection against photoyellowing. This suggests that hydroperoxide radicals are not involved in yellowing.

Another class of stabilisers is the hindered piperidines [71]:



These are a relatively new addition to the range of commercial ultraviolet stabilisers, and have proved to be by far the most effective systems for polymers. They do not appear to operate by mechanisms of optical screening or excited state quenching. Their effectiveness depends on their ability to form a stable nitroxyl radical, which then scavenges alkyl radicals produced during photooxidation. They also react with hydroperoxides. So far there is no report in the literature of these compounds being used for treatment of wool.

1.7.4. Reducing Agents

Having established that photoyellowing is an oxidation process, it is a logical step to attempt to decrease the rate and extent of the reactions involved with reducing agents. Several classes of reducing agents have been examined, which under certain conditions minimise the photoyellowing of wool. These include thiols **[107]**, thiocarboxyllic acids **[42,107]**, bisulphite **[3,42,108]**, phosphines **[42]**, and borohydride **[42,109]**.

One method used to assess "anti-yellowing" activity of these reducing agents was to irradiate fluorescently whitened wool in aqueous solutions of these reagents. Those found to decrease the extent of yellowing are listed in Table 1.2 **[42]**.

TABLE 1.2 The Effect of Immersion of Brightened Wool in Reducing Agent Solution on the Extent of Photoyellowing by Simulated Sunlight*

CONDITIONS	Yellowness Index of Fluorescently Whitened Wool		
	Pyrazoline (a)	Stilbene (b)	Bis-styryl biphenyl (c)
Before Irradiation	4.4	5.3	6.7
After Irradiation in:			
Water	10.4	16.0	13.6
0.1M Na_2SO_4	10.4	18.0	17.2
0.1M Na_2SO_3	2.2	3.5	3.7
0.1M $Na_2S_2O_3$	7.2	6.9	7.4
0.1M Thiourea	10.3	10.0	9.0
0.1M Cysteine	6.4	9.0	9.4
0.02M THPC	3.8	3.2	2.2
0.1M Thioglycollic Acid	5.6	4.8	1.5

* 2hr. exposure, 18" below a Phillips HOKI 2000W Hg arc lamp, fitted with a Corning glass filter 7740 to eliminate wavelengths below 295nm.

a. Sodium 1-(4' -sulphonyl) -3-phenyl pyrazoline. 0.1% owf.
b. Bis (triazinyl-amino) stilbene. 1.7% owf.
c. Uvitex NFW. 1% owf.

It can be seen that most reducing agents retarded photoyellowing under these conditions, while sodium sulphite, thioglycollic acid, (T.G.A.), and Tetrakis-hydroxymethyl phosphonium chloride, (T.H.P.C.)* actually resulted in further whitening of the samples.

* THP is the reducing species: $(CH_2OH)_4P^+Cl^- \rightarrow (CH_2OH)_3P + HCHO + HCl$

It is not known whether reducing agents function by promotion of the photobleaching process, inhibition of the yellowing process, (by reduction of photooxidation products), or possibly a combination of the two. Their effectiveness cannot be due to any screening of the wool from the light source, because none of the solutions significantly absorb light above 300nm, though some of the reagents, eg. T.H.P.C. or T.G.A., which are oxygen scavengers, may inhibit yellowing by depriving the wool of oxygen, since it has been shown that wool fails to yellow when irradiated in the absence of oxygen **[110]**.

To determine the effects of T.H.P.C., T.G.A. and sodium sulphite upon the photodecomposition of amino acids believed to be responsible for yellowing, samples of bleached wool, (whitened and unwhitened), were irradiated in the above solutions and hydrolysed for amino acid analysis by the Liu and Chang method **[111]**. This method of hydrolysis using p-toluene sulphonic acid, was chosen because it involves minimal tryptophan decomposition. The amino acids which underwent most significant destruction were tryptophan and histidine. Table 1.3 shows the results found **[42]**.

Table 1.3 Changes in Tryptophan & Histidine Contents of Bleached (B), and Fluorescently Whitened Wool, (FWA), After Irradiation in Reducing Agent Solutions

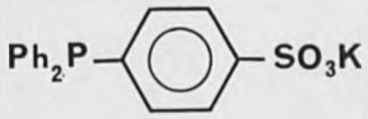
Wool Sample	Solution	Trp(μmole/g)		His(μmole/g)	
		Before hν	After hν	Before hν	After hν
B	Water	32	15	64	26
FWA	Water	33	2	65	21
B	0.1M TGA	35	26	65	58
FWA	0.1M TGA	35	17	64	53
B	0.1M Na_2SO_3	33	27	62	51
FWA	0.1M Na_2SO_3	33	23	63	37
B	0.1M THPC	17*	21	63	59
FWA	0.1M THPC	23*	23	63	57

* This method cannot be used to determine the tryptophan content of THPC treated wool.

Since it is believed that tryptophyl residues are primarily responsible for yellowing of wool [110], it is possible that these reducing agents achieve their protective effect by preventing the photodecomposition of tryptophan in wool. However, the level of protection of tryptophan residues achieved by these reagents is not sufficiently high to account fully for the excellent level of stabilisation observed. It is therefore probable that the major function of the reducing agents is to change the course of the photodegradative pathway so that colourless rather than yellow products result.

In the solid state, the above reducing agents have been found to be far less effective, (eg. after a pad/dry treatment). When fluorescently whitened wool is impregnated with THPC, better protection is afforded against wet irradiation that dry. The effect is reduced still further if the wool is washed after treatment. Some protection against wet yellowing is retained but all protection against dry yellowing is lost.

Because of the poor wash-fastness results obtained with THPC, the sulphonated triphenyl phosphine, VII, was examined. This was thought likely to be more substantive to wool.



VII

When applied at a level of 5% owf by the pad/batch method, some protection was conferred against wet yellowing, but the high loading markedly changed the handle of the fabric.

The observed effectiveness at high concentrations supports the theory that reducing agents are only effective yellowing inhibitors if

they are situated beside the photosensitive sites in the fibre. In the presence of water, diffusion to these sites could occur, explaining why wet-state yellowing is more easily retarded than dry.

1.7.5. Thiourea Formaldehyde

A process which has been found to be effective on untreated and fluorescently whitened wool is the treatment with thiourea formaldehyde mixtures [112115], or precondensates [116], followed by thermal curing. Using fairly high levels of treatment (10% owf thiourea and 20% owf formaldehyde), a four-fold improvement in lightfastness can be achieved for fluorescently whitened wool. The main deficiency of this process is that more than half of the protective effect is lost on the first laundering, though no further losses occur during subsequent washes. The treatment is also expensive at the effective levels of application.

The process was originally the subject of a Japanese patent [117] where Nakajo claimed that the thiourea-formaldehyde resin diminished the rate of photoyellowing by absorbing the U.V. component of sunlight. However, the resin has been found to have a minimal absorption in the region 295-315nm which wavelengths cause serious yellowing of the wool [112]. Furthermore, it was found that an aqueous solution of thiourea and formaldehyde gave almost the same protection against yellowing as the resin treatment. It appears therefore that the protection is due to some reaction of the wool with thiourea and formaldehyde. Formaldehyde alone gave some protection against yellowing of dry fabric but no protection when the fabric was exposed wet [112].

In an attempt to improve the washfastness of the treatment, mixtures of formaldehyde with substituted thioureas were tested and found to be almost as effective as thiourea for inhibition of yellowing, but protection was still diminished on rinsing.

Poor washfastness is a considerable drawback, because it is virtually essential to wash out residual reactants after treatment. Thiourea is a carcinogen suspect, and formaldehyde is know to cause dermatitis, thus restricting the commercial use of the treatment.

The mechanism by which thiourea-formaldehyde mixtures afford protection against yellowing remains obscure. Amino acid analyses of treated wool are virtually the same as for wool treated with formaldehyde alone. Yet formaldehyde treatment offers very little protection to wool. It has been found [112] that considerably more formaldehyde is bound to wool in the presence of thiourea than in its absence. Sulphur analysis of thiourea-formaldehyde treated wool showed that thiourea was also bound, so it does not appear to have a catalytic function. It seems likely that both thiourea and formaldehyde are covalently bonded to the wool, the latter being present presumably as methylol groups or as methylol thiourea derivatives.

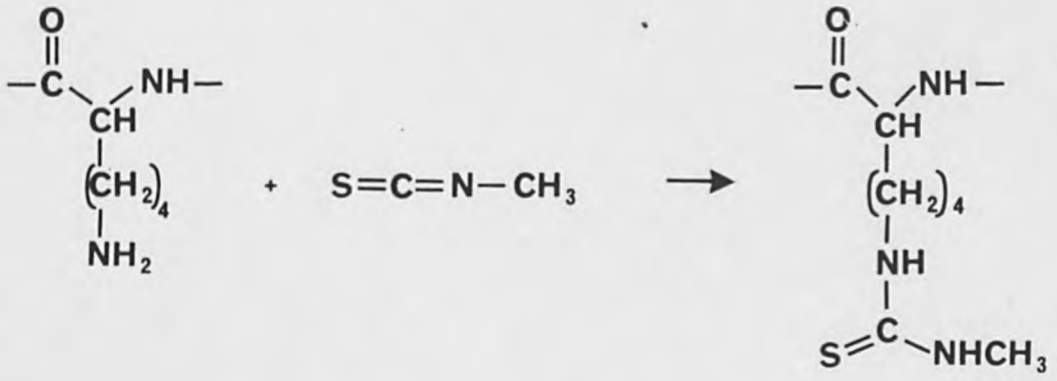
If the treatment could be modified to prevent loss of the protective effect occurring during initial rinsing, its commercial viability would undoubtedly increase, and may be able to achieve the photostability necessary for wool to compete with synthetic materials.

1.7.6. Chemical Modification

Various attempts have been made to modify sites in the wool keratin which are believed to be photosensitive, with a view to blocking the degradation mechanisms. These have generally been unsuccessful, but will be briefly described here.

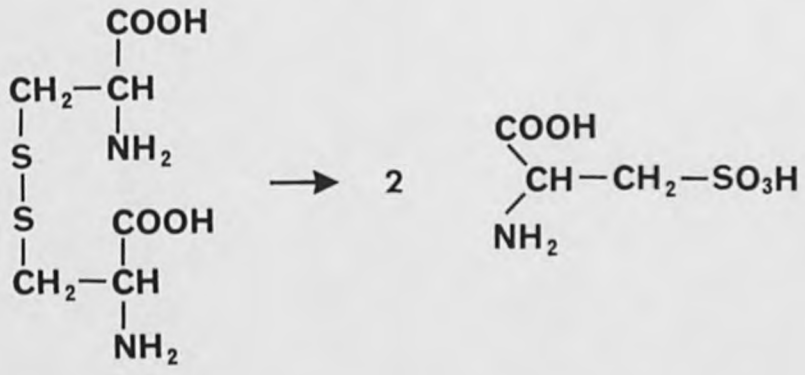
(i) Methyl Isothiocyanate

Subsequent to the discovery that the thiourea-formaldehyde treatment stabilised wool to yellowing, it was found that conversion of the lysyl amino groups to thioureido groups [118] by reacting with methyl isothiocyanate in DMF or DMSO also reduced yellowing without the need for a secondary formaldehyde treatment. The poor washfastness of the thiourea-formaldehyde treatment was no longer a problem.



The methyl isothiocyanate treatment confers a measure of photostability on both untreated and fluorescently whitened wool, but the degree of protection is less than that obtained with thiourea-formaldehyde. For this reason, and those of cost and toxicity of the reagent, this is not a commercially acceptable treatment.

(ii) The disulphide bond in cystyl residues is known to be a centre of attack during photooxidation **[119,120]**. Oxidation occurs through to cysteic acid:



This disulphide cleavage is of more relevance to phototendering than photoyellowing, but much work has been done to stabilise this linkage in the hope that an increase in photostability will ensue.

Treatment of wool with sodium carbonate or sodium cyanide converts cystyl residues to lanthionine [121-125].

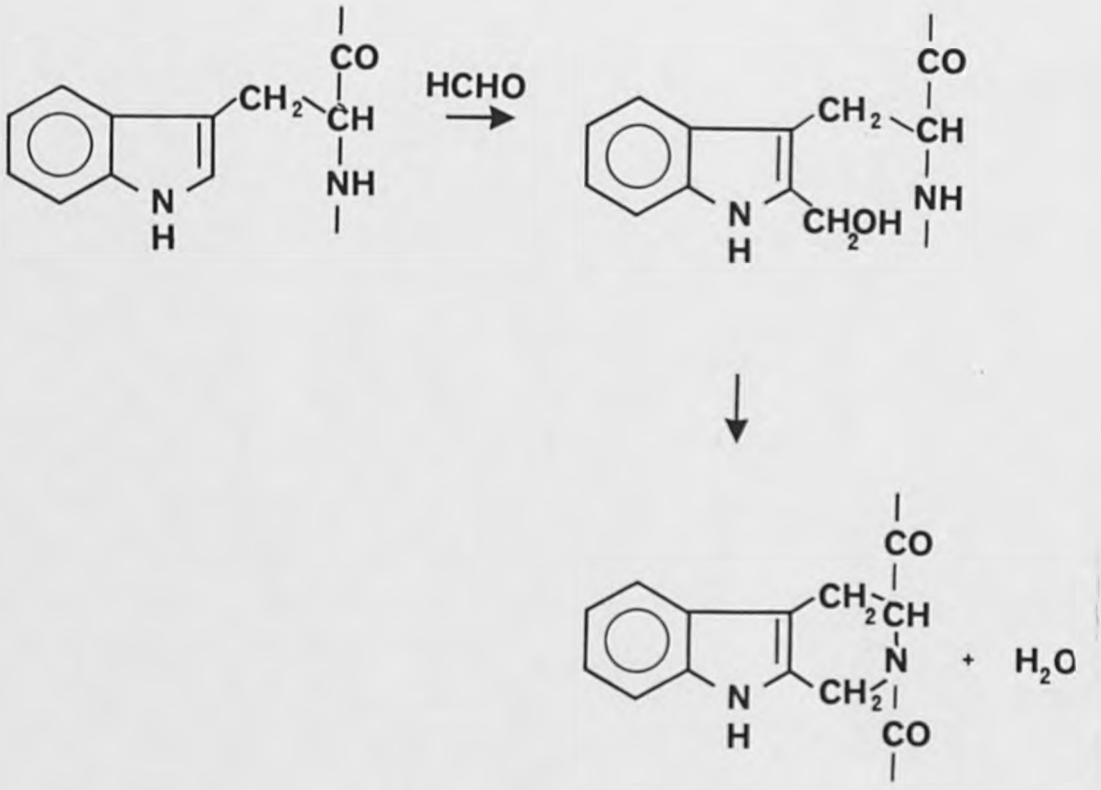
$$W - S - S - W \rightarrow W - S - W$$

Wool thus modified was found to photoyellow at least as fast as untreated wool, suggesting that decomposition of disulphide groups is not of major importance in the yellowing process [126]. Reaction with formaldehyde, under various conditions, inserts a methylene group [127-131]:

$$HCHO + RSH \longrightarrow RSCH_2OH$$

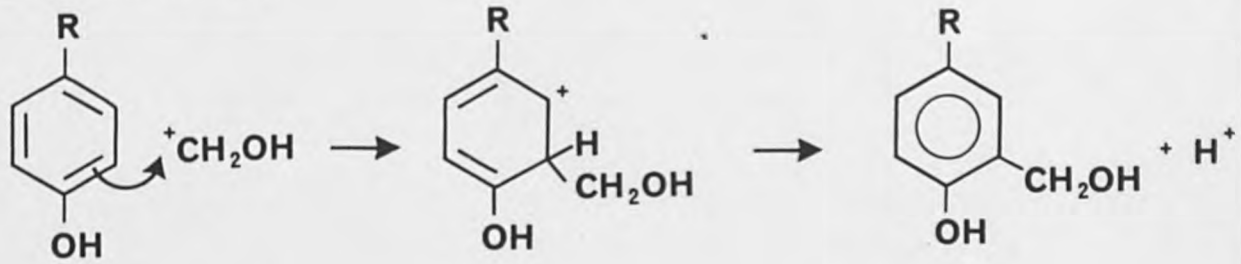
$$RSCH_2OH + RSH \longrightarrow RSCH_2SR + H_2O$$

Formaldehyde is also believed to react with tryptophan [27]:

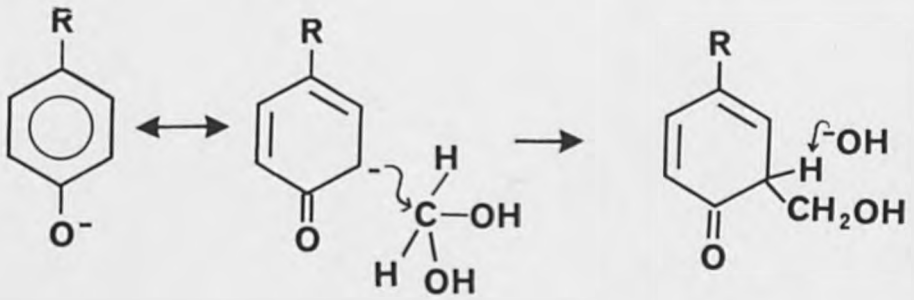


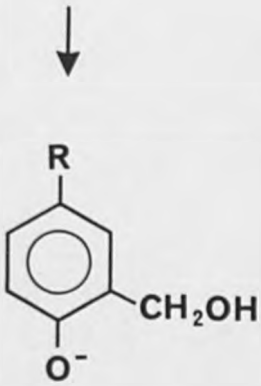
and with tyrosine:

Acid conditions



Alkaline Conditions



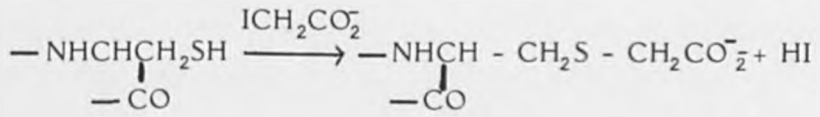


Reaction of thioglycollic acid with cystyl residues gives rise to the formation of mixed disulphides **[132,133]**:

$$W\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2W + H\text{-}S\text{-}CH_2\text{-}COOH \rightarrow W\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}COOH + W\text{-}CH_2\text{-}SH$$

Cystine — Thioglycollic Acid

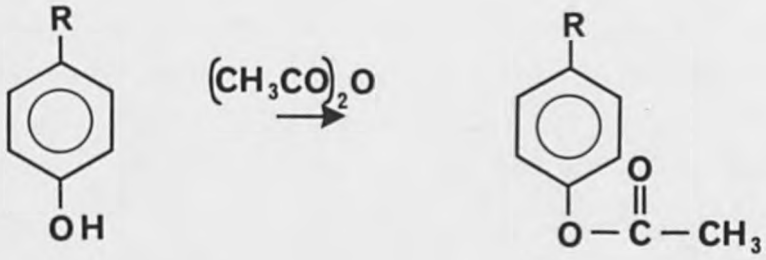
Another approach was to reduce the wool, either with sodium borohydride, or THPC **[134,135]**, or tri-n-butyl phosphine **[136,138]**, and then to crosslink with α , ω-dibromoalkanes **[139]**, or to block the resulting thiol groups with a reagent such as iodoacetate **[140]**:



None of these reactions have given rise to any improvement in lightfastness, but may well have use in the inhibition of phototendering.

(iii) Acetylation Wool has been treated with acetic anhydride, so as to acetylate tyrosylhydroxyl groups and lysyl amino groups [141]:

eg.



This was not found to protect wool against yellowing.

In summary, it appears that unless a treatment can be found which reacts with all amino acids involved in yellowing and modifies them to an extent such that they can no longer take part in the process, that this line of approach is unlikely to be successful in solving the photoyellowing problem. A useful survey entitled "The Chemical Reactivity and Modification of Keratin Fibres" was published by Leon in 1975 [142].

1.7.7. Impregnation with Potential Stabilisers

It is possible that a wide range of known compounds might have a protective effect on wool. The examination of substances in this manner dictates a pragmatic approach, rather than the calculated selection of potential stabilisers, and hence workers in this field have used very large numbers of test materials.

Kirkpatrick and Maclaren [143] surveyed 270 varied reagents for inhibitors of sunlight yellowing. All substances were padded onto the fabric and dried in air. After photoyellowing tests, 16 showed marginal protection against yellowing.

During the search for other substances, Tucker found that some organic sulphur-containing compounds conferred good protection [107]. In consequence, 31 such compounds were tested, again application was by the pad/dry technique. Generally, it was found that compounds containing sulphur in higher states of oxidation such as disulphide, sulphoxide, sulphone and sulphonic acid gave no protective effect against yellowing at all, the exception being thiourea dioxide which did give some improvement. Those compounds containing both free -COOH and free -SH groups offered the best protection, eg. (thioglycollic acid, $HSCH_2COOH$).

Hence, the results to date have identified only those compounds already known to be stabilisers for wool, as having any real promise; and only a handful of others as offering lower levels of protection. This work has at least eliminated many possible compounds, and provided information for further studies.

1.7.8. FWA/Polymer Treatments

The normal methods of application of a fluorescent whitening agent to wool result in the FWA being uniformly distributed throughout the fibre, and much of the whitener covalently bonded to the keratin. It has been established that the main reason for fluorescently whitened wool photoyellowing so much faster than untreated wool lies in the transfer of energy from the brightener either to the wool or the ground state oxygen, forming singlet oxygen within the fibre. It was suggested, therefore, that the yellowing of fluorescently whitened wool could be reduced by the application of the FWA to the wool surface in a polymer film [144-146]. This is known as "Surface Whitening". This method insulates the whitener from the wool and consequently avoids the sensitisation of yellowing that occurs in conventionally whitened wool.

Many whitener/polymer systems were found to lead to good whiteneing [144], though few provide white wool with a satisfactory stability to light, and few are as white as that obtained by conventional methods of application. The photostability of the surface-whitened fabric is influenced by the nature of the polymer as well as the FWA [144,145]. The systems with the best light-fastness give fabrics which yellow less rapidly in simulated sunlight

than conventionally whitened materials, the difference being very pronounced for exposure in the wet state. The whitening effect is generally found to be stable to washing, but not to dry-cleaning, though stability to dry-cleaning can be achieved by using polymers which can be cross-linked after application, the FWA being bound covalently to the polymer.

These FWA/polymer systems have the drawbacks that they confer an unacceptably harsh handle to the fibre, and whiten wool less effectively than conventional methods. This method may have a commercial prospect if the FWA can be incorporated into a shrink-resist polymer already in use, with acceptable handle and light-fastness properties.

1.8. Conclusions

This survey of the literature is by no means comprehensive due to the vast volume of work done in this area of wool chemistry over the past half century. Where relevant, introductions to specific fields described in this thesis will be given in the appropriate chapters.

It therefore suffices to observe that the photoyellowing problem is far from solved. Due to its intrinsic complexity, it is unlikely that any single treatment will completely inhibit yellowing, and it is even less likely that if such a treatment were found, it could be commercially viable. However, it is possible that a compromise will be achieved - a treatment that significantly retards photodegradation, and at the same time is of industrial use.

REFERENCES

1. J.B.Speakman, M.C. Hirst, Trans. Faraday Soc., 1933, 29, 148.
2. J.S.Ingham, J.Soc.Dyer.Col., 1954, 70, 227.
3. D.R.Graham, K.W.Statham, J.Soc.Dyer.Col., 1956, 72, 434.
4. C.H.Nicholls, M.T.Pailthorpe, J.Text.Inst., 1976, 67, 397.
5. F.G.Lennox, J.Text.Inst., 1960, 51, T1193.
6. H.F.Launer, Text.Res.J., 1963, 33, 258.
7. H.F.Launer, Text.Res.J., 1963, 33, 910.
8. F.G.Lennox, M.G.King, I.H.Leaver, G.C.Ramsay, W.E.Savige, Appl. Polym. Symp., 1971, No.18, 353.
9. B.Milligan, D.J.Tucker. Text. Res. J., 1962, 32, 634.
10. I.H.Leaver, G.C.Ramsay, Text.Res.J., 1969, 39, 730.
11. K.Gupta, B.K.Patnaik, V.B.Chipalkatti, Text.Res.J., 1968, 38, 107.
12. J.Cegarra, J.Gacen, Wool Sci. Rev., 1983, No.59, 1.
13. Anon., Wool Sci. Rev., 1966, No.30, 16
14. Anon., Wool Sci. Rev., 1967, No.31, 1.
15. H.Gold, The Chemistry of Synthetic Dyes Ed. K.Kenkatarman, Academic Press, New York, 1971, 5, 536.
16. A.K.Sarkar, Fluorescent Whitening Agents, Merrow Publ., Watford (UK), 1971.
17. H.F.Launer, Text Res.J., 1965, 35, 395.
18. L.A.Holt, A.S.Inglis, F.G.Lennox, Text.Res.J., 1966, 36 837.
19. I.H.Leaver, G.C.Ramsay, Text.Res.J., 1969, 39, 722.
20. E.M.Lock, G.C.Frank, J.R.Stevens, Text.Res.J., 1973, 43, 483.
21. M.J.Wall, G.C.Frank, Text.Res.J., 1971, 41, 38.
22. G.P.Norton, C.H.Nicholls, J.Text.Inst., 1960, 51, T1183.
23. P.Ponchel, M.Nguyen, Bull.Scient.I.T.F., 1984, 13, No.49.
24. J.Cegarra, J.Ribe, D.Vidal, J.F.Fernandez, J.Text.Inst., 1976, 67, 5.
25. K.McLaren, B.Rigg, J.Soc.Dyer.Col., 1976, 92, 337.
26. R.Meybeck, J.Meybeck, Photochem. Photobiol., 1967, 6, 355.
27. L.A.Holt, B.Milligan, Text.Res.J. 1977, 47, 620.
28. J.L.Hoare, J.Text.Inst., 1974, 65, 503.
29. L.A.Holt, B.Milligan, Aust.J.Biol.Sci., 1973, 26, 871.
30. A.S.Inglis, I.H.Leaver, F.G.Lennox, Proc.Int.Wool Text.Res.Conf., Paris, 1965, II, 121.
31. K.P.Ghiggino, Ph.D. Thesis, University of New South Wales, 1976.

32. F.G.Lennox, R.J.Rowlands, Photochem. Photobiol, 1969, 9, 359.
33. L.A.Holt, B.Milligan, J.Text.Inst., 1976, 67, 269.
34. L.A.Holt, B.Milligan, D.E.Rivett, F.H.C. Stewart, Biochim.Biophys.Acta, 1977, 499, 131.
35. L.A.Holt, B.Milligan, W.E.Savige, J.Text.Inst., 1977, 68, 124.
36. B.Milligan, L.A.Holt, Aust.J.Chem., 1974, 27, 195.
37. N.A.Evans, D.E.Rivett, P.J.Waters, Text.Res.J., 1976, 46, 214.
38. I.H.Leaver, Aust.J.Chem., 1977, 30, 87.
39. I.H.Leaver, Text.Res.J., 1968, 38, 729.
40. L.A.Holt, B.Milligan, Aust.J.Biol.Sci., 1974, 27, 23.
41. I.H.Leaver, G.C.Ramsay, Photochem.Photobiol., 1969, 9, 359.
42. L.A.Holt, B.Milligan, L.J.Wolfram, Text.Res.J., 1974, 44, 846.
43. I.H.Leaver, Photochem.Photobiol., 1978, 27, 451.
44. E.G.Bendit, J.Text.Inst., 1960, 51, 544.
45. J.I.Dunlop, C.H.Nicholls, Nature, 1965, 205, 1310.
46. S.V.Koner, Fluorescence & Phosphorescence of Proteins & Nucleic Acids., Plenum Press, New York, 1967.
47. G.M.Bhatnagar, L.C.Gruen, Aust.J.Biol.Sci., 1968, 21, 1325.
48. F.W.S.Teale, Biochem.J., 1960, 76, 381.
49. J.W.Longworth, Excited States of Proteins & Nucleic Acids, Plenum Press, New York, 1971.
50. F.W.S.Teale, G.Weber, Biochem.J., 1957, 65, 476.
51. K.P.Ghiggino, C.H.Nicholls, M.T.Pailthorpe, J.Photochem., 1975, 4, 155.
52. I.H.Leaver, Photochem.Photobiol., 1978, 27, 439.
53. A.S.Inglis, F.G.Lennox, Text.Res.J., 1963, 33, 431.
54. J.A.Maclaren, Text.Res.J., 1963, 33, 773.
55. A.S.Inglis, F.G.Lennox, Text.Res.J., 1965, 35, 104.
56. L.A.Holt, B.Milligan, D.E.Rivett, Biochem., 1971, 26, 3559.
57. L.A.Holt, A.S.Inglis, F.G.Lennox, Text.Res.J., 1967, 37, 490.
58. D.Hildebrand, Zeitschrift ges.Textilindustries, 1959, 5, 180.
59. R.S.Asquith, L.Hirst, D.E.Rivett, Appl.Polym.Symp., No.18, 1971, 33.
60. R.Nilsson, P.P.Merkel, D.P.Kearns, Photochem.Photobiol., 1972, 16, 177.
61. M.T.Pailthorpe, J.P.Bonjour, C.H.Nicholls, Photochem.Photobiol., 1973, 17, 209.

62. A.R.Deschreider, M.Renard., Bull.Inst. Agron.Sta.Rech.Gemblouse Hours Ser., 1955, 23, 269.
63. R.S.Asquith, D.E.Rivett, Biochim.Biophys.Acta. 1971, 252, 111.
64. G.Matsuda, Nagusoki Igakkai Zasshi, 1953, 28, 438.
65. M.Sun, S.Zigmann, Photochem. Photobiol., 1979, 29, 893.
66. I.H.Leaver, F.G.Lennox, Photochem.Photobiol., 1965, 4, 491.
67. M.Nakagawa, H.Watanabe, S.Kodato, H.Okajima, T.Hino, J.L.Flippen, B.Witkop, Proc.Natl.Acad.Sci., 1977, 74, 4730.
68. W.E.Savige, Aust.J.Chem., 1971, 24, 1285.
69. W.E.Savige, Proc.Int.Wool Text.Res.Conf.Aachen, 1975, II, 570.
70. B.Milligan, Proc.Int.Wool Text. Res.Conf.Pretoria, 1980, V, 167.
71. C.H.Nicholls, Developments in Polymer Photochemistry Appl.Sci.Publ., 1980, 125.
72. Y.Kunita, W.Gordy, J.Chem.Phys., 1961, 34, 282.
73. P.Douzon, M.Ptak, L.Ropars, Nature, 1963, 197, 1105.
74. J.I.Dunlop, C.H.Nicholls, Photochem Photobiol., 1965, 4, 881.
75. J.J.Windle, Text.Res.J., 1962, 32, 963.
76. R.S.Asquith, D.E.Rivett, Text.Res.J., 1969, 39, 633.
77. R.S.Asquith, L.Hirst, D.E.Rivett, Text.Res.J., 1970, 40, 285.
78. G.M.Bhatnagar, L.C.Gruen, Text.Res.J., 1969, 39, 938.
79. I.H.Leaver, Photochem.Photobiol., 1975, 21, 197.
80. I.B.C. Matheson, J.Lee, Photochem.Photobiol., 1979, 29, 879.
81. I.H.Leaver, Text.Res.J., 1978, 48, 610.
82. H.F.Launer, Text.Res.J., 1965, 35, 813.
83. H.F.Launer, Text.Res.J., 1966, 36, 606.
84. F.G.Lennox, A.S.Inglis, L.A.Holt, Text.Res.J., 1966, 36, 837.
85. F.G.Lennox, M.G.King, Text.Res.J., 1968, 38, 754.
86. H.F.Launer, Text.Res.J., 1971, 41, 211.
87. H.F.Launer, Text.Res.J., 1971, 41, 311.
88. D.M.Lewis, I.W.S.Product Development Report 156., 1973.
89. Wool Science Review., 1970, No.39, 27.
90. M.G.King, J.Text.Inst., 1971, 62, 251.
91. B.Ranby, J.F.Rabek, Photodegradation, Photooxiation & Photostabilisation of Polymers Wiley, New York, 1975.
92. N.S.Allen, J.F.McKellar, Macromol.Rev., 1978, 13, 241.
93. W.G.Rose, M.K.Walden, J.E.Moore, Text.Res.J., 1961, 31, 495.
94. J.Cegarra, J.Ribe, P.Miro, J.Soc.Dyer.Col., 1972, 88, 293.

95. P.J.Waters, N.A.Evans, Text.Res.J., 1978, 48, 251.
96. I.H.Leaver, P.J.Waters, N.A.Evans, J.Polym.Sci.Polym.Chem., 1979, 17, 1531.
97. P.J.Waters, N.A.Evans, L.A.Holt, B.Milligan, Proc.Int.Wool Text. Res. Conf. Pretoria, 1980, V, 195.
98. N.A.Evans, P.J.Waters, Text.Res.J., 1981, 51, 432.
99. N.A.Evans, I.H.Leaver, J.Rosevear, P.J.Waters, J.F.K.Wilshire, Aust. Patent Application PF7151/82, 1982.
100. A.B.Abdullah, L.W.C.Miles, Text.Res.J., 1984, 54, 415.
101. B.Milligan, L.A.Holt, Polym.Degr.Stab., 1983, 5, 339
102. B.Milligan, L.A.Holt, Polym.Degr.Stab., In Press.
103. W.S.Simpson, C.T.Page, Proc. Int. Wool Text.Res. Conf., Pretoria, 1980, V, 183.
104. M.R.Eftink, G.A.Ghiron, J.Phys.Chem., 1976, 80, 486.
105. C.H.Nicholls, Unpublished work.
106. N.S.Allen, Polymer.Photochem., 1981, 1, 243.
107. J.E.Tucker, Text.Res.J., 1969, 39, 830.
108. J.L.Hoare, R.G.Steward, Text.Res.J., 1969, 39, 884.
109. L.J.Wolfram, I.W.S. Product Development Report 159, 1973.
110. I.H.Leaver, G.C.Ramsay, Photochem. Photobiol., 1969, 9, 531.
111. T.-Y. Liu, Y.H.Chang, J.Biol.Chem., 1971, 246, 2842.
112. B.Milligan, D.J.Tucker, Text.Res.J., 1964, 34, 681.
113. G.N.Evans, B.Milligan, Text.Res.J., 1966, 36, 927.
114. W.E.Savige, Text.Res.J., 1968, 38, 101.
115. L.C.Gruen, A.S.Inglis, Text.Res.J., 1968, 38, 1062.
116. R.W.Cowgill, Arch.Biochem Biophys., 1963, 100, 36.
117. N.Nakajo, Japanese Patent 1197, March 5th, 1951, (Chem.Abstr., 1952, 46, 11700).
118. J.B.Caldwell, B.Milligan, Text.Res.J., 1970, 40, 194.
119. P.Alexander, M.Fox, Proc.Int.Wool Text.Res.Conf.Australia, 1955, c, 35.
120. P.Alexander, D.Carter, C.Earland, J.Soc.Dyer.Col., 1951, 67, 23.
121. W.G.Crewther, L.M.Dowling Proc.Int.Text.Res.Conf.Paris, 1965, II, 431.
122. L.M.Dowling, W.G.Crewther, Text.Res.J., 1964, 34, 1109.
123. K.Ziegler, Proc.Int.Wool Text.Res.Conf.Paris, 1965, II, 403.
124. W.G.Crewther, L.M.Dowling, A.S.Inglis, J.A.Maclaren, Text.Res.J., 1967, 37, 736.

125. R.S.Asquith, P.Miro, J.J.G.Domingues, Text.Res.J., 1968, 38, 1057.
126. A.Kirkpatrick, J.A.Maclaren, Text.Res.J., 1964, 34, 1082.
127. R.N.Reddie, C.H.Nicholls, Text.Res.J., 1971, 41, 841.
128. R.N.Reddie, C.H.Nicholls, Text.Res.J., 1971, 41. 303.
129. R.N.Reddie, C.H.Nicholls, Die Makromolekulare Chemie, 1972, 153, 153.
130. R.N.Reddie, C.H.Nicholls, Text. Res.J., 1975, 45, 805.
131. I.C.Watt, R.Morris, Text.Res.J., 1970, 40, 952.
132. R.S.Asquith, A.K.Puri, Text.Res.J., 1969, 39, 95.
133. R.S.Asquith, A.K.Puri, Text.Res.J., 1970, 40, 273.
134. L.S.Bajpoi, C.S.Whewell, J.M.Woodhouse, Nature, 1960, 187, 602.
135. A.D.Jenkins, L.J.Wolfram, J.Soc.Dyer.Col., 1963, 79, 55.
136. J.Maclaren, Text.Res.J. 1971, 41, 713.
137. H.Lindley, R.W.Cranston, Biochem.J., 1974, 139, 515.
138. M.Friedman, A.T.Noma, Text.Res.J., 1970, 40, 1073.
139. A.E.Brown, M.Harris, Ind.Eng.Chem., 1948, 40, 316.
140. D.R.Goddard, L.Michaelis, J.Biol.Chem., 1934, 106, 605.
141. B.Milligan, L.J.Wolfram, J.Text.Inst., 1973, 64, 170.
142. N.H.Leon, Text. Progress, 1975, No.1, 1.
143. A.Kirkpatrick, J.A.Maclaren, Text.Res.J., 1967, 37, 510.
144. L.A.Holt, B.Milligan, L.H.Wolfram, Text.Res.J., 1975, 45, 257.
145. B.Milligan, L.A.Holt, I.H.Leaver, Proc.Int.Wool Text.Res.Conf.Aachen, 1975, III, 607.
146. L.A.Holt, I.H.Leaver, B.Milligan, Text.Res.J., 1976, 46, 539.

CHAPTER 2

A Treatment for Stabilizing Wool

CHAPTER 2

A TREATMENT FOR STABILIZING WOOL

A STABILISING TREATMENT FOR WOOL

2.1 Introduction

As long ago as 1956 it was known [1] that the irradiation of wool treated with a reducing agent gave rise to less yellowing than when untreated wool was irradiated. At that time it had been found that when sodium bisulphite was applied either during or after the application of a fluorescent whitening agent, an improvement in the light fastness was observed. It was not until 1974, however, that wool was irradiated in solutions containing reducing agents [2]. It was then found that if fluorescently whitened wool was immersed in aqueous solutions of certain reducing agents during exposure to simulated sunlight, photoyellowing was greatly retarded, and in some cases prevented altogether. The reducing agents tested included sodium sulphite, tetrakis-hydroxymethane-phosphonium-chloride (T.H.P.C.), sodium borohydride, thioglycollic acid and a sulphonated triphenyl phosphine. Of these, THPC, thioglycollic acid and sodium sulphite were the most effective, the whiteness of the wool actually being increased by the treatment. Amino acid analyses showed that the reducing agents protected the amino acids tryptophan and histidine from decomposition in both whitened and unwhitened wool, and this was suggested as the method by which yellowing was inhibited. The same workers examined the effect of reducing agents upon the photodecomposition of a fluorescent whitening agent. A stilbene whitener was used, and the results showed that there was very little effect on either the extent or the pathway of photodegradation.

Impregnation of wool with reducing agents is very much less effective than irradiation in reducing agent solutions for reducing yellowing. The effect is fair for wet-state irradiation, but poor for dry exposure. After washing, almost all protection is lost.

Thus it was concluded that the protective effect of reducing agents was probably mainly due to their ability to inhibit photodecomposition of amino acids. An alternative suggestion was that the course of the photodegradation pathway is changed, so that colourless rather than yellow products result.

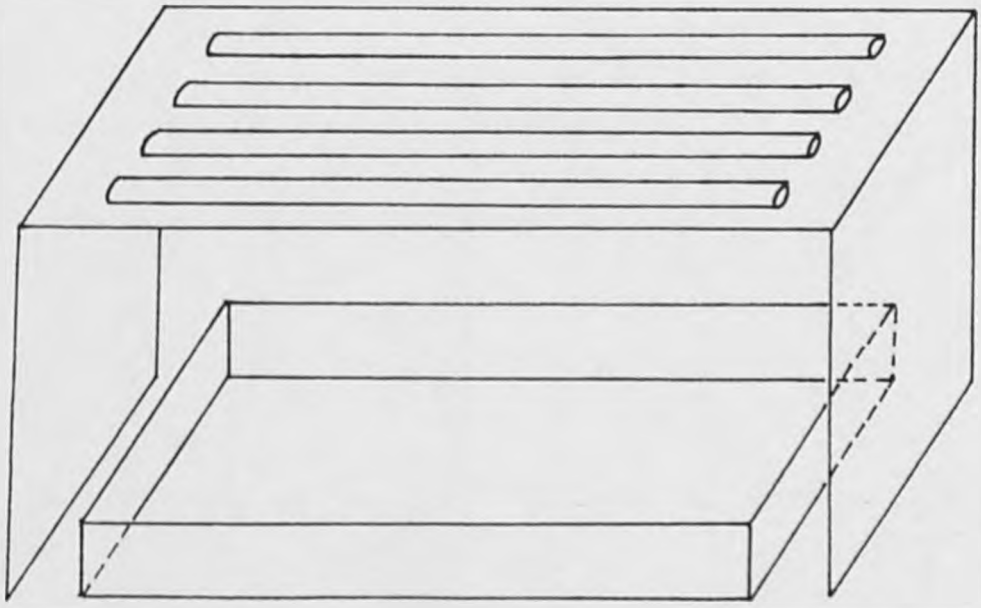
2.2 Experimental Methods Used

2.2.1 Method for Irradiation of Wool Fabric

The wool fabric used was Salts serge, buffered to pH7 by immersion in sodium acetate solution (0.5M) overnight, followed by rinsing in cold tap water. Samples (10g) approximately 15cm x 15cm, were immersed in 2 litres of aqueous solution contained in a flat, open stainless steel tray 60cm x 20cm x 5cm, constructed at the City University. The wool was covered by 2cm of solution during the irradiation period.

The light source consisted of 4 x 15W 350nm Thorn fluorescent tubes, mounted on a metal frame 15cm above the surface of the solution, with open sides to allow an air flow to cool the lamps. See Fig.2.1.

Fig 2.1 Photoyellowing Apparatus



Wool samples being treated were allowed to fully "wet out" before irradiation was commenced.

A somewhat similar system was used to measure the photostability of the samples before and after treatment. Strips of wool 15cm x 5cm were supported on glass plates of similar dimensions. One half of the sample was covered with aluminium foil, and the other half irradiated for 24 hours under distilled water. The fabric was then dried in air, and the Yellowness Index value determined.

Yellowness indices were measured using a Zeiss RFC3 reflectance spectrophotometer. Readings were taken at 20nm intervals with barium sulphate as a reference. A Xenon lamp was used as the light source. The instrument was coupled to a computer programmed to calculate Yellowness Index **[3]** and Whiteness Index **[4,6]** values (ASTM D1925), using the formulae given below:-

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

$$W = [(100-Y)^2 + K(X-Z)^2]^{\frac{1}{2}} \quad \text{Where } k=5.5$$

It should be noted that the different wool batches used showed some colour variations. The Y.I. of the untreated wool used in an experiment was therefore always measured as a control.

2.2.2 Preparation of Oxidatively Bleached Wool

The bleaching agent was hydrogen peroxide. This method was used to prepare all samples of peroxide bleached wool used throughout this work.

1) Weighed out 5g dm^{-3} Tubotex PC Dissolved and diluted to approximately half the desired volume.

2) Added hydrogen peroxide to make overall concentration up to 2.8 vol. ie. diluted 100 vol. H_2O_2 by 1:40.

eg. 7.5ml of 100 vol. in 300ml
20.0ml of 100 vol. in 800ml

3) Adjusted to pH8, using formic or acetic ačid.

4) Heated to 40°C in a Jeffreys Dyemaster for 5 hours.

NOTE:

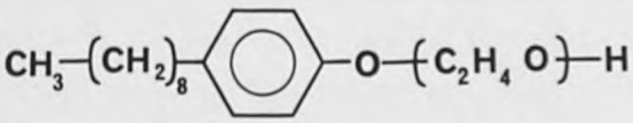
a) Use of higher pH or higher temperature results in serious fibre damage.

b) Used liquor to wool ratio of 30:1

c) Pre-wetted the wool with Lissapol N and rinsed thoroughly.

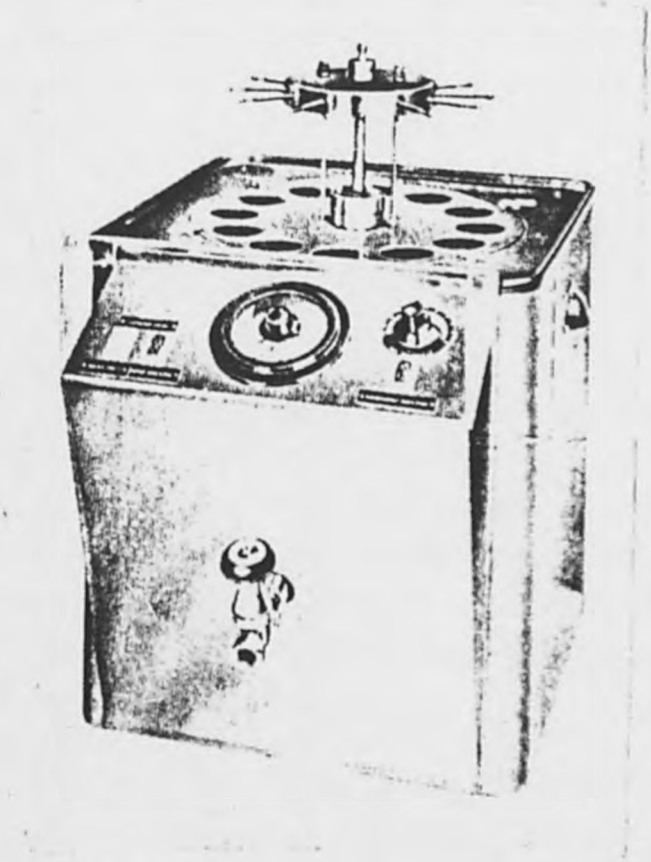
Tubotex PC is a silicate stabilizer, manufactured by CHT (Tubingen) to replace the older phosphate stabilizer Tubotex C [5]. Tubotex PC is a blend of sodium oxide, silica, alkalis and sequestering and wetting agents [6].

The wetting agent, Lissapol N, 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
Lissapol NX - 40% Active

The reaction is carried out in a Jeffreys Dyemaster. This consists of a cylindrical tank which serves as a water bath, and is thermostatically controlled. For temperatures higher than 100°C, ethylene glycol is used. A top-plate is fitted, with a circular array of round holes to allow tubes of reactants 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 speed of 30 or 60 cycles per minute, thus agitating the fabric and the reactants very effectively. The rate of increase in temperature can also be preset, 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 peroxide bleach is carried out in alkali because it is the peroxide anion which is the actual bleaching agent:

$$H_2O_2 \overset{H_2O}{\rightleftharpoons} H_3O^+ + {}^-OOH$$

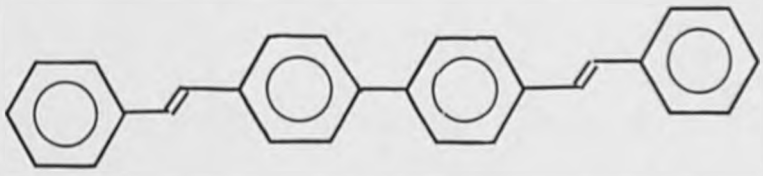
At the same time, wool is hydrolysed by alkali, and to prevent excessive fibre damage, pH8 must not be exceeded. Wool bleached in this manner usually gains a good degree of whiteness, but photoyellows approximately four times faster than untreated wool.

During peroxide oxidation of wool the disulphide bonds in cystine residues are oxidized to form cysteic acid residues. This decrease in cross-linking is accompanied by a decrease in the sulphur content, a shortening of the peptide chains, and a variation in the acid-base properties of the fibre.

2.2.3. Preparation of Fluorescently Whitened Wool

A fluorescent whiteneing agent is a colourless compound which absorbs near U.V. light (typically with maximum between 340-380nm) and fluoresces in the blue region of the spectrum. Wool treated with such a compound is very much whiter even than bleached wool, these substances being used to achieve the "whiter than white" effects claimed by soap powder manufacturers. Unfortunately, the application of an FWA to wool decimates the photostability, and the resultant propensity to yellowing severly restricts their commercial use with wool.

For much of this work, one particular FWA has been chosen because of the excellent whiteness which it imparts to wool. This is Uvitex NFW, manufactured by Ciba-Geigy. The exact structure of this compound is not available, but it is known to be a bis-Styryl biphenyl derivative:



It is likely to be a disulphonic acid derivative, for reasons of water solubility and reactivity towards wool. The following method has been used throughout this work for the preparation of fluorescently whitened wool, regardless of FWA used, unless stated to the contrary.

1) Weighed out 6gdm^{-3} Blankit 1N. (Sodium dithionite + 1% EDTA as stabilizer. Manufactured by BASF). Dissolved in water and made up to half the final volume.

2) Added citric acid, 1% owf. (on weight of fibre). ie. 1g per 100g wool treated.

3) Added Uvitex NFW, 1% owf. (A 1% solution was made up from the concentrate supplied by the manufacturer. For χg of wool, χml of 1% solution was used).

4) Added a few drops of Lissapol N.

5) Made up to final volume, and heated to 80°C in Jeffreys Dyemaster. Maintained this temperature for 1 hour.

6) Rinsed wool in cold water, and dried in air at room temperature.

The presence of Lissapol N was essential if an even application was to be achieved.

2.2.4. Irradiation of Wool in Reducing Agent Solutions

A reducing agent known to react with more than one site in the fibre is sodium borohydride **[7,8]**. Initially, wool was irradiated in a 5% aqueous solution, but extensive fibre damage was incurred due to disulphide and peptide bond cleavage. It was found that the tensile strength of the fabric could be partially restored by an after-treatment with 20% formaldehyde solution at 50°C for 2 hours reforming the inter-fibre cross links **[9,10]**:

$$2R\text{-}SH + HCHO \longrightarrow R\text{-}S\text{-}CH_2\text{-}S\text{-}R + H_2O$$

RSH=cystyl residue Ljenkolic Acid

The resultant wool, however, was found to have a "boardy" finish, rendering the treatment unacceptable. The borohydride concentration was therefore reduced to 2% w/v, continuing to subsequently treat with formaldehyde. This gave a marked increase in whiteness to untreated wool, without the usual loss of photostability observed for bleached wool.

TABLE 2.1 Effect of Irradiation of Wool in the Presence of Reducing Agents

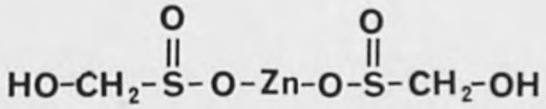
WOOL SAMPLE	Yellowness Index of Wool Irradiated for 24 hours at 350nm in the Presence of Reducing Agents					
	BLANK	Sodium Borohydride 2%	Sodium Borohydride 0.5%	Sodium Dithionite 2%	T.H.P.C. 2°C	Tri-n-butyl Phosphine 2%
Untreated Wool	28.5	20.3	26.8	28.8	23.2	28.0
Photoyellowed	32.6	23.3	30.7	32.7	26.7	32.7
Δ Y.I.	4.1	3.0	3.9	3.9	3.5	4.7
Peroxide Bleached Wool	16.8	14.5	16.5	14.9	14.8	16.7
Photoyellowed	32.0	27.5	31.6	30.6	28.9	31.5
Δ Y.I.	15.2	13.0	15.1	15.7	14.1	14.8
Optically Brightened Wool	0.2	-0.5	-0.1	0.1	-0.8	0.0
Photoyellowed	31.1	24.3	29.1	33.4	27.0	30.8
Δ Y.I.	30.9	24.8	29.2	33.3	26.2	30.8

The effect was not so great for bleached or optically brightened wool. See Table 2.1.

When the borohydride concentration was reduced to 0.5% w/v., the effect was almost lost. Other reducing agents were also assessed in the same manner, but with very little protective effect. In the case of sodium dithionite, no yellowing was observed during exposure to the reducing agent, but no protection was conferred against subsequent irradiation. This was probably due simply to U.V. screening by the dithionite solution (U.V. cut off 390nm), and hence no reduction of oxidation products is likely to have occurred.

2.3 Blankit D

Having achieved a moderate stabilization effect with sodium borohydride, but very little with other reducing agents tested, attention was centred upon Blankit D, manufactured by BASF* [11]. Blankit D consists of 80% zinc formaldehyde sulphoxylate and 20% stabilizers and complexing agents [12].



It is also known as zinc hydroxymethane sulphinate. Blankit D and sodium dithionite are effective as reductive bleaching agents, Blankit D being rather more stable to low pH and higher temperatures, [13]. It is soluble in water up to 350g/l at 25°C, forming a solution of pH4, with a U.V. cut-off at 255nm.

* Badische Anilin - & Soda-Fabrik Aktiengesellschaft

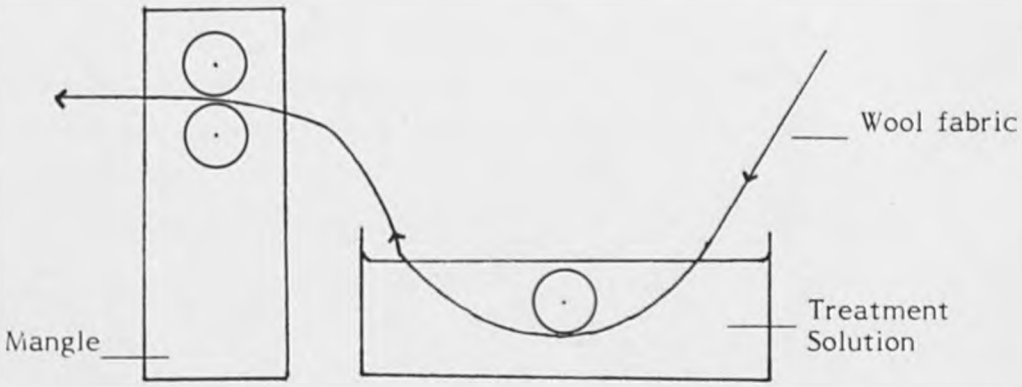
2.3.1 Treatment of Wool with Blankit D

Wool was irradiated in a 2% w/v (ie. 20g dm^{-3}) solution of Blankit D, as described for other reducing agents. It was found that a good degree of whiteness was obtained, equivalent to that achieved with a peroxide bleach, without apparent loss in photostability, as measured by change in Y.I. after subsequent 24 hours yellowing. Higher concentrations of reducing agent were examined, up to 10%, but the increases in whiteness were very small, and large increases in concentration were required to obtain them. For this reason, it was considered unnecessary to exceed a concentration of 20g dm^{-3}. The results are shown in Table 2.2.

Table 2.2 Irradiation of Untreated Wool in the Presence of Aqueous Solutions of Blankit D

BLANKIT D CONCENTRATION	INITIAL Y.I.	Y.I. AFTER PHOTOYELLOWING	Δ Y.I.
0	30.2	35.4	5.20
20g/l	16.3	20.9	4.6
40g/l	15.8	20.7	4.9
60g/l	14.7	18.9	4.2
100g/l	13.7	16.9	3.20

Fig 2.2 Schematic Representation of the Padding Process



The apparatus above 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; ie. for $\acute{x}$g of wool, xg of liquor is absorbed. In this way, if a 2% solution is used, the uptake of Blankit D is known to be 2% owf. A wetting agent, such as Lissapol N, is always included in aqueous solutions to facilitate sufficiently rapid absorption by the fabric as it passes through. The wool travels at a constant speed, dictated by the preselected speed of the rollers, and hence a very even treatment may be obtained by this method. 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 in this state overnight. When the fabric is removed, it is rinsed in cold water to remove excess reagent, and dried in air at room temperature. The combined process is the "pad/batch" treatment.

To determine whether these results were due solely to the bleaching properties of Blankit D, wool fabric was treated with a range of concentrations by the pad/batch method. This involved drawing the wool a solution of Blankit D, and passing it through a mangle to remove excess liquid. See Figure 2.2. The yellowness indices were again measured after treatment with Blankit D, and after a subsequent photoyellowing test (as described in section 2.2.1). The change in yellowness index during the photoyellowing step (Δ Y.I.) was taken as a measure of photostability. The results of the pad/batch treatments are shown in Table 2.3.

Table 2.3 Treatment of Wool with Blankit D by the pad/batch Method

BLANKIT D CONCENTRATION	INITIAL Y.I.	Y.I. AFTER PHOTOYELLOWING	Δ Y.I.
0	30.2	35.4	5.20
20g/l	23.4	30.1	6.7
40	23.2	28.9	5.7
60	21.1	27.6	6.5
100	20.2	25.7	5.5

These clearly show that although wool is whitened by the pad/batch treatment, the bleaching effect is not of the same order as obtained with the samples irradiated under aqueous solution. Neither is there any increase in photostability. From this it is concluded that the irradiation step is essential to achieve the high degree of bleaching observed.

For wool bleached chemically with alkaline peroxide, the effect of this treatment is very much reduced. If urea (10% w/v) is added to the Blankit D solution to denature and swell the fibre, an increase in the protective effect is observed, but the overall change in stability and whiteness is small.

Fluorescently whitened wool does not show much change in whiteness during irradiation in Blankit D solutions. Some wool samples appeared slightly whiter, others slightly yellower after the treatment, the differences being due either to variations in the level of whitener applied or to differences in the wool itself, from one batch to another. However, a 25% improvement in photostability is consistently observed. If urea is included in the solution,

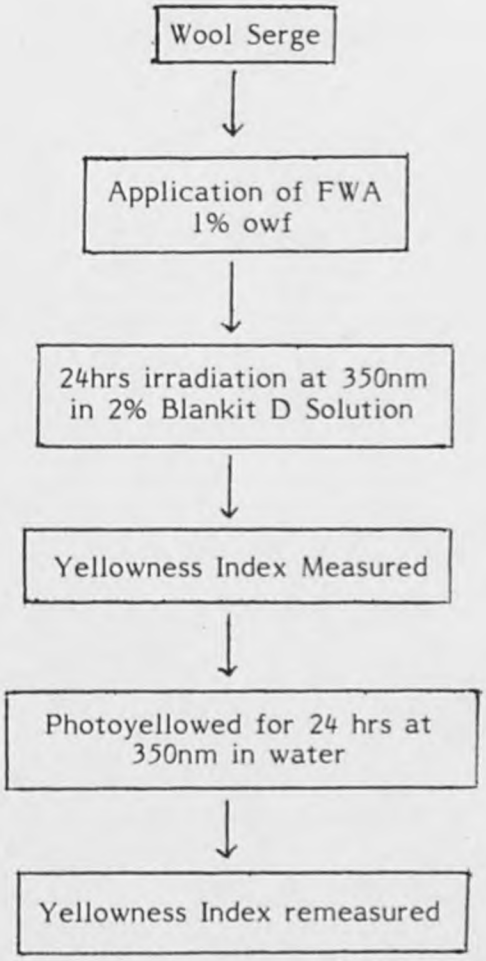
the whiteness becomes slightly worse than the control sample, but the photostability is improved by 40%. These results are given in Table 2.4.

Table 2.4 Comparison of the Effects of Irradiation in Blankit D Solution upon (i) Untreated (ii) Bleached and (iii) Fluorescently Whitened Wool

WOOL SAMPLE	Y.I.	Y.I. AFTER SUBSEQUENT PHOTOYELLOWING TEST	Δ Y.I.
(i) Untreated Wool	29.9	34.2	4.3
Untreated Wool, Irradiated in 2% Blankit D Solution	14.6	20.4	5.8
Untreated Wool, Irradiated in 2% Blankit D Solution Containing 100g/litre Urea	12.6	16.5	3.9
(ii) Peroxide Bleached Wool	14.2	27.8	13.6
Bleached Wool, Irradiated in 2% Blankit D Solution	14.9	27.9	13.0
Bleached Wool, Irradiated in 2% Blankit D Solution Containing 100g/litre Urea	11.8	23.1	11.3
(iii) Fluorescently Whitened Wool (Uvitex NFW)	4.2	28.1	23.9
Fluorescently Whitened Wool Irradiated in 2% Blankit D Solution	5.4	23.5	18.1
Fluorescently Whitened Wool Irradiated in 2% Blankit D Solution Containing 100g/litre Urea	7.8	22.2	14.4

The sequence of wool treatments used to obtain the above results was as shown in Fig.2.3.

Fig 2.3 Treatment of Wool with Blankit D



Steps 1 and 2 were now reversed, to assess whether the effect of the Blankit D treatment was maintained after application of an FWA. A sample of fluorescently whitened wool was photoyellowed as a control, the results being shown in Table 2.5.

Table 2.5 The Effect of Irradiation in Blankit D Solution Prior to FWA Application

SAMPLE	Y.I.AFTER FWA APPLIED	Y.I. AFTER PHOTOYELLOWING	Δ Y.I.
Untreated Serge	6.5	33.6	27.1
Serge Irradiated in 2% Blankit D for 24 Hours	4.2	34.9	30.7

From these it can be seen that the pre-treatment with Blankit D yields a slightly whiter wool, once the brightener has been applied, but no increase in photostability ensues. Thus it is concluded that for the protective treatment to be effective, it must take place subsequent to the whitening process.

2.3.2 Discussion

It has been observed that the irradiation of untreated wool in 2% Blankit D solution causes the wool to be whitened and stabilized to further photo-oxidation. This effect has been shown to be very small for peroxide bleached wool, but of significant magnitude for fluorescently whitened wool. It is therefore necessary to establish the nature of the reactions occurring, and the mechanism by which these inhibit photoyellowing.

Five possible reaction pathways were envisaged :-

(i) UV screening, preventing damaging radiation reaching the fibre.

(ii) Promotion of photobleaching process.

(iii) Interaction with excited states in the fibre.

(iv) Oxygen scavenging, removing an essential reactant for photooxidation.

(v) Inhibition of photoyellowing process, possibly by reaction with species responsible for yellowing.

Any one or more of the above pathways could be responsible for the observed results. Further experiments were therefore carried out to verify these possibilities.

2.4 Investigation of the Interaction of Blankit D with Wool

2.4.1 U.V. Screening

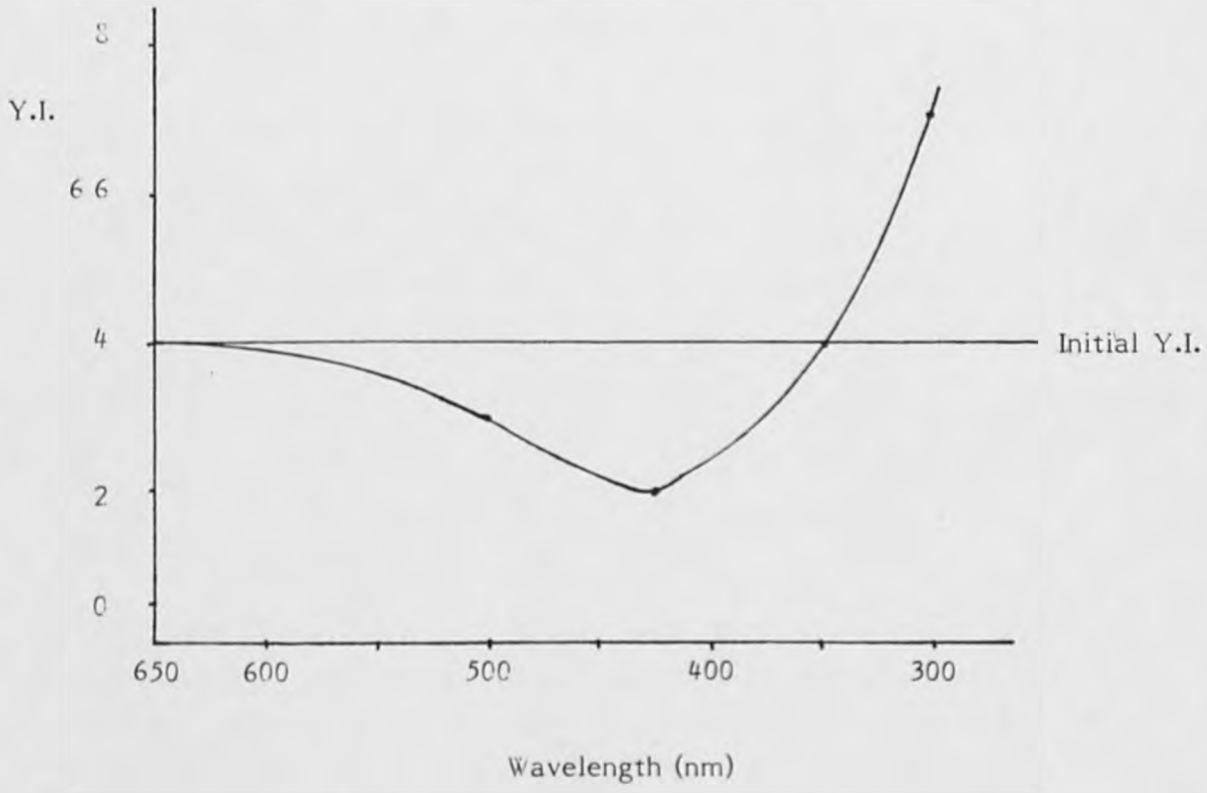
The suggestion that Blankit D functioned as a U.V. screen to the wool in solution, and to a lesser extent on the fibre during subsequent irradiations, is reasonable, because by removing the U.V. component of the incident light responsible for photoyellowing, the photobleaching process above would operate, resulting in a whitening of the fibre. Residual Blankit D, either trapped or bonded to the wool, would confer a measure of protection against further exposure to light and the inherent yellowing effect.

Experimentally, it was simple to test this proposal. The U.V. spectrum of Blankit D was run as a 2% solution in distilled water, and the cut-off observed at 255nm. It was clear, therefore, that the solution was transparent to the damaging radiation between 290-360nm, and hence a screening mechanism could not apply.

2.4.2. Promotion of Photobleaching

Pretreatment of wool with such compounds as sodium hydrosulphite, thiourea formaldehyde resins or thioglycollic acid and its zinc salts, has been shown to accelerate the photobleaching effect [14,15] Blankit D is the zinc salt of a reducing agent incorporating formaldehyde so it is quite feasible that a similar interaction occurs with wool. King [15] found that the action spectrum for wool treated with thioglycollic acid is as shown in Fig. 2.4.

Fig 2.4 Action Spectrum of Wool Impregnated with Thioglycollic Acid, Redrawn from King [15].



From Fig.2.4 it can be seen that maximum bleaching occurs still with blue light, and that the increase in photobleaching observed is attributable to the extension into the U.V. region of wavelengths giving rise to photobleaching. Thus it would seem probable that if this is the mechanism for the observed bleaching effect with Blankit D, then an increase in efficiency of the photobleaching reaction should be seen when wool is irradiated by visible light in the presence of 2% Blankit D solution.

Wool serge was therefore irradiated in three separate reactors, fitted with 4 x 300nm tubes, 4 x 350nm tubes and 4 x "cool daylight" tubes respectively. In each case, 2% Blankit D solution was present but the wool batch used for this experiment had been buffered to pH7, and was whiter than that used previously. After 24 hours irradiation in the solution, the wool was photoyellowed (as described previously, with 350nm light for 24 hours and dried in air). The results are shown in Table 2.6.

Table 2.6 Comparison of the Whitening Effect Obtained by Irradiation at 3 Different Wavelengths in the Presence of Blankit D.

Wavelength of Irradiation in Blankit D Solution	Initial Y.I.	Y.I.After Photoyellowing	Δ Y.I
Control Sample	21.3	26.2	4.9
300nm	26.0	32.2	6.2
350nm	16.0	20.4	4.4
"Cool Daylight"	20.1	26.2	6.1

These results show clearly that very little bleaching occurred using the visible light irradiation, and no increase in photostability was conferred. This evidence does not eliminate the possibility that whitening occurs by the promotion of the photobleaching process, but does render it unlikely to have more than a minor contribution.

2.4.3 Interaction with Excited States in the Fibre

Considerable evidence has been reported in the literature for the involvement of singlet and triplet excited sates in the photoyellowing of wool [16]. This has been reviewed in Chapter 1.5. It is possible that by quenching the excited states in wool the formation of singlet oxygen (by reaction of ground state oxygen with triplet excited states) could be retarded, thus inhibiting reactions responsible for yellowing.

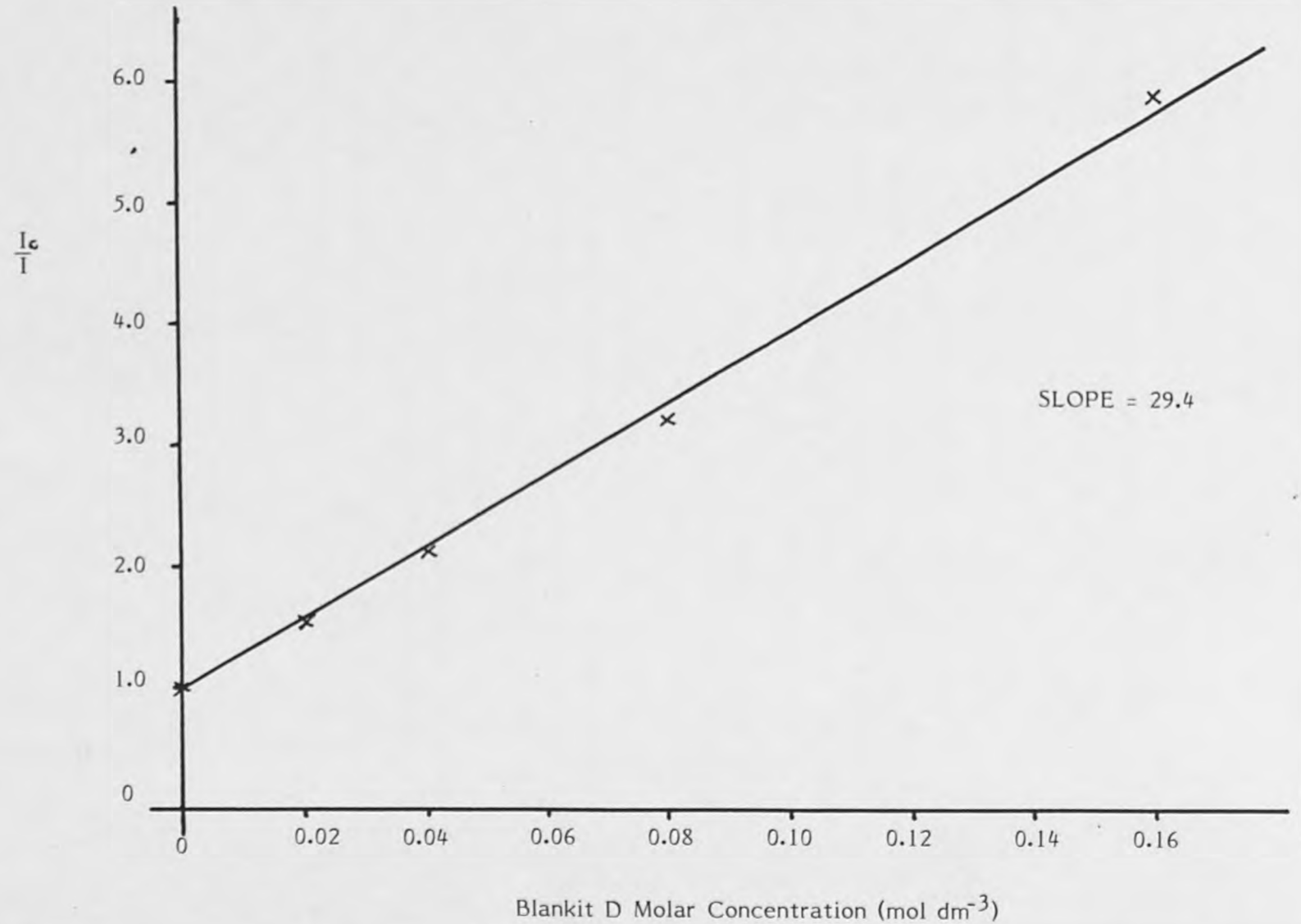
Tryptophyl residues are known to be those most responsible for yellowing of wool, and the existence of excited states of tryptophan in wool has also been reported. The interaction of Blankit D with the excited states of tryptophan was therefore considered to be a reasonable model system for experimental purposes.

The fluorescence of N-Acetyl Tryptophan (used as a better model for tryptophan in the peptide, and because of its higher water solubility) was measured in a quartz cuvette on a Perkin Elmer MPF4 spectrofluorimeter. A stock solution of OD=0.1 (at 290nm) was made up in distilled water, and to this solution, varying quantities of Blankit D were added in separate flasks, to give Blankit D concentrations of 0, 0.02M, 0.04M, 0.08M and 0.16M respectively. The excitation wavelength was set at 290nm, and the fluorescence of each of the solutions measured, under identical conditions, at 358nm, as shown below:-

Table 2.7 Quenching of Tryptophan Fluorescence by Blankit D

N-AcTrp Concentration	Fluorescence Intensity (as a % of fsd)	I_o/I
0	90.2	1.000
0.02M	57.5	1.569
0.04M	42.8	2.107
0.08M (≃2% w/v)	27.8	3.241
0.16M	15.4	5.857

Fig 2.5 Stern-Volmer Plot of N-Acetyl Tryptophan in Water, Quenched by Blankit D



A Stern-Volmer plot of these results is given in Fig.2.5. Clearly the fluorescence of tryptophan is strongly quenched by Blankit D. It may be that while the singlet excited state of tryptophan is quenched, inhibiting the formation of yellow photooxidation products, a second reaction occurs with tryptophan forming colourless products. The tryptophan content would thus be depleted in treated wool, as may be other species responsible for the formation of the yellow colour, thus giving rise to the increase in photostability observed.

2.4.4. Oxygen Scavenging

The favoured mechanism for photoyellowing postulates the quenching of triplet excited states with ground state oxygen, forming singlet excited oxygen which then goes on to react with amino acids in the fibre to produce yellow products **[16]**. This clearly implicates the presence of oxygen. It has been further shown that in the absence of oxygen, yellowing does not occur **[18]**. Thus if wool is irradiated in the presence of an oxygen-scavenging solution the photoyellowing reactions would be expected to be retarded.

In order to determine whether photooxidation was stopped by the presence of Blankit D solution, the photodegradation of sodium 4-(3-phenyl-2-pyrazolin-1-yl) benzene sulphonate tryptophan in aqueous solution was followed by scanning U.V. spectroscopy. A solution was made up in a quartz cuvette to OD=1.2, (at 330nm) and aerated with oxygen for 3 minutes. Having run the initial spectrum (t=o), the solution was irradiated by a circular array of 16x8W (FT8TS/BLB) Sylvania Black light tubes with a λmax of 350nm. The photooxidation was followed by running the U.V. spectrum at frequent intervals to ascertain the time taken for degradation to occur. This is shown as Figure 2.6 on the next page.

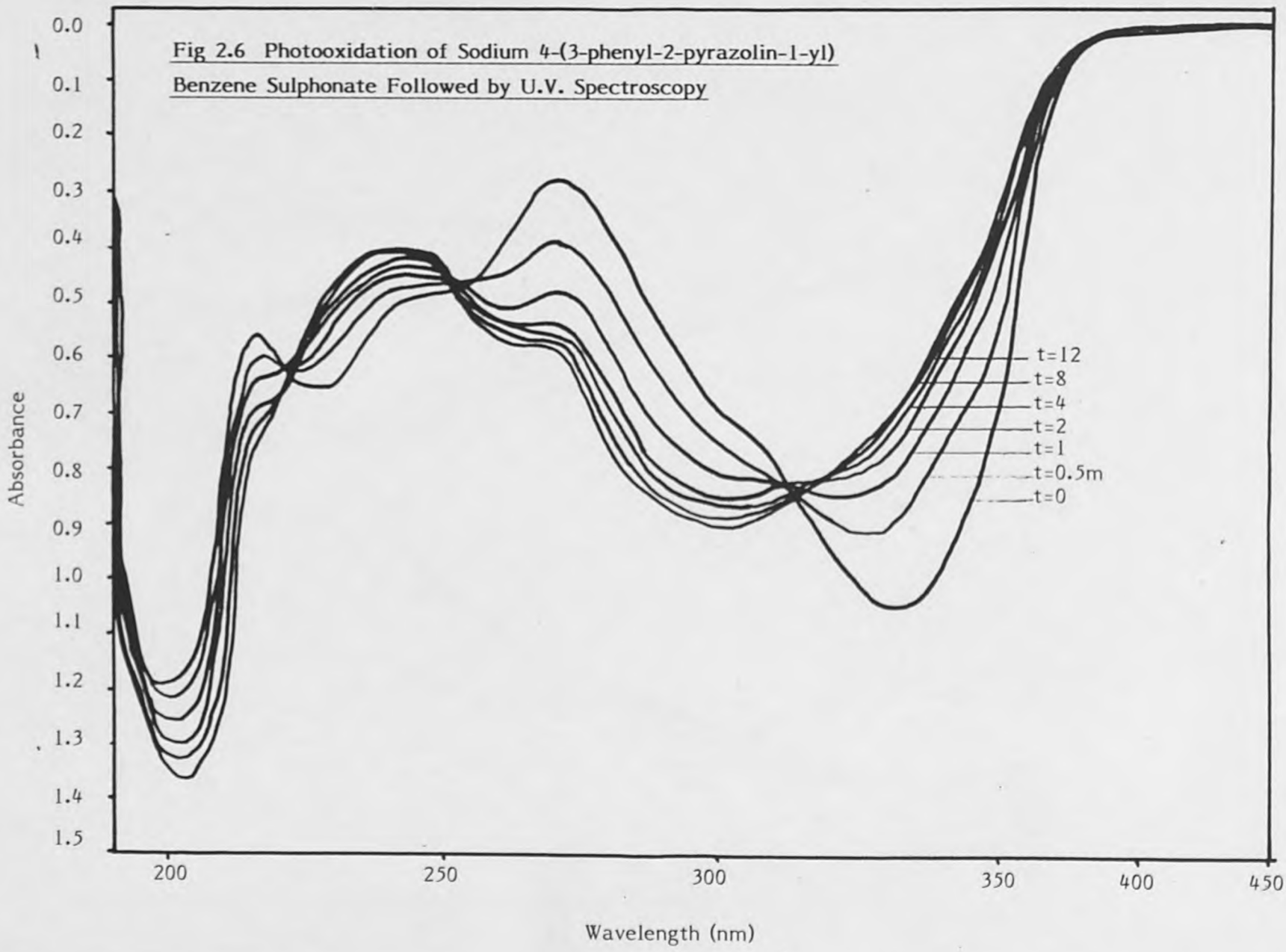
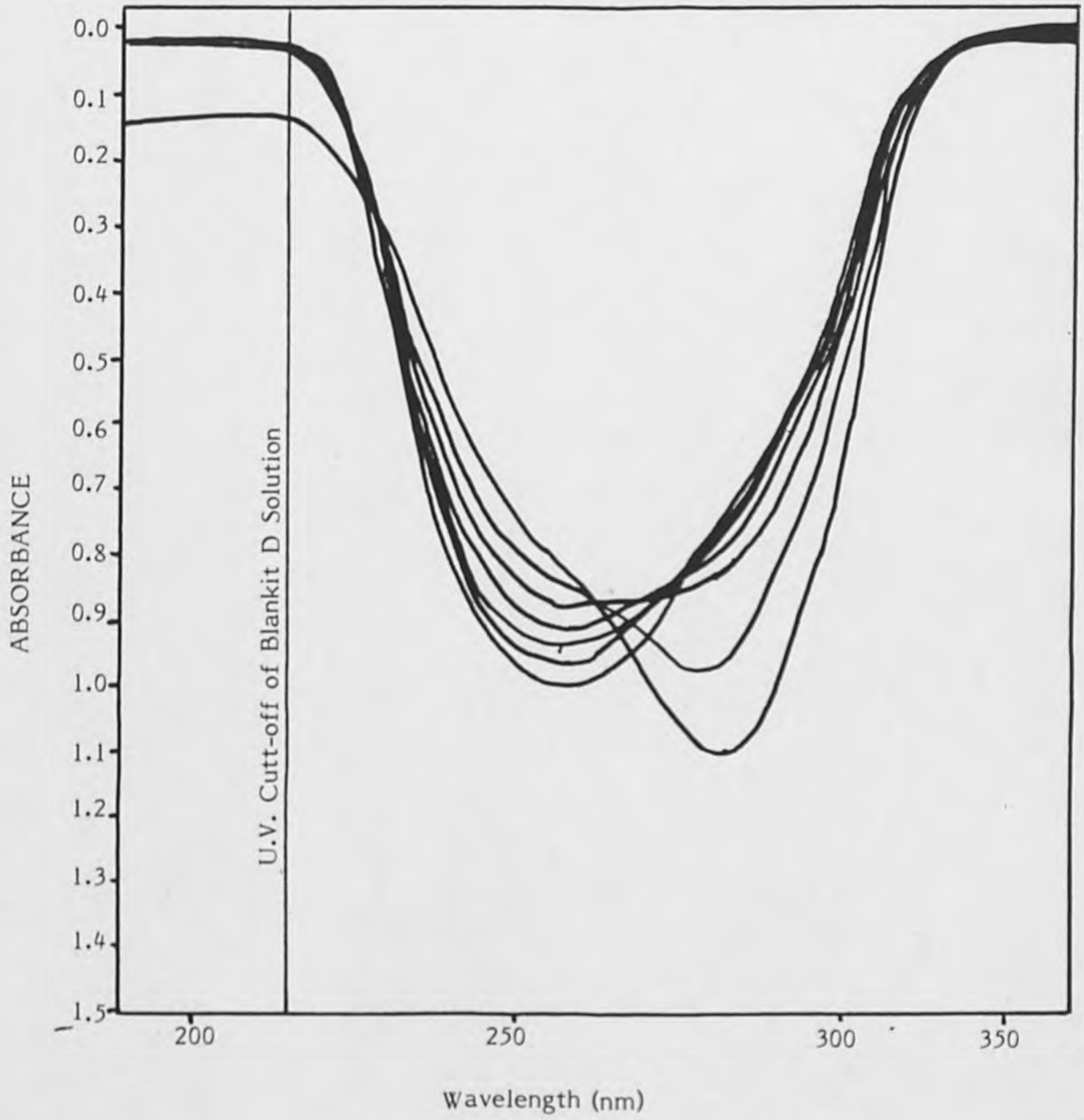


Fig 2.6 Photooxidation of Sodium 4-(3-phenyl-2-pyrazolin-1-yl) Benzene Sulphonate Followed by U.V. Spectroscopy

It is clear from Fig 2.6 that as the photooxidation proceeds, degradation products are formed, having an absorption in other regions of the spectrum.

Another solution was made up with a similar pyrazoline concentration, but in this case 2% w/v Blankit D was also present. The solution was aerated with oxygen for 3 minutes as before, and irradiated in the 350nm reactor used in the last experiment. Scanning UV spectra were again run at frequent intervals, and these are shown in Fig 2.7:-

Fig 2.7 Photooxidation of Sodium 4-(3-phenyl-2-pyrazoline-1-yl) Benzene Sulphonate in 2% Blankit D Solution



The pyrazoline used was known to be photooxidized by 350nm light. Comparison of Figures 2.6 and 2.7 showed that there was no significant difference in the rate of photooxidation, and no reason to believe that the products were not the same. No information could be obtained from the low wavelength end of the spectrum shown in Figure 2.7, due to the absorption from the Blankit D solution, but it could nevertheless be concluded that the presence of the reducing agent had not scavenged oxygen to any appreciable extent. It was thus reasonable to expect that photooxidation of tryptophan solutions would also occur in the presence of Blankit D, even if via a different reaction pathway and yielding different products.

2.4.5 Inhibition of the Photoyellowing Process

It is possible that Blankit D retards the normal processes of photoyellowing by some means not discussed above. One such possibility may take the form of a reaction, photochemical or otherwise, with the species in wool which are responsible for photoyellowing, such that the normal decomposition processes can no longer occur.

This is not easy to test experimentally, because of the limited knowledge available about the photosensitive sites existing in wool. The evidence for involvement of tryptophyl residues has already been discussed in chapter one. Other amino acids have been found to give coloured photooxidation products in solution, but have only undergone very slight degradation in wool when the fibre had yellowed quite appreciably. The reactions of Blankit D with other species present, such as that giving rise to the long wavelength phosphorescence emission, cannot be determined until such components are identified. The reactions of Blankit D with N-acetyl tryptophan which have been shown to occur are described later in this chapter.

2.5 Experiments with Model Compounds

Having obtained information regarding the means by which Blankit D retards the photoyellowing of wool, attention was now centred upon Blankit D itself, with a view to determining which component or combination of components is necessary for the photostabilization processes to proceed.

The chemical composition of Blankit D has already been given as 80% Zinc hydroxymethane sulphinate and 20% stabilizers [12]. Four components are apparent, viz.,

(i) Zinc
(ii) Formaldehyde
(iii) Reducing agent (ie. sulphinate)
(iv) Stabilizers

The effects of these individual parts of Blankit D were studied in relation to the photoyellowing problem. In some cases, similar work has been reported in the literature, and this is discussed in the relevant sections.

2.5.1 The Effect of Zinc Ions on Wool

For many years, work has been carried out to investigate the reactions of Cu^{2+} ions with wool, [19-22] primarily with a view to developing a shrinkproofing treatment. These studies have ascribed the reactions mainly to complex formation between the Cu^{2+} ion and the free carboxyl groups in wool, [20], and also to an interaction with the disulphide bonds [21]. One problem with this treatment is that the treated wool is pale green in colour, and another that ferric and cupric ions have been found to cause an increase in photoyellowing [23]. Zinc ions, in contrast, are colourless. McPhee reported that metal ions do react with wool in the presence of sulphite [24]:

$$RS\text{-}SR + M^{\oplus} + SO_3^{2-} \rightarrow RSSO_3^{\ominus} + RSM$$

Zinc ions could influence the equilibrium by mercaptide formation with thiol groups formed in the wool from the sulphite reaction, or by forming complexes with $-S^{\ominus}$ and $-NH_2$ groups, depending on the steric arrangements of the groups in wool. The presence of the zinc would thus allow the forward reaction to proceed to a greater extent than in its absence.

Cleavage of the disulphide bond would be expected to increase the fluorescence of tryptophyl residues, [24a], and thus enhance the rate of yellowing, if the singlet oxygen mechanism is true. McPhee, however, did not comment upon the effect of this treatment on photoyellowing. More recently Nicholls has found [25] that the deposition of Zn^{2+} ions on wool causes a marked increase in lightfastness of triarylmethane dyes, and at a level of 5% or 10% owf stabilizes wool to yellowing. However, no-one has ever reported irradiating wool in a solution of Zn^{2+} ions.

A 10g sample each of untreated and fluorescently whitened wool were immersed in a 1.5% w/v solution of zinc chloride. This particular zinc salt was selected (a) because of its high water solubility , in contrast to the sulphate or sulphite salts, and (b) because the chloride ion is not present in Blankit D. The wool was irradiated in the presence of the zinc ions for 24 hours, after which time, both samples had yellowed

as normally observed in water. See Table 2.8. This shows that neither whitening nor stabilization had accrued from the treatment. Manganese $^{2+}$ ions were also investigated. A pale pink solution is formed, but Mn^{2+} is known to be a stabilizer for some inorganic species, eg. titanium compounds. The results obtained by irradiating wool in a 2% w/v solution of manganese acetate for 24 hours are also shown in Table 2.8. Clearly, manganese ions do not stabilize wool under these conditions. Whereas the zinc chloride samples were a creamy yellow colour after irradiation, the manganese acetate specimens were strongly yellow.

Table 2.8 Irradiation of Wool in Zinc and Manganese Ions

Wool Sample/Solution	Y.I.Before Treatment	Y.I.After Treatment	Δ Y.I.
Untreated serge in 1.5% $ZnCl_2$ aq	21.3	24.2	2.9
FWA serge in $ZnCl_2$ aq	1.5	24.7	23.2
Untreated serge in 2% Manganese Acetate aq	21.4	34.1	12.7
FWA serge in Manganese Acetate Solution	1.5	35.7	34.2

Considering the extent to which these samples yellowed during treatment it was not considered necessary to subsequently test their photostabilities.

The possibility still remained, however, that zinc ions would only have a stabilizing effect in the presence of a reducing agent. An attempt was made to make up a solution of zinc chloride and sodium borohydride, but the latter decomposed very vigorously leaving a white, insoluble solid, presumed to be zinc oxide (solubility 0.0016g 100ml cold water) or zinc hydroxide (v.slightly soluble). Wool was therefore reduced by a pad/batch treatment with 2% sodium borohydride, and then irradiated at 350nm in zinc chloride solution as before. This experiment yielded similar results to those obtained for zinc chloride alone. Finally, wool samples were irradiated in a solution containing 2% zinc chloride and 2% sodium dithionite

as reducing agent. Again, no effect was observed, but in this instance this was probably due to UV screening by the dithionite.

All the wool samples irradiated in the presence of zinc or manganese ions were examined using X-ray fluorescence to determine whether the metal ions had been taken up by the fibre. The samples were thoroughly rinsed first to remove excess reagent from the wool, so the results were expected to indicate the levels of material which were in some way bound to the fibre, either covalently, or by ionic interactions.

The X-ray fluorescence studies were carried out using a Jeol JEM100B electron microscope with scanning system, fitted with an EDAX 9100/60 Energy Dispersive X-ray Fluorescence Analyser (E.D.X.F.A.). Electron excitation was used, and the emitted X-rays detected with a semi-conductor junction device. This generated a conduction pulse for each X-ray photon, the height of the pulse being related to the energy of the X-ray, thus indicating the element from which it was emitted. The lower detection limit is of the order of 1% of the bulk of the sample, but in the case of localized surface impurities, it is possible to focus down with the microscope and detect lower concentrations.

The technique is semi-quantitative, calculating elemental analyses as a percentage of the total elements present. The lower atomic detection limit is sulphur, but all elements of higher atomic weight can be determined. Below this limit, the accuracy is unacceptably low. Sulphur is present in the amino acids cystine, cysteine and methionine in wool, and may in some cases be used as an internal standard. However, many treatments of wool involve sulphur-containing reagents, which cause the sulphur content of the wool to change, thus restricting its usefulness in this capacity. The percentage results obtained refer to atomic percentage and not by weight ie. 20% Mn and 80%S indicates that manganese and sulphur are present in the empirical ratio MnS_4. The accuracy of these percentages is of the order of ±15-20%. eg. 30%S means 24-36% sulphur. This is a very high error for X-ray fluorescence techniques, but is an inherent problem resulting from the uneven fibre geometry.

The wool samples were examined whilst on the copper probe of the electron microscope. This lead to the existence of a slight background level of copper in some samples, but in no case was this more than 1 or 2%. The results obtained are shown in Table 2.9. The % ratios listed were computed using a ZAF program:

Z= Atomic number. A correction factor is used to normalize variations in the intensities of X-rays generated from elements of different atomic numbers.

A= Absorption effect. A copper X-ray could be absorbed by a Nickel atom, for example:

A Nickel X-ray could not excite a copper atom because it is of too low energy.

F= Fluorescence. X-rays generated by one element can be absorbed by another and re-emitted at a different frequency.

Having corrected for these three possible occurrences, the % values of the elements present are obtained.

The results presented in Table 2.9 show that when wool was irradiated in zinc chloride solution, there was an uptake of zinc and chlorine by the fabric, in the ratio of Zn:2Cl, thus suggesting that possibly residual reactant is trapped in the fibre, rather than a reaction product. When sodium dithionite was present, there was an apparent decrease in zinc absorption. This could be due either to the UV screening effect of the dithionite if a light catalysed reaction with zinc is occurring, or to a reaction between the reducing agent and the wool:

$$W\text{-}SS\text{-}W + {}^{\ominus}O_2S.SO_2{}^{\ominus} \rightleftarrows WS^{\ominus} + WSSO_2.SO_2{}^{\ominus}$$

This would clearly increase the sulphur content of the fibre, giving rise to an apparent decrease in the percentage zinc present.

The results obtained for wool treated with Blankit D were also included for comparison. The amount of zinc absorbed in this case was considerably

TABLE 2.9 X-Ray Fluorescence Data for Wool Treated with Zn^{2+} and Mn^{2+} Ions

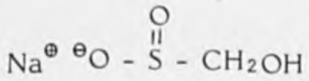
WOOL SAMPLE	SOLUTION USED FOR IRRADIATION	ELEMENTS DETECTED (%)		
		S	Mn	
Untreated	Manganese Acetate	79.5	20.5	
FWA	Manganese Acetate	81	19 *	
		S	Cl	Zn
Untreated	Zinc Chloride	47	36	15
FWA	Zinc Chloride	48	37	15 *
		S	Cl	Zn
Untreated	$Na_2S_2O_4$ + $Zn\ Cl_2$	66.5	22	11.5
FWA	$Na_2S_2O_4$ + $Zn\ Cl_2$	73	10	17
		S	Zn	
Untreated	2% Blankit D	94	6	
FWA	2% Blankit D	94	6	
FWA, Reverse Side	2% Blankit D	94	6	

* These results are the same, within experimental error.

lower, and was the same for both the irradiated and the reverse sides of the fabric. The sample was sufficienty thick for measurement to be of surface characteristics, so it was not possible to be observing the irradiated side of the wool through the bulk of the fabric. Thus it was concluded that when zinc is absorbed it is either because of residual Blankit D trapped in the fibre, or because of a dark reaction. Thus it seems unlikely to be the zinc which is affording the observed protective effect.

2.5.2 Comparison with Rongalit C

Rongalit C is the sodium salt of hydroxymethane sulphinic acid also manufactured by B.A.S.F:



$$Na^{\oplus}\ {}^{\ominus}O - \overset{O}{\overset{\|}{S}} - CH_2OH$$

Both Rongalit C and Blankit D have been used as reducing agents during the application of fluorescent whitening agents and found to improve the photostability of the treated wool [26].

There are two principal differences between this compound and Blankit D : (i) it is the sodium salt, instead of the zinc salt, and will thus have a higher degree of dissociation in solutions and (ii) it forms an aqueous solution of pH8, in contrast to pH4 in the case of Blankit D

Initially, samples of untreated and optically brightened serge were irradiated in the presence of a 2% Rongalit C solution for 24 hours, and in parallel, in a 2% Blankit D solution. The results, given in Table 2.10, showed that Rongalit C does not exhibit the whitening effect to the same extent as Blankit D, although a slight increase in whiteness is observed for untreated wool.

Table 2.10 Comparison of the Whitening Effects of Rongalit C and Blankit D

WOOL SAMPLE	REDUCING AGENT	INITIAL Y.I.	Y.I. AFTER TREATMENT	Δ Y.I.
Untreated	Rongalit C	19.6	18.3	-1.3
Fluorescently Whitened	Rongalit C	1.5	11.3	9.8
Untreated	Blankit D	19.6	12.1	-7.5
Fluorescently Whitened	Blankit D	1.4	5.0	3.6

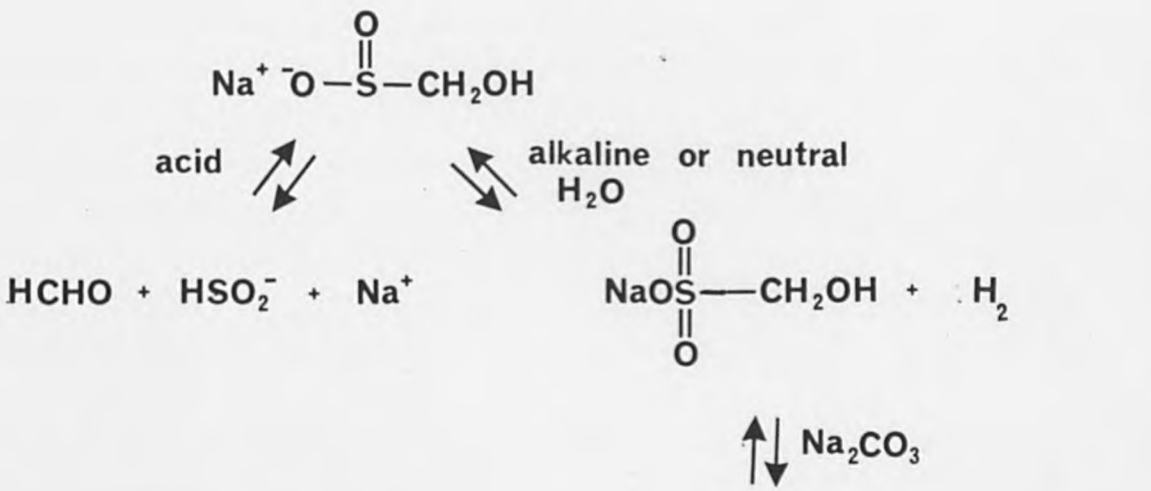
The photostability of these samples was then tested, irradiating in water for 24 hours with 350nm light. See Table 2.11.

2.11 Comparison of the Photostability of Wool Irradiated in Blankit D and in Rongalit C

SAMPLE	Y.I.	Y.I. AFTER PHOTOYELLOWING	Δ Y.I.
Untreated serge	19.6	24.4	4.8
Untreated, 2% Rongalit C	18.3	20.6	2.3
Untreated, 2% Blankit D	12.1	17.0	4.9
FWA Treated	1.7	30.3	28.6
FWA, 2% Rongalit C	11.8	25.4	13.6
FWA, 2% Blankit D	5.4	13.3	7.9

Clearly, an increase in photostability is observed with the Rongalit C treatment, but again this is inferior to that obtained with Blankit D. These results are believed to be explained by the dissociation/decomposition

pathways of hydroxymethane sulphinates shown below:



$$HCHO + Na_2SO_3 + CO_2 + NaOH$$

Under the alkaline conditions of Rongalit C solution, (pH8.0), there will be at least partial oxidation of the sulphinate to the sulphonate moiety, thus decreasing the reducing properties of the reagent.

In contrast, under acid conditions dissociation yields formaldehyde and the reducing species, which may be the combination required for the stabilizing of wool. If this is the case, then the partial activity of Rongalit C may be due to the presence of a small percentage of sodium sulphite and formaldehyde. An attempt was made to acidify a solution of Rongalit C, but the reagent was immediately precipitated out. Thus it appears, that although Rongalit C and Blankit D are structurally similar, their reactivity with wool is different.

2.5.3 Treatment of Wool with Formaldehyde

Wool is known to undergo reactions with formaldehyde at a number of sites in the fibre. Of particular interest have been the reactions with cystyl, [27-29], tyrosyl, [30,31] lysyl, [32] and tryptophyl [30,31] residues, details of which are summarized in Chapter 1. All reactions reported have been in the absence of light, however, and it was necessary to determine whether a species as reactive as formaldehyde would interact with wool photochemically (ie. a reaction with either excited states or photoproducts of the wool).

To this end, samples of untreated and fluorescently whitened wool were irradiated in a 2% solution of formaldehyde (made up by diluting a 40% solution 1:20) for 24 hours, and also in water as a control. The results, given in Table 2.12, indicate that while such reactions may be occurring, they do not influence either the whiteness or the lightfastness of the untreated wool. However the lightfastness of the fluorescently whitened wool does show a 20% improvement.

Table 2.12 The Effect of Irradiation of Wool in Formaldehyde Solution

Wool Sample	Y.I. Before Treatment	Y.I. After Treatment	Δ Y.I.
Untreated Serge/HCHO	21.5	27.5	6.0
Untreated Serge (Control)	20.6	24.7	4.1
FWA Serge/HCHO	1.3	24.1	22.8
FWA Serge (Control)	1.7	30.3	28.6

The conclusion may therefore be drawn that although formaldehyde is known to undergo many reactions with wool, none of these are responsible for conferring the protective effect. It still remains, however, that formaldehyde may be involved in a more complex raction pathway, when present in the Blankit D solution. This is discussed later.

2.5.4 The Effect of Formaldehyde Bisulphite

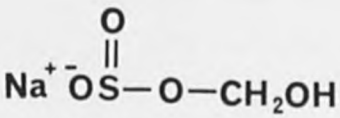
Sodium formaldehyde bisulphite is formed by the reaction of formaldehyde with sodium bisulphite:

$$HCHO + NaHSO_3 \rightarrow HOCH_2SO_3Na$$

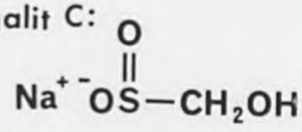
It is believed to react with aromatic amines as shown below:

$$Ar\ NH_2 + HO\ CH_2SO_3^{\ominus}Na^{\oplus} \rightleftarrows Ar\ NH\ CH_2SO_3^{\ominus}Na^{\oplus} + H_2O$$

It is also a related compound to the hydroxymethane sulphinates, having the structure:



Cf. Rongalit C:



There was therefore the possibility that sodium formaldehyde bisulphite would have a similar effect on wool as did Blankit D, or Rongalit C.

Samples of untreated and fluorescently whitened wool were therefore irradiated in the presence of a 2% aqueous solution of sodium formaldehyde bisulphite for 24 hours, with control samples irradiated in water at the same time. The resultant yellowing is quantified in Table 2.13 and shows that virtually no protection was provided by the bisulphite, the treated wool yellowing as fast as the control samples.

Table 2.13 The Effect of Irradiation in Sodium Formaldehyde Bisulphite Solution

Wool Sample	Y.I.Before Treatment	Y.I.After Treatment	Δ Y.I.
Untreated Wool	25.9	29.7	3.8
Untreated Wool (Control)	26.5	30.4	3.9
FWA Treated Wool	1.3	26.3	25.0
FWA Treated Wool (Control)	1.2	29.4	28.2

Had stabilization been achieved, it may have been that the indolic nitrogen of tryptophyl residues was reacting with the bisulphite, according to the reaction pathway given above. Whilst the above results do not eliminate that possibility, they do indicate that this cannot be the explanation for the effect of Blankit D.

2.5.5 The Elimination of the Stabilizers - Decrolin

It was found that B.A.S.F. also manufacture zinc formaldehyde sulphoxylate without the stabilizers present in Blankit D, under the trade name Decrolin. Assessment of this reagent in comparison with Blankit D would reveal any contribution of the stabilizers in protecting the wool.

Wool samples were irradiated in a 2% Decrolin solution and a 2% Blankit D solution respectively. This would mean that a higher concentration of zinc hydroxymethane sulphinate was present in the Decrolin solution, but this has already been shown to have little effect on the extent of bleaching and protection, at this level of reducing agent concentration.

It was found that very similar results were obtained for both reagents. This suggests that the stabilizers will only promote the whitening process by prevention of the decomposition of the reducing agent, rather than by reacting themselves with the wool. The yellowness indices of the treated samples were measured and are given in Table 2.14.

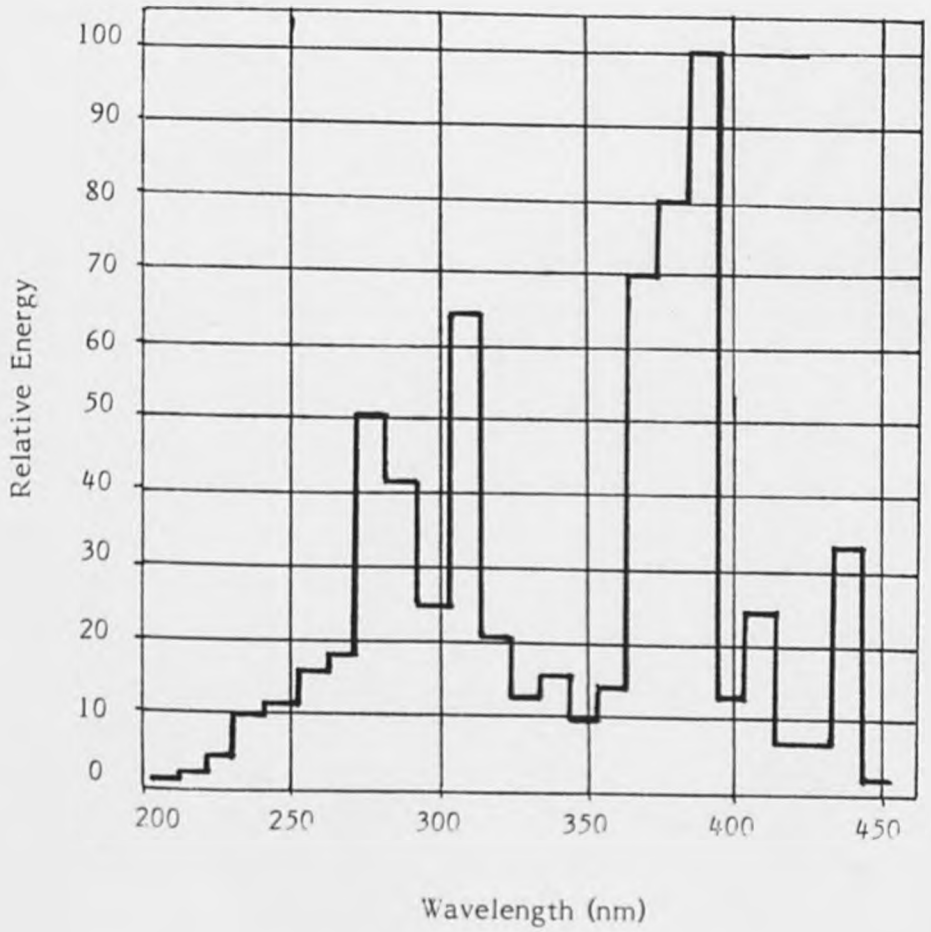
Table 2.14 Comparison of the Effects of Blankit D and Decrolin

Sample	Y.I.Before Treatment	Y.I.After Treatment	Δ Y.I.
Untreated Serge Blankit D	18.5	12.5	-6.0
Untreated Serge Decrolin	18.9	13.7	-5.2
FWA Treated Serge Blankit D	1.4	6.8	5.4
FWA Treated Serge Decrolin	1.1	6.8	5.7

2.6 Use of U.V. Curing Lamp

The whitening effect obtained so far by irradiation of wool in the presence of a Blankit D solution was too slow to be commercially viable. An attempt was therefore made to accelerate the process using a Thorn 91-7480 1800W U.V. cure metal halide discharge lamp. The typical spectral output of the lamp is given below:

Typical Spectral Power Distribution of Ultraviolet Curing Lamp



This lamp clearly gives a 30 fold increase in power output over the 4 x 15W lamps used previously. It was hoped that a whitening effect could be generated in this way in minutes rather than hours.

Samples of untreated wool were irradiated in 2%, 5% and 10% Blankit D solutions for periods of 1, 2, 5, 10 and 30 minutes. The change in yellowness during this period was measured, and the samples then irradiated in water with the 15W lamps to determine the photostability.

Table 2.15 Curing Lamp Irradiation of Wool in Blankit D Solution

Irradiation time (mins)	Y.I.	Change in Y.I. During Irradiation	Y.I. After Photoyellowing	Photostability Δ Y.I.
a) 2% Solution				
0	25.9	-	30.3	4.4
1	23.6	-2.3	28.1	4.5
2	23.0	-2.9	26.4	3.4
5	22.0	-3.9	26.2	4.2
10	22.3	-3.6	27.2	4.9
b) 5% Solution				
0	22.3	-	27.1	4.8
1	21.3	-1.0	26.8	5.5
2	20.4	-1.9	25.9	5.5
5	19.1	-3.2	26.6	7.5
10	17.2	-5.3	24.0	6.8
30	19.1	-3.2	25.7	6.6
c) 10% Solution				
0	25.6	-	29.3	3.7
1	23.2	-2.4	28.7	5.5
2	21.1	-4.5	28.7	7.6
5	19.9	-5.7	28.0	8.1
10	17.6	-8.0	27.5	9.9
30	17.9	-7.7	24.6	6.7

One problem that was encountered during the curing lamp irradiations was that the temperature of the solution was raised from 17°C to 85°C in 30 minutes. This is known to have a marked effect on the yellowing properties of the fibre. Wool has been shown to yellow three or four times as fast at 75°C as at 35°C [33]. At higher temperatures, thermal yellowing occurs together with photoyellowing giving rise to a more complex reaction system. It was found that if wool was irradiated for 20-30 minutes in Blankit D solution, the resultant wool was dark yellow/brown in colour, with a Yellowness Index of 40-50. However, after standing in the reducing agent solution for a further 20 minutes, the colour was almost completely bleached out, giving rise to a wool which was sometimes whiter than the initial colour of the fabric. This suggests that in the case of the curing lamp at least, two or more distinct processes are occurring; namely photooxidation of the wool to give yellow products, and a dark reaction involving the reduction of these photoproducts to colourless species. It appears that by increasing the power of the lamp used, the rate of the photooxidation is increased, in this case to such an extent that it occurs faster than the dark reaction, thus giving rise to the temporary presence of yellow products. Two further observations were made. The first was that if the wool irradiated for 30 minutes in 10% Blankit D solution was rinsed thoroughly immediately the lamp was turned off, only minimal bleaching occurred, presumably due to the presence of residual reducing agent trapped within the fibre. This shows that there are at least two separate reactions occurring, and that the yellow species is not simply a transient intermediate. To confirm this, wool was irradiated under the curing lamp in distilled water, whereupon a permanent yellow/brown colour developed. However, the second observation was that when the yellowed wool (irradiated in water) was subsequently placed in a 10% Blankit D solution in the dark, only partial bleaching occurred. Thus it may be that the initial yellow photoproducts are further photooxidized in the absence of reducing agent to give a species which cannot be bleached out with a reducing agent.

As can be seen by inspection of Table 2.15, a measure of photobleaching is achieved by this curing lamp process, but the extent is considerably less than that gained when the 15W lamps were used (Table 2.4). Also, the photostability was found to be worse after treatment rather than better.

It therefore appears that the process cannot be satisfactorily accelerated using this type of light source. It may be that this is partly due to the spectral output of this particular lamp (diagram) which has a relatively large emission at around 300nm, and only a small component at 350nm. Thus although the overall power of the lamp is greater, the majority of the emitted photons are of energies which have been shown to give only minimal whitening, or even yellowing (Table 2.6)

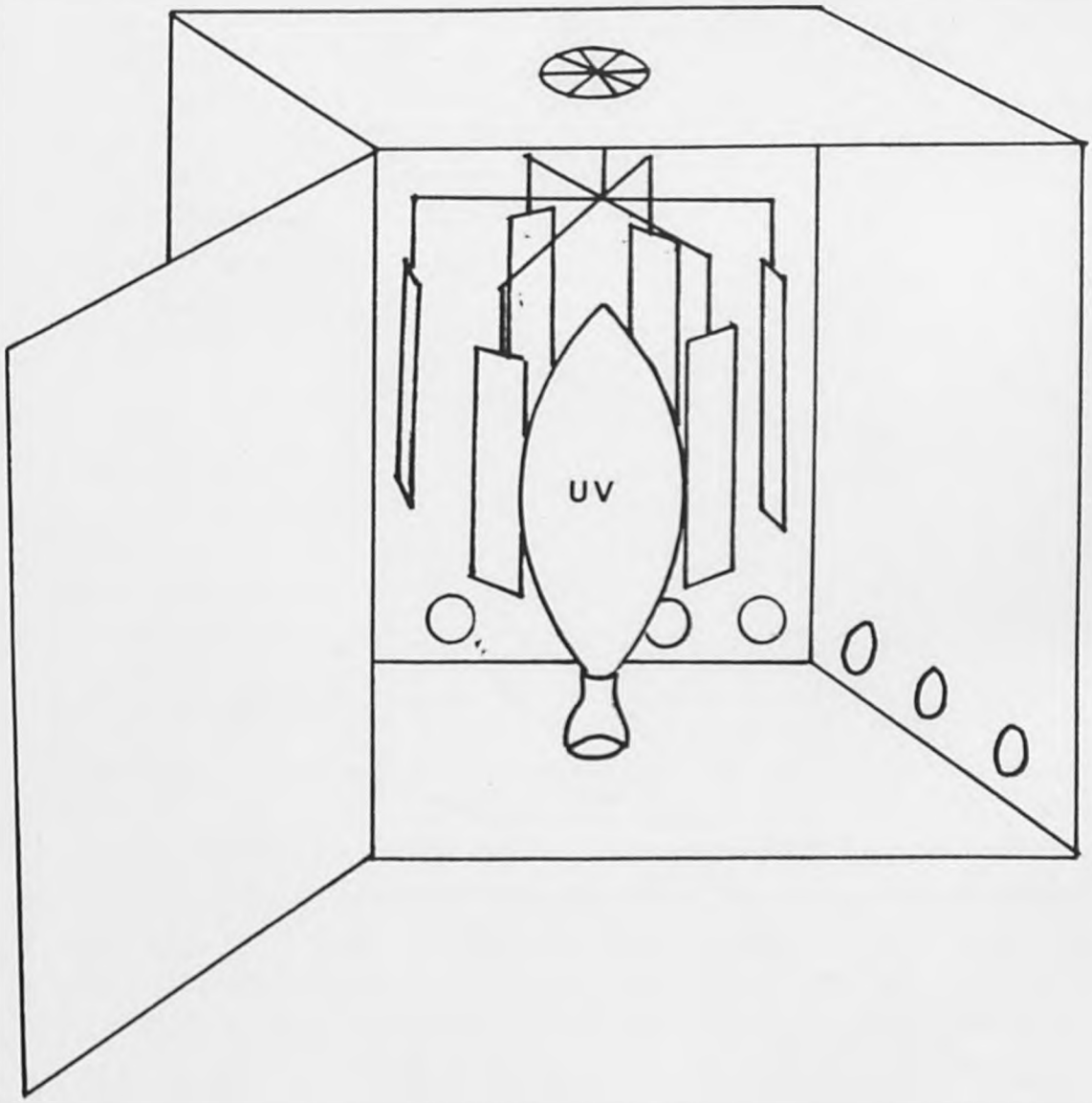
From a commercial point of view, this treatment is of limited practical use because of the heat generated by the lamps. Wool would need to be irradiated on both sides, and even if a lamp of appropriate spectral output could be found, the heat would cause the wool to dry out and be thermally damaged. The cost of implementing the treatment is not justified by the limited results achieved.

2.7 Comparison of Wet and Dry Yellowing

When previous workers tried irradiating wool in reducing agent solutions, [2], they also tried padding the reagents onto wool and irradiating the dried fabric. This showed that in the dry state, reducing agents are very much less effective inhibitors of yellowing, probably because of the inherent decrease in diffusion rates. However, these reports discuss only the protection conferred whilst the wool was in the presence of the reducing agent. In the case of Blankit D treatment, we are considering stabilization to subsequent irradiation, probably as a result of photochemical modifications to the fibre, and hence diffusion is unlikely to directly affect the protective effect observed. One would therefore not expect a marked difference in the levels of protection against wet and dry yellowing.

Initially, wool was exposed to the 350nm tubes used for yellowing in the wet state (see section 2.2.1). However, after 3 weeks the level of yellowing was still fairly small. For this reason, a Philips air cooled mercury vapour lamp was used, as shown in Figure 2.8. The wool samples were half-covered with aluminium foil, as before, and mounted on metal plates suspended from a series of radially positioned overhead rods. The lamp was encased in a fan cooled box.

Fig 2.8 Dry-Yellowing Apparatus



After 3 weeks irradiation, an appreciable degree of yellowing had occurred to all samples. The yellowness indices were measured, and from these the photostabilities (Δ Y.I.) calculated. These are shown in Table 2.16.

Table 2.16 Comparison of Wet & Dry Yellowing, After Blankit D Treatment

Wool Sample	Initial Y.I.	Y.I. After Photoyellowing	Δ Y.I.
(1) DRY			
Untreated Serge	21.6	29.5	7.9
Untreated Serge, hν in 2% Blankit D	14.5	24.4	9.9
Peroxide Bleached	13.9	30.0	16.1
Fluorescently Whitened	-1.5	29.1	30.6
FWA Treated, then hν in 2% Blankit D	2.6	28.2	25.6
(2) WET			
Untreated Serge	21.4	26.6	4.2
Untreated Serge, hν in 2% Blankit D	14.7	19.8	5.1
Peroxide Bleached	13.9	27.2	13.3
Fluorescently Whitened	-1.5	25.9	27.4
FWA Treated, then hν in 2% Blankit D	2.1	20.6	18.5

The spectral output of the two light sources used was not the same and it has already been shown that this effect shows a marked wavelength dependence (Table 2.6). It was not expected, therefore, that the stabilizing effect would be duplicated exactly for wet and dry yellowing under these conditions. However, as can be seen from Table 2.16, the Blankit D treatment had conferred a measure of photostability to the wool in both cases, albeit to a lesser extent in the dry state.

2.8 Attempts to Synthesize Longer Chain Aldehyde Sulphinates

It is possible that part of the protective influence of the Blankit D treatment against subsequent photoyellowing is due to the residual zinc hydroxymethane sulphinate trapped in the fibre. If this is so, it would explain the better photostability observed for irradiation in this wet state rather than dry. It is therefore possible that by increasing the level of reagent left in the fibre, yellowing could be further retarded.

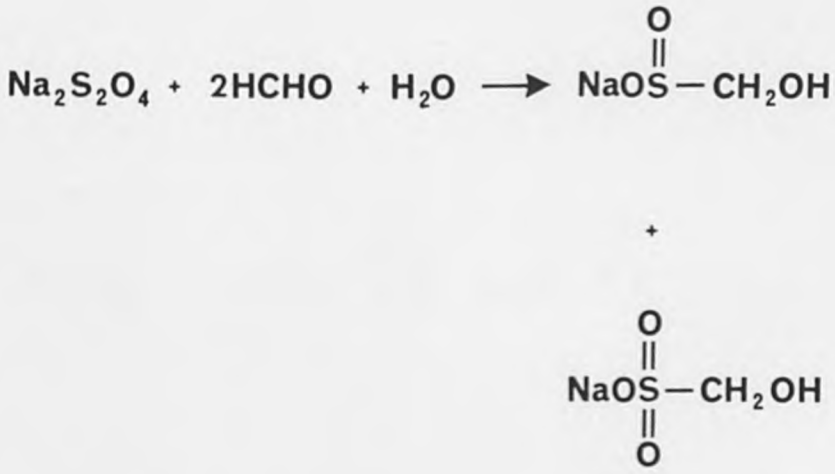
Two potential means of achieving this end were apparent. The first was to covalently bond the Blankit D molecule to the wool, and the second to use an analogue of Blankit D which was more hydrophobic or less water soluble.

The first idea was rejected for two reasons. If diffusion increases the extent of protection against yellowing in the wet-state, then covalently bonding the reducing agent will effectively achieve the opposite. Also, unless the sulphinate already forms an adduct with the wool protein, it would be necessary to add a substituent to the Blankit D molecule which would react with the fibre. Because of the relative simplicity of the original structure, this would almost certainly inhibit the stabilizing reaction pathways.

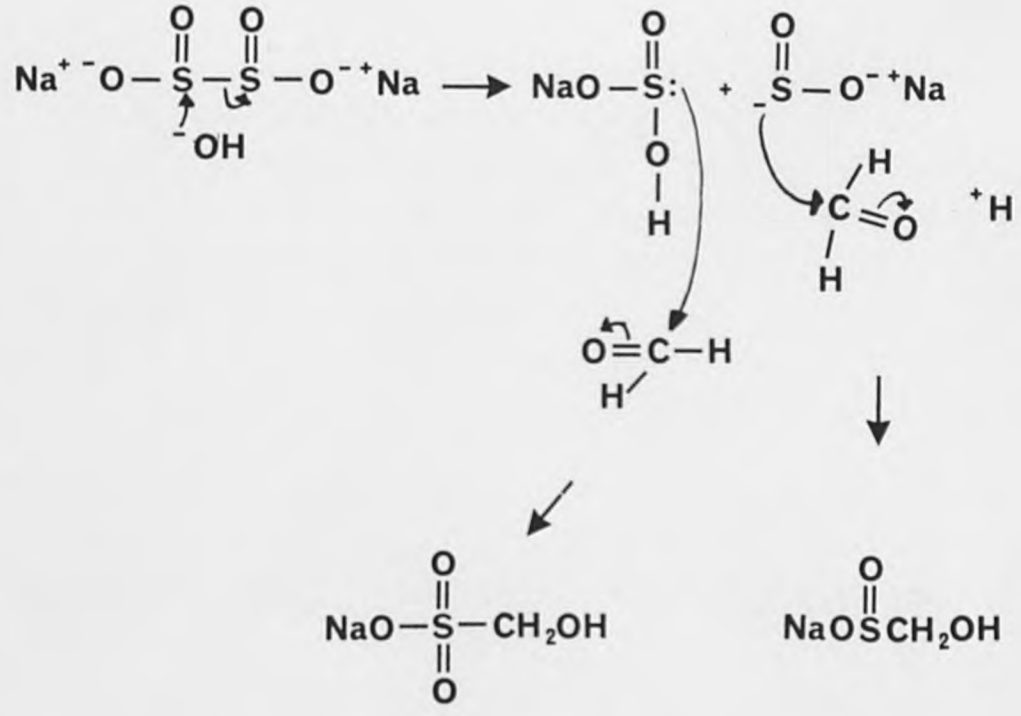
Two approaches were followed to decrease the water solubility of the sulphinate. The first was to exchange the zinc ion for calcium. After the irradiation step was complete, but before rinsing, the wool was placed in a 10% w/v solution of calcium chloride for 1 hour, with regular agitation. The photostability of the samples were then tested, and compared with that of the control samples, where the calcium chloride step was omitted. It was found that there was no significant difference between them. Hence it was concluded that either the calcium had not exchanged with the zinc, or this change did not improve the effectiveness of the Blankit D.

The second, and potentially the most useful approach was to attempt to synthesise a molecule, similar to Blankit D in structure, but replacing the formaldehyde with a longer chain aldehyde, hopefully introducing

surfactant properties. If the reducing power of Blankit D could be achieved, the molecule would have an increased affinity for the wool, and be more hydrophobic than the formaldehyde sulphinate. Sodium hydroxymethane sulphinate is made by the action of formaldehyde on sodium dithionite:

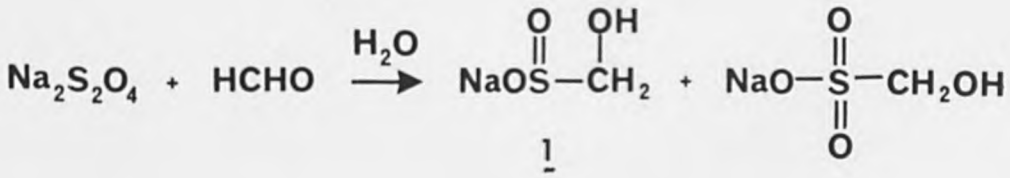


Clearly only the sulphinate will be a reducing species. A proposed mechanism for the above reaction is shown below:

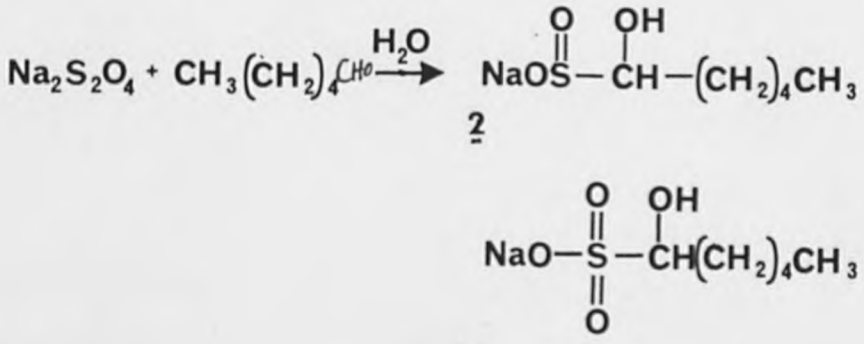


Experimental

(i) Sodium dithionite (17.4g, 0.1M) and formaldehyde (18ml, 38%, ≈0.2M) were dissolved in 300ml water. This was stirred at room temperature for 18 hours, and the resultant colourless liquid stored in the dark at 0°C.



(ii) Sodium dithionite (17.4g 0.1M) was dissolved in 100ml distilled water. To this was added Hexaldehyde (20.0g, 0.2M) and a further 200ml water. Two immiscible layers were present, so the reaction was carried out in an ultrasonic bath, at 35°C, for 2 days. Two layers were still present at the end of the reaction, but much of the smell of hexaldehyde had disappeared.



The crude products (1) and (2) were each diluted to 1 litre, and placed in open trays in two of the reactors shown in Figure 2.1. In the third reactor, was 1 litre of 1.1% Rongalit C, calculated to be equivalent to the theoretical yield of sodium hydroxymethane sulphinate from reaction (i). In both solutions was placed a sample each of untreated and fluorescently whitened wool, followed by 24 hours irradiation with 350nm light. The changes in yellowness of each sample is shown in Table 2.17, together with photostabilities to subsequent irradiation.

Table 2.17 Comparison of the Stabilizing Effects of Rongalit C and Synthetic Analogues

SAMPLE	Y.I. After Irradiation in Reducing Agent	Effect of Treatment Δ Y.I.	Y.I. After Photoyellowing	Photostability Δ Y.I.
Untreated Serge (Y.I. = 21.5)	-	-	25.8	4.3
Untreated Wool in Rongalit C	18.7	-2.8	21.0	2.3
Untreated Wool in 1	19.1	-2.4	23.0	3.9
Untreated Wool in 2	20.1	-1.4	24.5	4.4
FWA Wool (Y.I. = 2.5)	-	-	27.2	24.7
FWA Wool in Rongalit C	11.2	8.7	22.4	11.2
FWA Wool in 1	12.4	9.9	26.6	14.2
FWA Wool in 2	15.8	13.3	27.1	11.3

NOTE: $\underset{\sim}{1}$ = $Na\text{-}O\text{-}\overset{\overset{O}{\|}}{S}\text{-}CH_2OH$

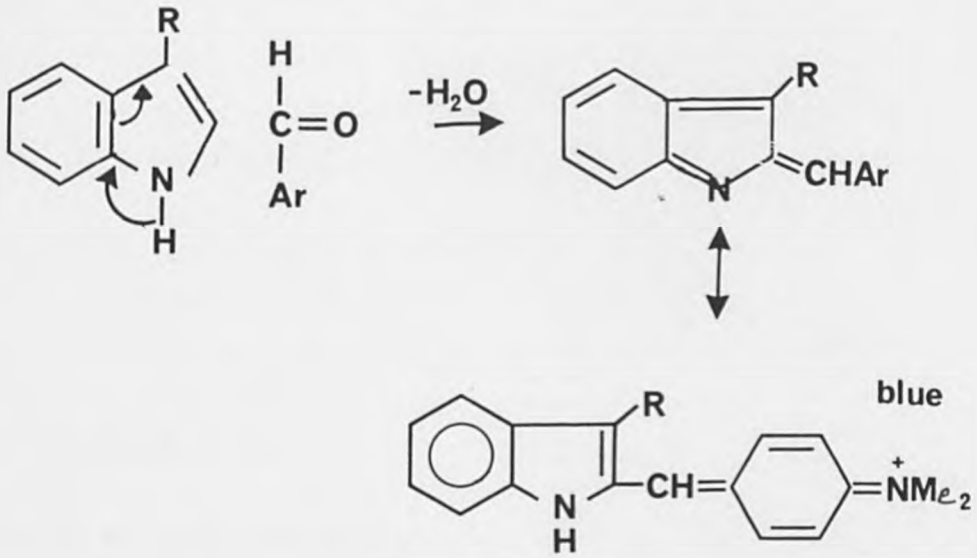
$\underset{\sim}{2}$ = $Na\text{-}O\text{-}\overset{\overset{O}{\|}}{S}\text{-}CH_2OH\ \text{-}(CH_2)_4\ CH_3$

It was found that the effect of the synthesized hydroxymethane sulphinate was almost as good as Rongalit C, and that the longer chain aldehyde product was rather less successful as a stabilizer. None were as effective as Blankit D, of course, but zinc dithionite was not found to be readily available. It is used by BASF in the manufacture of Blankit D [34,35], where it is made by the reaction of a slurry of zinc dust in water with liquid SO_2 [36]. However, the results with sodium dithionite suggest that the introduction of longer chain aldehydes reducesthe protective effect. This may be for steric reasons, if the formaldehyde reacts with wool under reducing conditions.

2.9 Colorimetric Tryptophan Analysis of Wool

The photoyellowing of wool has been shown (in Chapter 1) to be strongly connected with the photooxidation of tryptophyl resides. It was therefore desirable to assess the effect of the Blankit D treatment on the tryptophan content of wool. Conventional amino acid analysis techniques were unsuitable because the hydrolysis procedures almost totally destroy the tryptophyl residues. For this reason, a colorimetric method was adopted. A number of such procedures have been described in the literature, [37-47] most involving the hydrolysis of wool with hydrochloric or sulphuric acids, followed by reaction with an aldehyde to give a coloured compound. The time taken, and the reproducibility achieved vary considerably, these two factors being considered when this method was selected. The application and suitability of these methods to the analysis of modified wool samples is discussed in the above references. Graham and Stathan [43] also observed a marked correlation between the colour of modified wool and the colour of tryptophan when modified in the same way.

The method chosen was that of Cegarra & Gacen, [48] involving hydrolysis of the wool with sulphuric acid, followed by reaction with Ehrlich's reagent, p-dimethylaminobenzaldehyde.



The colour does not develop if the tryptophan is substituted in the 2-position. The significance of this becomes apparent later in this chapter.

Sulphuric acid was used in preference to hydrochloric acid because the latter has been found to give low results [46]. The particular method chosen for this work gives acceptably reproducible results after only two hours hydrolysis, as opposed to up to 7 days in other methods. Ehrlich's reagent was selected by Cegarra & Gacen [48], because it did not give secondary colorations when used with chemically modified wool, such as chlorinated or peroxide bleached fabric.

Experimental Procedure

All samples were analysed in triplicate, and the mean result taken.

1) Preparation of Wool Samples

a/ The wool was washed at room temperature in each of the following solvents, in the order given:

Ether; Ethanol; Distilled water; Ethanol; Ether

b/ The samples were dried in air at room temperature, and then further dried to constant weight in an oven at 105°C.

2) Preparation of Stock Solutions

a/ Ehrlich's reagent. 5.00g p-dimethylaminobenzaldehyde was dissolved in 100ml 10% w/v sulphuric acid (made up by diluting 98% acid 1:10 with water).

b/ Sulphuric acid, 18N. 98% H_2SO_4 was diluted 1:1 with distilled water.

c/ Sodium nitrite, 0.01M. 0.69g were dissolved in 1 litre.

d/ Tryptophan solution, 600mg/litre. 0.60g tryptophan was dissolved in 1 litre of 0.03N sulphuric acid, (made up by diluting 18N acid 1:600).

3) Preparation of Tryptophan Calibration Solutions

a/ 100mg/litre. 100ml of 600mg/litre solution were diluted to 600ml.
b/ 225mg/litre. 225ml of 600mg/litre solution were diluted to 600ml.
c/ 400mg/litre. 400ml of 600mg/litre solution were diluted to 600ml.

Method of Analysis

NOTE: All reagents must be added in the order, and exact amounts specified if reproducible results are to be achieved.

(i) For calibration solutions, 1.0ml of each tryptophan solution was measured out into separate 50ml volumetric flasks (in triplicate).

(ii) For wool samples, 0.06g wool (dry weight) was weighed out into a 50ml volumetric flask (in triplicate).

To each flask was added 2.0ml of 18N sulphuric acid and 1.0ml of p-dimethylaminobenzaldehyde. The flask solutions were mixed thoroughly, and a further (1.0ml of 18N sulphuric acid added, followed by further mixing. The flasks were lightly stoppered, and placed in a water bath, thermostatted at 70°C±0.1°C, for 2 hours, shaking every 15 minutes. The flasks were then removed, and to each flask, 2.0ml of 0.01M sodium nitrite was added. The total volume of liquid was made up to 50ml with distilled water, and mixed thoroughly. Each flask was then placed in a second water bath set at 60°C ± 0.1°C for 2 hours, shaking every 15 minutes. Finally the solutions were filtered through a No.3 sinter, and allowed to cool.

The optical density of each solution was measured at 585nm using a Cecil variable wavelength UV spectrometer. The intensity of the blue colour formed decreases with time, so the optical densities were always measured within an hour after removal from the water bath.

A calibration graph was drawn, based upon the four known tryptophan solutions, from which the optical densities of the unknown solutions were converted into concentration values. A good straight line was obtained for the range of tryptophan solutions studied, indicating a linear relationship between the optical density of the solutions and the concentration of tryptophan present under these experimental conditions. See Figure 2.9.

The following wool samples were analysed by this method, each one in triplicate:-

1) Untreated wool
2) Peroxide bleached wool
3) Fluorescently whitened wool
4) Fluorescently whitened wool, photoyellowed 3 days
5) Blankit D treated wool

The results, given in Table 2.18, were calculated using the following method:-

Consider a 200mg dm^{-3} standard solution. 1ml (as used in the analysis) contains $\frac{200}{1000}$ mg. ie. 0.0002g. An optical density corresponding to 200mg dm^{-3} therefore indicates the presence of 0.0002g tryptophan in that flask.

Therefore, if we use 0.06g wool and obtain an optical density corresponding to 200mg dm^{-3}, it follows that the % tryptophan = $\frac{0.0002}{0.06} \times 100\%$ = 0.33% Trp.

In each case, the results given in Table 2.18 are the mean values calculated from the triplicate analyses.

Fig 2.9 Colorimetric Tryptophan Analysis of Wool

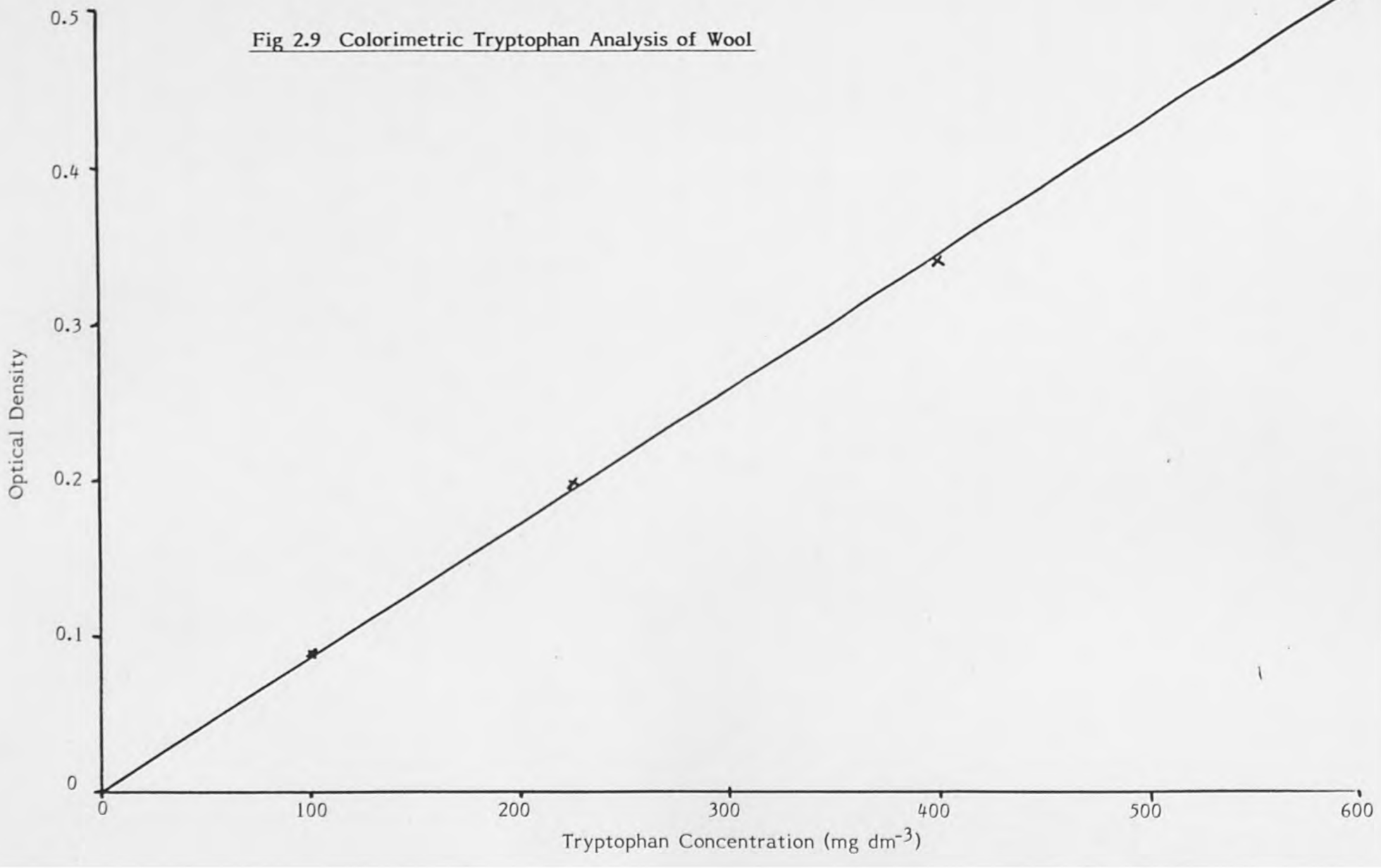


Table 2.18 Colourimetric Tryptophan Analysis of Wool

SAMPLE DESCRIPTION	TRYPTOPHAN %
Untreated Wool	0.81
Peroxide Bleached Wool	0.76
Fluorescently Whitened Wool	0.64
Fluorescently Whitened Wool photoyellowed for 3 days	0.56
Blankit D Treated Wool	0.28

These results were consistent with those obtained by Cegarra and Gacen, [48], where untreated wool had a tryptophan content of approximately 0.75%, varying with the type of wool used. The fluorescently whitened wool was irradiated for 3 days to give severe yellowing. It is doubtful whether lesser degrees of yellowing would give rise to a significant change in tryptophan content using this method of analysis, since whilst only surface degradation occurs, analysis is of the bulk of the sample.

It is clear from Table 2.18 that small changes in tryptophan content occurred during bleaching and brightening treatments, but the Blankit D process appears to have modified 60-70% of all tryptophyl residues in the fibre. This is perhaps a little surprising, because it was only one surface of the fibre which was irradiated during the treatment. This suggests either that tryptophan reacts with Blankit D as a dark reaction, or that singlet oxygen, formed at sites near the wool surface, diffuses into the fibre and oxidizes tryptophyl residues which are subsequently reduced by the Blankit D. The first possibility is rendered unlikely by HPLC studies of N-Acetyltryptophan and Blankit D (described later) which show little evidence of reaction when left standing in the dark for several days.

If the second were true, one would expect to see a similar change in tryptophan content of wool irradiated in the absence of Blankit D, unless that process gives rise to residues which still undergo the reaction with Ehrlich's reagent. Whatever the reason, however, it is clear that tryptophyl residues are extensively modified by Blankit D, in such a way that both the 2- and 3- positions on the indole ring are substituted.

2.10 Studies on the Degradation of N-Acetyl Tryptophan in Solution Monitoring the Reaction by U.V. Spectroscopy

Having determined that tryptophyl residues were markedly affected by the Blankit D treatment, tryptophan photodecomposition was studied in aqueous solution and compared with that of tryptophan in 2% Blankit D solution. N-Acetyltryptophan was used for solubility reasons.

2.10.1 Experimental Results

A solution of N-Acetyltryptophan was made up to OD 1.4 at 290nm. The solution was placed in a quartz cuvette, and aerated with oxygen for 3 minutes. The UV spectrum of the solution was run on a Perkin Elmer 402 scanning UV spectrometer, following which, the cuvette was placed in a photochemical reactor consisting of a circular array of 16x8W (RPR 3000A) Rayonet tubes of λmax 300nm, and irradiated for 1 minute. The UV spectrum was rerun, and the solution irradiated for a further minute. This process was repeated until the photodegradation appeared to have reached completion, spectra having been run after 0,1,2,4,6,10,20,40 and 80 minutes. These are shown in Figure 2.10. The resultant solution was clearly yellow. The above sequence was repeated, with the N-acetyl-tryptophan dissolved in 2% w/v Blankit D solution. In this case, spectra were run after 0,1,2,4,8,16,32 and 64 minutes. The resultant solution was colourless, and the spectra are shown in Figure 2.11. Finally the photodecomposition of N-acetyltryptophan was run again in water, and when after 80 minutes the solution was visibly yellow, 0.1g Blankit D was added (0.1g in 5ml is equivalent to 20g/litre). The solution was slightly decolourized, but the U.V. absorption remained very similar to that shown in Figure 2.10.

From these two spectra it is clear that extensive photodegradation occurs in both cases, but the products formed are different. In the absence of Blankit D, the products have a very broad absorption band stretching out into the blue region of the spectrum, whereas in the presence of Blankit D, only the residual tryptophan absorption is observed. Because of the strong absorption of Blankit D below 265nm, no information can be obtained about the products absorbing in this region. However, this experiment has confirmed that irradiation in the presence of Blankit

Fig 2.10 Photodegradation of N-acetyl Tryptophan Followed by U.V. Spectroscopy

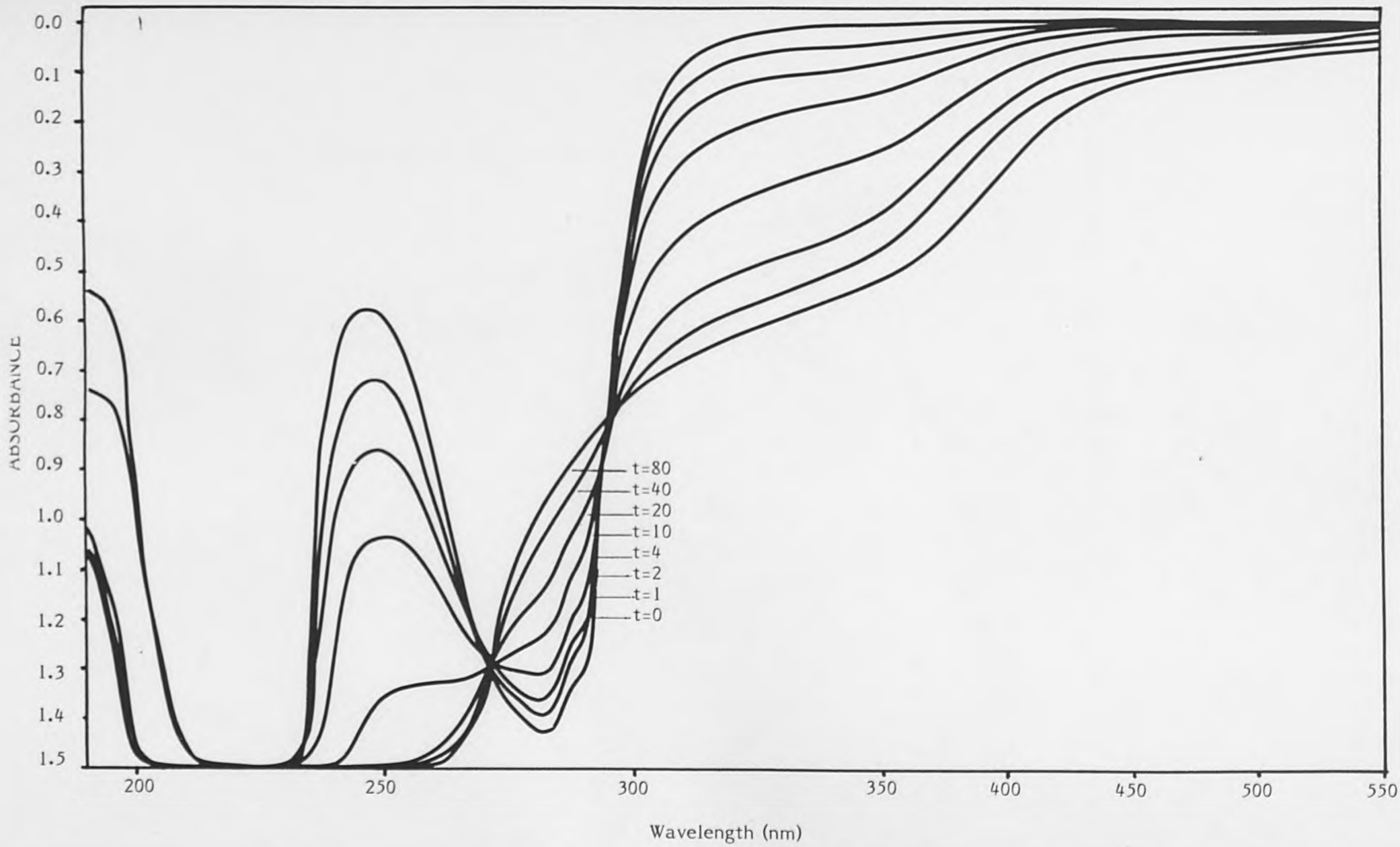
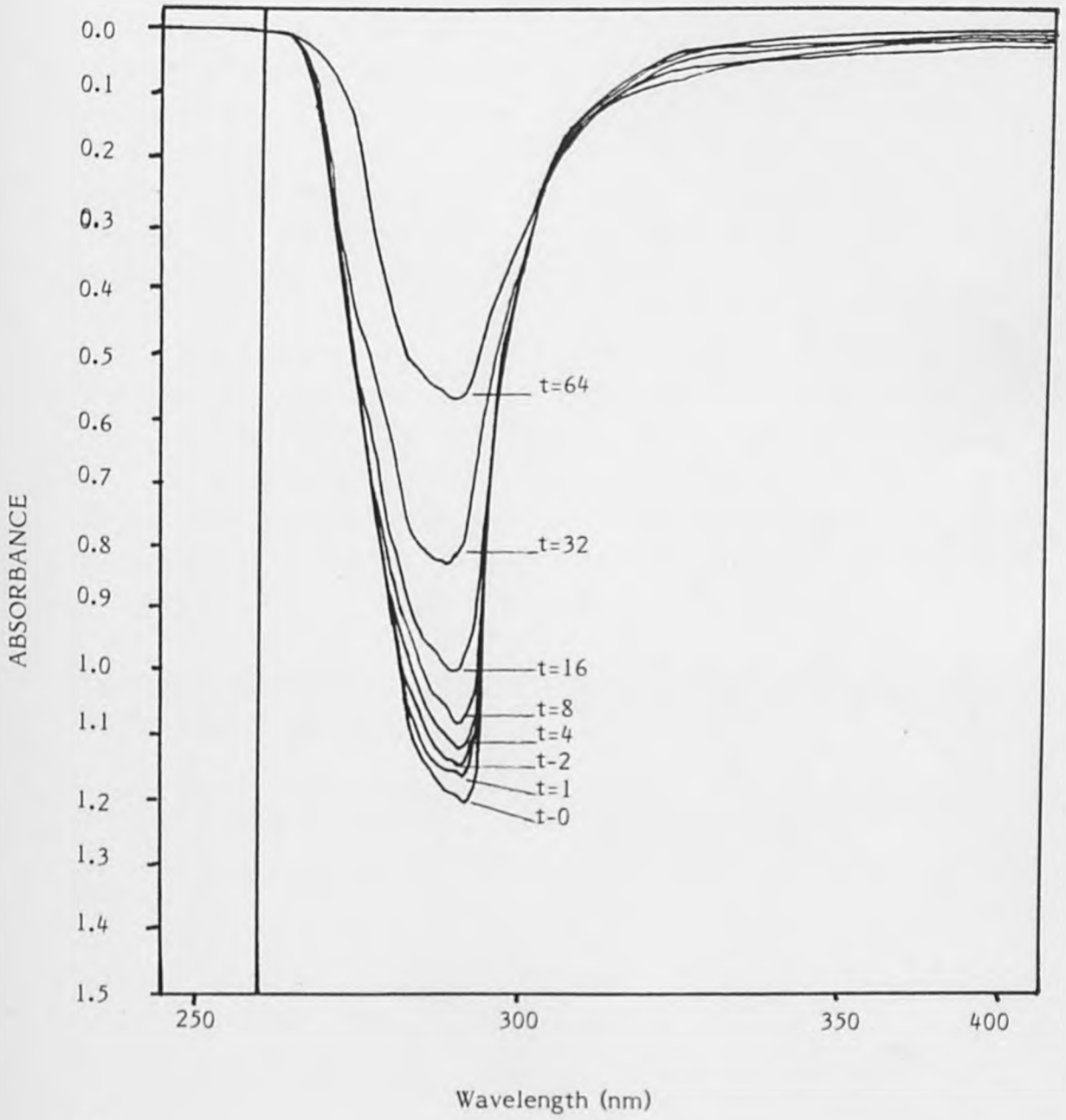


Fig 2.11 Photodegradation of N-acetyl Tryptophan in 2% Blankit D Solution



D solution does modify a large percentage of tryptophan present, in agreement with the results obtained from the colorimetric determination of wool.

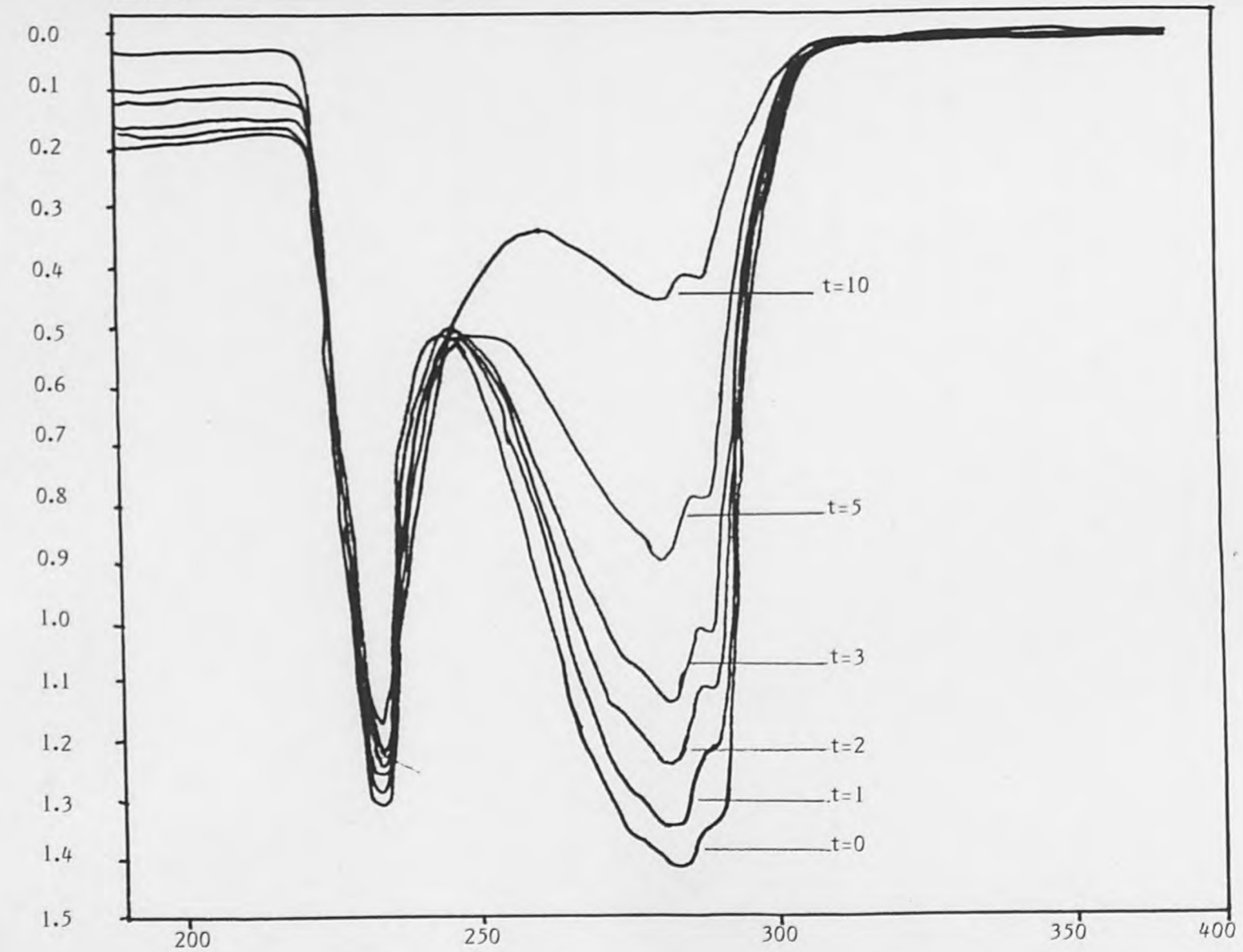
To assess the significance of the Blankit D acting as a reducing agent, these experiments were repeated using sodium borohydride and T.H.P.C., both of which are known reducing agents for wool and have been found to decrease the rate of photoyellowing of the wool fibre. In the presence of the borohydride solution, N-acetyl tryptophan was found to photodegrade quite rapidly to yield colourless products, as shown in Figure 2.12. However, in the case of T.H.P.C., virtually no decomposition occurred at all, even after 2 hours of irradiation at 300nm. This marked difference in stability was attributed to the oxygen-scavenging properties of T.H.P.C., which would thus inhibit the photooxidation pathway. The results obtained with sodium borohydride lend credance to the suggestion that the reducing power of Blankit D plays a significant part in the mechanism followed to achieve the photostabilization effect.

The decrease in optical density of the N-acetyl tryptophan solutions was plotted against time, and the initial rates calculated. These are shown in Table 2.19, and indicate that initially, Blankit D slows down the rate of degradation whereas sodium borohydride has the opposite effect. However, only initial rates can be determined, due to the overlap of the product absorption bands with those of the tryptophan (illustrated in Figure 2.10).

Table 2.19 Comparison of the Initial Rates of Photooxidation in Different Reducing Agent Solutions.

N-Acetyl Tryptophan Solutions	Initial Rate of Decomposition (OD units per minute)
Water	0.035
Blankit D 2% aq.	0.018
T.H.P.C. 2% aq.	0.006
Sodium Borohydride 2% aq.	0.099

Fig 2.12 The Photodegradation of N-acetyl Tryptophan in the Presence of Sodium Borohydride Solutions



2.11 Use of HPLC to Follow N-Acetyl Tryptophan Degradation

2.11.1 Introduction to HPLC

In order to elucidate the photodegradation pathway of N-Acetyl tryptophan in aqueous solution, it was necessary to obtain information concerning the products formed, and to observe how the degradation in Blankit D solution differed from that in water. For this purpose, High Performance Liquid Chromatography, (HPLC) was chosen.

HPLC is a technique which has been developed during the last 25 years as the liquid analogue of gas chromatography. The stationary phase, which may be a solid surface, a liquid, an ion-exchange resin or a porous polymer, is packed in a metal or glass column and the liquid mobile phase is forced through under pressure, thus achieving much more rapid separations than were possible using conventional liquid chromatography. HPLC has several other advantages over older methods:

i) The columns can be used many times without regeneration;
ii) Resolution far exceeds that of conventional column chromatography;
iii) Reproducibility is greatly improved, the technique being less dependent upon the operators' skill;
iv) The instrumentation lends itself to automation; and
v) Preparative liquid chromatography is possible on a much larger scale.

At this point it is pertinent to note briefly the terminology of HPLC, as used in this thesis. The sample is dissolved in a SOLVENT, and ELUTED from a packed column, where the column packing is the STATIONARY PHASE, and the ELUENT is the MOBILE PHASE. Ideally, the eluent should be used as solvent, but this is frequently not the case. In such circumstances, the sample components must be soluble in the eluent to avoid blocking the column.

Several modes of chromatographic separation are used. In many cases, more than one mode will operate simultaneously for a given column. Four of these are discussed below:

1) Adsorption Chromatography

This is the original form of classical column chromatography, and was developed by Tswett in 1903-6 [49]. Separation is effected with a liquid mobile phase, and a solid stationary phase which reversibly adsorbs the solute molecules.

2) Partition Chromatography

The technique was developed by Martin & Synge in 1941, and involves a liquid stationary phase which is either dispersed onto a finely divided inert support or chemically bonded to the support material. The sample reaches equilibrium between the stationary and mobile phases according to its partition coefficient. This leads to a differential rate of migration, and separation occurrs.

3) Ion-Exchange Chromatography

I.E.C. has been used for over thirty years, and involves the substitution of one ionic species for another. The stationary phase consists of a rigid matrix, the surface of which carries a net positive or negative charge. The mobile phase is a solution of selected counter ions, which are attracted and held by the stationary phase. These counter ions may then exchange with ions of similar charge in the sample. The relative ability of sample ions to exchange with those from the eluent buffer at charged sites on the stationary phase results in some components being retained longer than others and is thus the mechanism of separation.

4) Gel-Permeation Chromatography

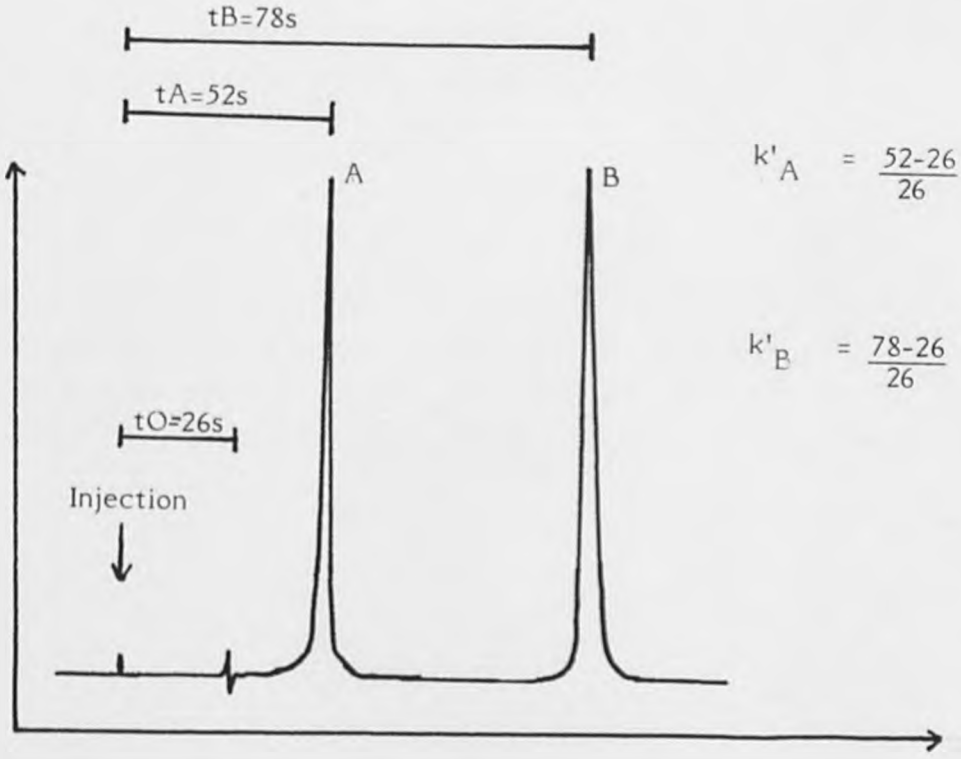
This method was introduced by Pharmacia in 1959, when a dextran gel in bead form was marketed under the name of Sephadex. Also known as size exclusion chromatography the technique separates substances according to their molecular size and shape. Small molecules enter freely into the pores of the stationary phase, and are thus retained on the column, whilst very large molecules are excluded and move rapidly through the column. Molecules are thus eluted in order of decreasing molecular size.

In the early days of HPLC development, the silica packing material was typically of particle size between 40-50μ. It has since been found that the column efficiency is increased by reducing the particle size, reaching a maximum value at 3μ. This, however, leads to practical difficulties, firstly because it is not easy to achieve good column packing with such fine particles, and secondly because very high back pressures are incurred. 3μ silica is thus used only for short columns, and 5μ packing material accepted as a compromise for most other applications.

Whereas in Thin Layer Chromatography (T.L.C.) the relative retention times of spots are quoted, for HPLC peaks the column capacity ratio, k' is used,

$$k' = \frac{tr - to}{to}$$

where 'tr' is the retention time of a given peak, and 'to' is the retention time of the unretained solvent peak.



$$k'_A = \frac{52-26}{26} = 1.0$$

$$k'_B = \frac{78-26}{26} = 2.0$$

Strictly speaking, retention volumes should be quoted in calculations, rather than retention times. However if constant flow rates can be assumed, retention times are more convenient to use and can be obtained directly from the chromatogram. The k' values are used in preference to retention times alone because they remain constant, while the latter vary with flow rate and temperature.

Another important chromatographic parameter is the equilibrium distribution coefficient, K.

$$K = \frac{Cs}{Cm}$$

Where Cs is the sample concentration in the stationary phase, and Cm is the sample concentration in the mobile phase. For a component to travel along the column, it must be in the mobile phase. Hence the rate of migration is inversely poportional to its distribution coefficient, K. Unless two sample components have different K values, no separation can be achieved.

The efficiency of a chromatographic system may be defined from a single peak by the "number of theoretical plates", N.

$$N = 16 \left(\frac{tr}{w} \right)^2$$

Where tr is the retention time of the peak and w is the peak width at the base line, measured in units of time. Under a given set of conditions, N is approximately constant for different bands in the chromatogram, and is therefore a measure of column efficiency. See Fig. 2.13.

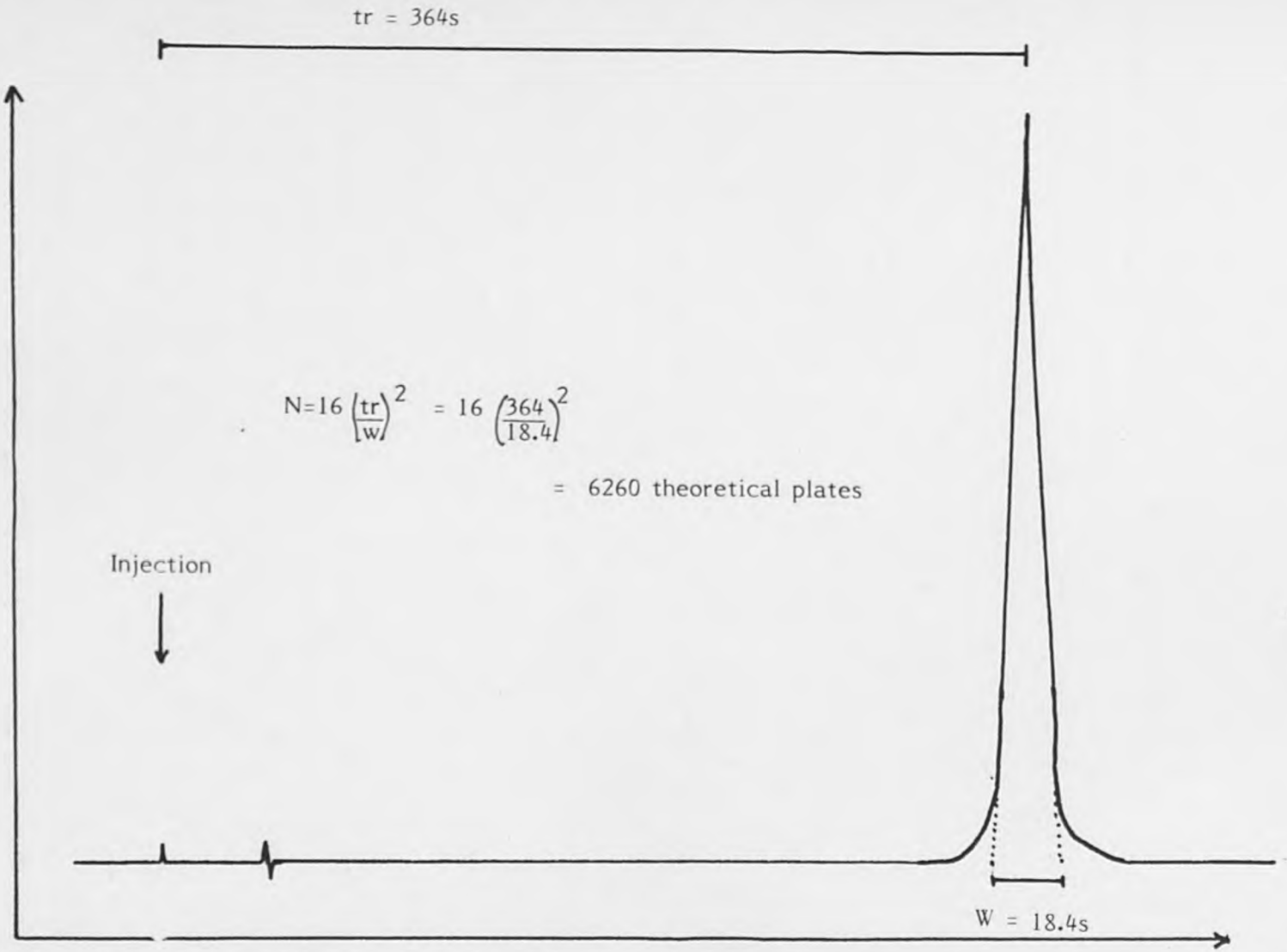


Fig 2.13 Calculation of Column Efficiency

Another useful parameter is the Height equivalent to a theoretical plate (H.E.T.P.)

$$H = \frac{L}{N}$$

Where L is the length of the column. The plate height varies with the linear velocity of the eluent, (or for a given column, with flow rate), a maximum efficiency being achieved usually between 0.1 - 0.5ml/min. Since separation time is also important, a compromise is made and columns are normally used at considerably higher flow rates.

The resolution of two chromatographic peaks, Rs, is a measure of their separation, and is calculated from the equation:

$$Rs = 2\left(\frac{t_2 - t_1}{w_1 + w_2}\right)$$

Where t_1 and t_2 are the retention times of the peaks and w_1 and w_2 are the peak base widths for two gaussian peaks. Effective baseline separation is achieved when Rs is at least 1.5. Three factors contribute to the resolution, namely column selectivity, α, the capacity factor, k', and the column efficiency, N. The selectivity, α, is defined as the ratio of the k' values of the two peaks of interest:

$$\alpha = \frac{k'_2}{k'_1}$$

Obviously α must not equal unity. H may be optimized by increasing the temperature **[50]**, or by altering the mobile phase composition. Such changes will also affect the k' values of other components of the sample, and a compromise situation may be necessary. The column efficiency may be improved by increasing the column length or decreasing the stationary phase particle size.

The apparatus used in classical liquid chromatography was very simple, consisting of a solvent reservoir, a wide-bore glass column and a series of collecting tubes for the eluent. To achieve the higher efficiency and resolution obtained with HPLC., a faster liquid flow rate must be used.

It must also be possible to achieve faster equilibration of the sample between the stationary and mobile phases, so that the resolution keeps pace with the increased flow rates. These are obtained by pressurizing the mobile phase and using a finer particle size column packing.

Shorter columns must be used to avoid excessive back pressures resulting from the decreased permeability of the columns. Further, the column diameter may be reduced to increase the linear velocity for a given flow rate. It follows from this that smaller samples are required to prevent overloading these columns, and detection systems used must be capable of monitoring micro-quantities resolved from very small samples.

A typical H.P.L.C. instrumentation is shown in Figure 2.14.

The main components of the chromatograph are a high pressure pump, a column/injector system and a detector. The pump is usually of a constant volume design, such that the flow rate produced is unchanged by variations in eluent viscosity, or by settling or swelling of the column packing material. This is important because flow changes cause non-reproducible retention times, poor resolution and an unstable baseline. Two types of constant volume pump are normally used, namely the syringe pump and the reciprocating pump.

The syringe pump consists of a syringe, the plunger of which is driven by an electrically powered motor and pressurizes a given volume of eluent, typically 50-250ml. The pump delivers a pulse-free flow of solvent at high pressure (up to 7500 psi). Its main disadvantage is that it has a finite capacity, and hence this type of pump is normally used on automated instruments for routine multiple analyses, such as quality control work. The reciprocating pump uses electrically driven pistons to pressurize either the eluent directly, or hydraulic fluid, acting via a diaphragm. A pulsating flow of eluent is produced (usually between 25 and 100 strokes per minute, though one instrument produces up to 18 pulses per second) giving a time-averaged flow rate, dependent upon the pumping rate and the stroke length of the piston. This type of pump has the advantage of continuous delivery, and by making the pump volume very small, baseline noise can be minimized. At low pressures, however, a pulse damper is required.

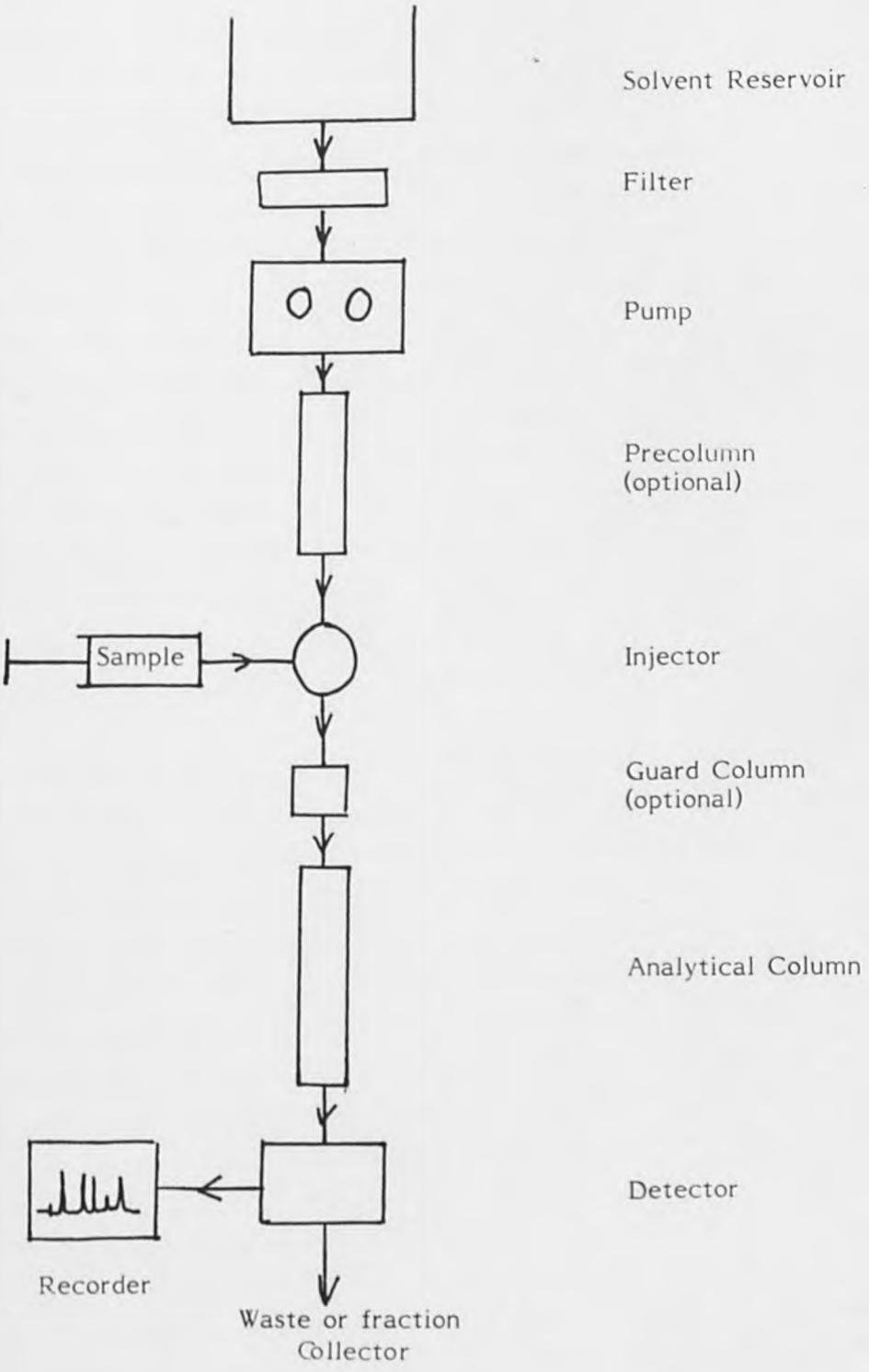
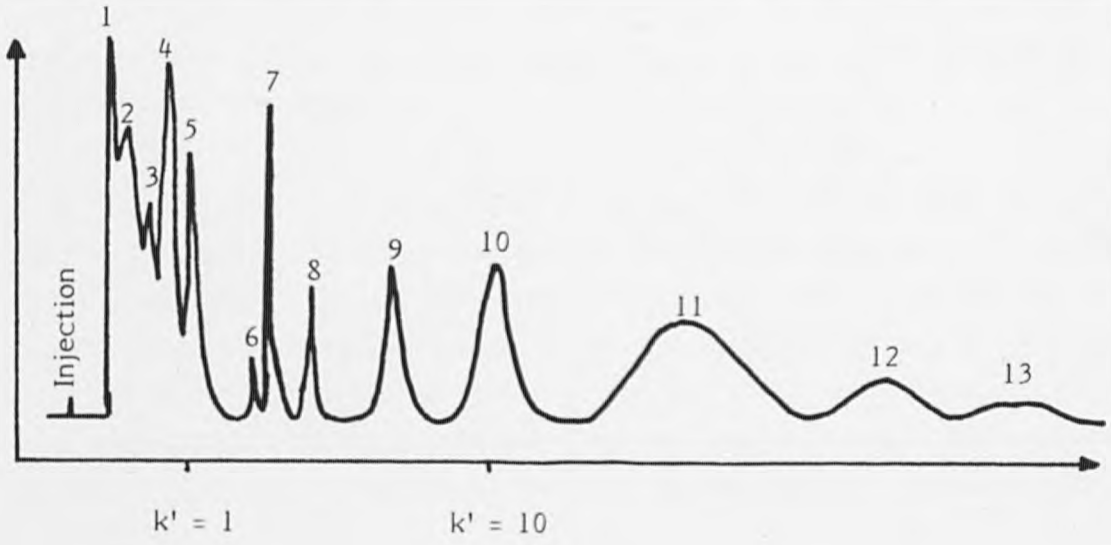


Fig 2.14 Typical HPLC Instrumentation Layout

The column/injector system is where the efficiency of the system can easily be lost. The most important considerations are the column dimensions, the means by which the column packing is terminated at each end and the type of injection system used. For normal analytical HPLC, a 4.6mm internal diameter (id) column is used, between 10 and 25cm long. Recently, microbore columns have been developed using only 1.0mm id. Larger columns are used for preparative scale separations. End fittings should have zero dead volume for maximum efficiency, and connecting tubing from the column to the detector likewise. Injection can be made either directly, using a syringe, or by means of a valve. It has been shown [51] that the most efficient chromatography can be expected if the solute, introduced centrally at the top of the column as a point injection, does not reach the wall of the column as it migrates down. However, direct syringe injections can disturb the packing, and are not practical at high pressure operation. Hence, valve injection is almost invariably used today. The sample is injected into a loop at atmospheric pressure. Once the valve is operated, the pressurized eluent flows through the loop and sweeps the sample onto the column.

Two basic types of detection system have been developed for liquid chromatography, involving (i) the differential measurement of a property common to both sample and mobile phase (eg. refractive index, conductivity or dielectric constant), or (ii) the measurement of a property that is specific to the sample, either with or without the removal of the mobile phase before detection (eg. U.V. absorption, fluorescence, polarographic or radio activity detectors). U.V. detection is by far the most commonly used, measuring the change in U.V. absorption as the solute passes through the flow cell in a tranparent solvent. If a variable wavelength instrument is available, monitoring close to the sample absorption maximum optimizes the sensitivity. For molecules of high extinction coefficient (E = 10000 - 20000), a lower detection limited of one part in 10^9 may be achieved. Fluorescence detectors are even more sensitive, but are obviously restricted in their application to molecules which are natural fluorescers, or which form fluorescent derivatives. Refractive Index dectectors are also widely used, but are far less sensitive, and require a considerable degree of temperature stability(± 0.001°C at high attenuation).

In an ideal HPLC separation, all the components of the sample should elute with capacity factor in the range $1 < k' < 10$ under a given set of conditions. Unfortunately, the range is often wider than this, yielding a chromatogram such as that drawn below:



This is known as the General Elution Problem, and is common to all forms of chromatography. The problem is usually overcome using the gradient elution technique, where the composition of the mobile phase is varied continuously throughout the analysis. Using a weaker eluent better resolution of peaks 1-4 could be obtained, and as the eluent strength increased, the later peaks would become sharper and the retention times reduced. This method is incompatible with the use of refractive index detectors, but is quite satisfactory whena U.V. detector is selected.

2.11.2 Equipment Used

The equipment used for this work was as follows:-

A Rheodyne Injection Valve
Waters M1000A Reciprocating Pump
Cecil Variable Wavelength U.V. Detector
Perkin Elmer Fluorescence Detector

Two columns were used, both packed at the City University with Shandon ODS-hypersil 5μ. One was 12.5cm long, 4.6mm internal diameter; the other was 30cm long, 9.4mm i.d. and was used for the semi-preparative scale separations. At all times, a guard column was used, packed with Co-Pell ODS (Whatman).

Solvents were always filtered at source, and samples filtered prior to injection using a Gelman 0.45μ disposable membrane filter unit. All solvents were Fisons HPLC grade, and were degassed by vacuum/ultrasonification techniques prior to use. Separations were always carried out at ambient temperature, no column oven being available. Prior to use, each column was equilibrated with the mobile phase for half an hour at 2ml min^{-1}, and flushed after use with methanol for half an hour at the same flow rate.

2.11.3 Photodegradation in Water

The initial problem was to determine the optimum eluent composition, which would elute the N-acetyl tryptophan with a capacity factor (k') of around 2.5.

Once this could be achieved, many of the degradation products could be separated and observed without the need for gradient elution.

The HPLC system was fitted with the 12.5cm x 0.46mm ODS column, and the UV detector set at 290nm, (λ max of N-acetyl tryptophan = 278nm). Methanol:Water was selected as the mobile phase, and trial injections of a fresh 0.15% w/v solution of N-Acetyl tryptophan were run for a range of eluent compositions. It was found that the peaks were very

broad and tailing, and this was attributed to the equilibrium:-

$$R\text{-}COOH \rightleftharpoons RCOO^{\ominus} + H^{\oplus}$$

Addition of 10% v/v glacial acetic acid to the mobile phase resulted in much sharper peaks being observed, having displaced the above equilibrium over to the left. It was finally concluded that the optimum eluent composition at this stage was 70% water:20% methanol: 10% glacial acetic acid. Using a flow rate of 2.0ml min^{-1} , the capacity factor of N-acetyl tryptophan was calculated to be 2.520.

A solution of N-acetyl tryptophan (0.15%, w/v) was made up in distilled water, and irradiated in a quartz photolysis tube for 24 hours using the circular array of 16x8 Watt (RPR 3000A) Rayonet tubes at 300nm. This caused extensive photodegradation to occur, giving rise to a brown solution with a brown/black sediment in the bottom. A 20ml sample of this solution was filtered using a glass syringe fitted with a 0.45μ disposable membrane filter to remove the particulate material. This solution was then injected into the HPLC system (20μl) and run at a flow rate of 2.0ml min^{-1}.

The resultant chromatogram is shown in Fig. 2.15. It can be clearly seen that there were a large number of products, all present in very low concentrations. This was not surprising, since it has already been shown in Chapter 1 that a number of breakdown pathways exist, giving rise to a diversity of products.

The photodecomposition procedure was repeated using as a light source a circular array of 16x8W (FT8 75/BLB) Sylvania black light tubes with a λmax of 350nm. Because of the low molar extinction coefficient of tryptophan at this wavelength, and to a lesser degree, the lower energy of the radiation, this was not expected to cause such rapid degradation . Again the irradiation period was 24 hours, and on this occasion, the resultant solution was a pinkish brown, with considerably less sediment in the tube. A filtered sample of this solution was run on the HPLC, the chromatogram being shown in Fig.2.16. This also showed many small product peaks, with no major product, suggesting again that several reaction pathways were followed. Addition of Blankit D to this solution reduced the coloration slightly but did not result in any observable change in the chromatogram

Fig 2.15 N-acetyl Tryptophan Solution, Irradiated at 300nm

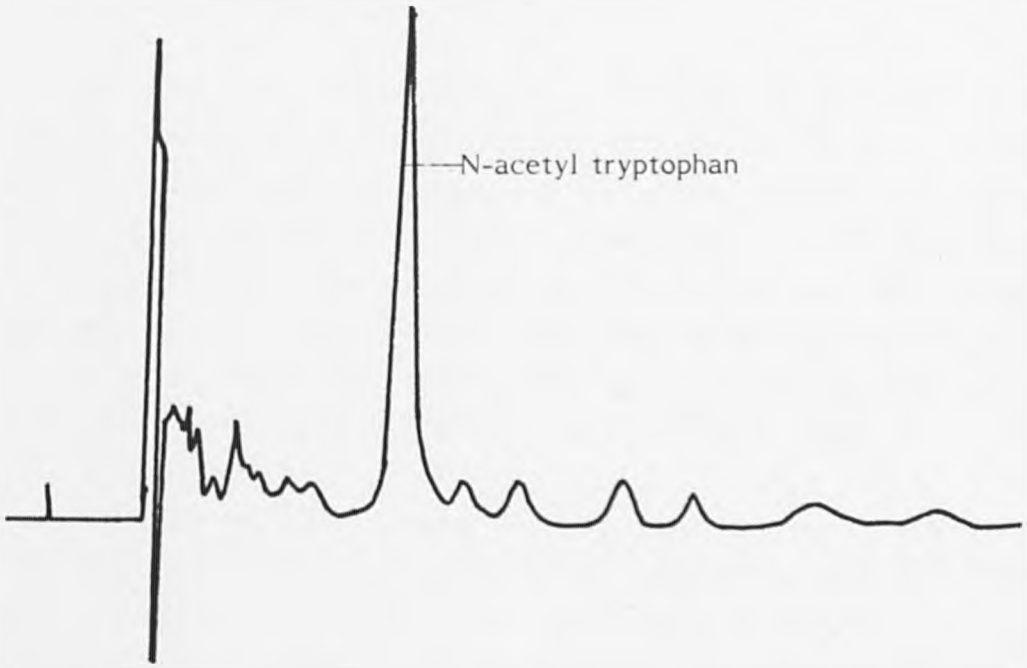
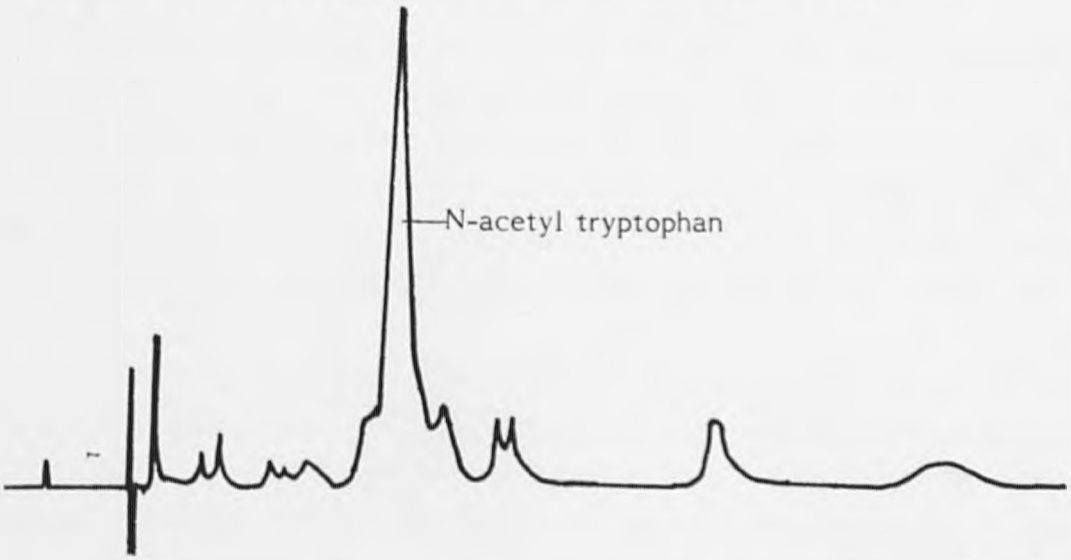


Fig 2.16 N-acetyl Tryptophan Solution, Irradiated at 350nm



No colour change at all was seen when Blankit D was added to the solution irradiated at 300nm.

2.11.4 Photodegradation in Blankit D Solution

By way of a direct comparison, the photodegradation of N-acetyl tryptophan was repeated in solutions of Blankit D (2.0% w/v). Irradiating at 350nm, the extent of decomposition was very limited, and no colour change was observed, even after 7 days exposure. The chromatogram of the solution is shown as Figure 2.17, from which it can be seen that very few product peaks existed, the majority of the starting material remaining unchanged. As before, this was not surprising because, unlike wool, the solution had a very low absorption of light at 350nm.

The use of 300nm lamps, however, gave a very different result. The most noticeable change to the solution was the formation of a metallic grey coating on the inside of the quartz tube after only a few hours, and a grey/brown sediment in the bottom. The solution was otherwise only slightly discoloured after 24 hours irradiation. When left to stand for a few hours, either in daylight or in the dark, most of the grey deposit disappeared, but was reformed after further 300nm irradiation. The solution was filtered and run on the HPLC, giving the chromatogram shown in Figure 2.18. This shows one very interesting feature, namely a single product peak many times larger than all the rest.

This indicated that in the presence of Blankit D, the photodegradation appears to be channelled into one particular pathway, with one major product being formed. The implication of this is that the stabilization of wool to photoyellowing by irradiation in Blankit D solution operates by blocking the decomposition pathways which lead to coloured products, and directing the reaction towards this new species, not previously seen.

The rate of formation of this product, and its stability to ultraviolet light were studied next. The photodegradation was repeated, taking samples after 2,4,6,8,10,24,48,72 and 96 hours. Analysis of these samples by HPLC showed that the product formation commenced immediately, a small peak being observed after two hours irradiation. This increased steadily

Fig 2.17 N-acetyl Tryptophan in Blankit D Solution, Irradiated at 350nm

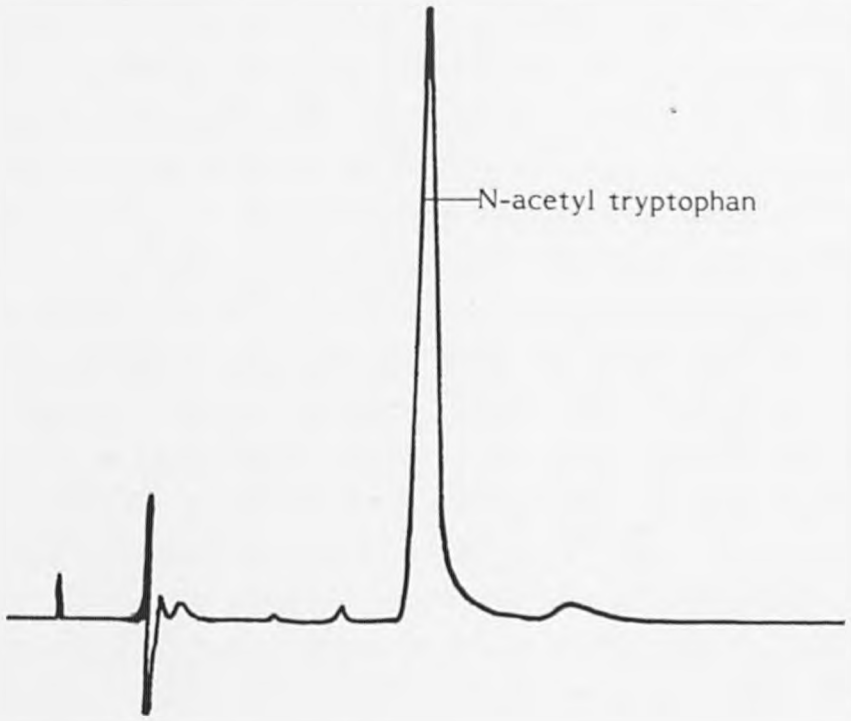
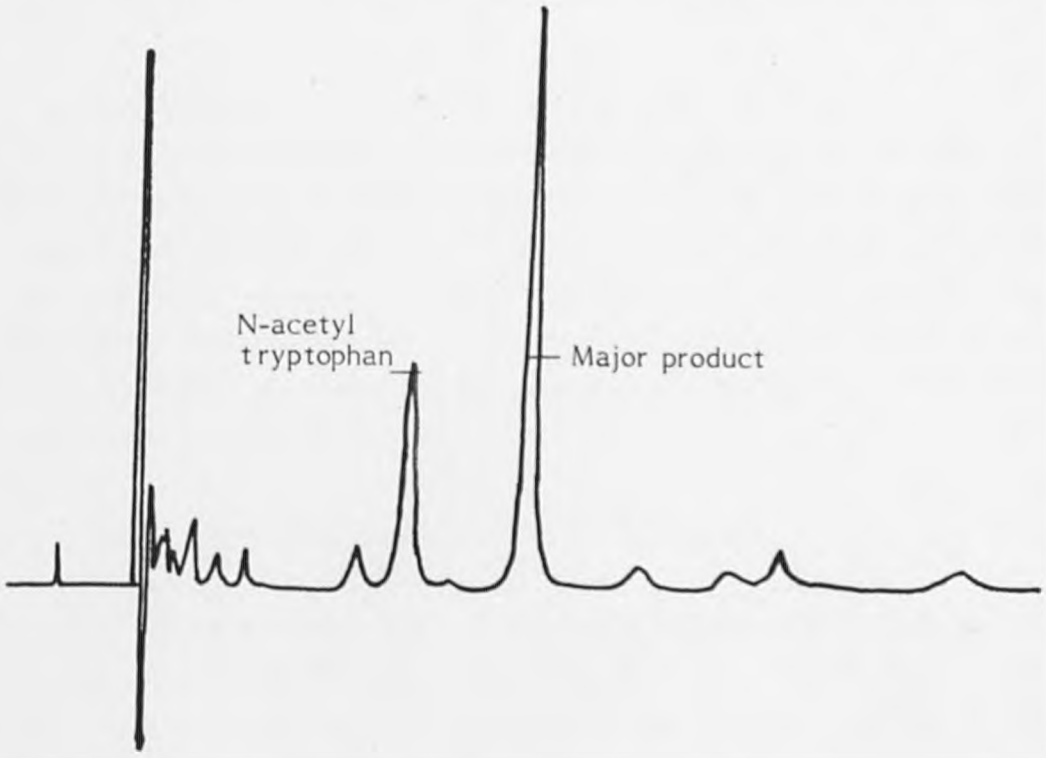


Fig 2.18 N-acetyl Tryptophan in Blankit D Solution, Irradiated at 300nm



to a maximum after 48 hours, when approximately 80% of the original N-acetyl tryptophan had decomposed. After this period, the new peak was slowly degraded, but less rapidly than the rate of disappearance of the starting material. The presence of the metallic layer on the inside of the tube (formed within the first 2-4 hours of irradiation at 300nm) rendered more precise kinetic studies impossible. Its existence greatly reduced the transmission of light into the tube, and consequently it was necessary to illuminate the reaction intermittently, allowing a two-hour interval every six hours to allow the layer to settle out or re-dissolve. The only metal known to be present in the system was zinc in the Blankit D (Zinc formaldehyde sulphoxylate). The colour of the deposit was similar to that of powdered zinc, and this was assumed to be the source. By way of confirmation, a solution of Blankit D (2.0% w/v) was irradiated at 300nm for 24 hours in the absence of tryptophan, whereupon a grey deposit was again formed on the walls of the quartz tube, albeit far less intense. This clearly identified the Blankit D as the source of the metal, but also suggested that the zinc formaldehyde sulphoxylate was undergoing a reversible photodecomposition reaction in the 300nm light, possibly yielding a species which was reactive towards the tryptophan, liberating free metallic zinc. This process was believed to be reversible because the zinc was taken back into solution shortly after the lamps were turned off.

The retention time of the product peak was slightly longer than for N-acetyl tryptophan. Using reversed phase partition chromatography, this indicated that it was slightly less polar than the starting material. The U.V. absorption was examined by stopping the pump and observing the change in detector signal as the wavelength was manually scanned. Repeating this process for the tryptophan peak showed that, within the high level of approximation necessary with the method used, the two species contained very similar chromophores.

2.11.5 Photodegradation in Rongalit C Solution

A comparison was made at this stage, between the effects of reaction in the presence of Blankit D and of Rongalit C. It had been found that the latter offered only minimal protection to wool, whereas a marked

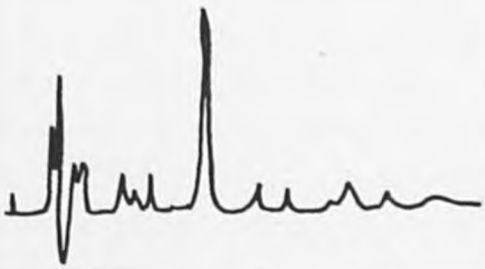
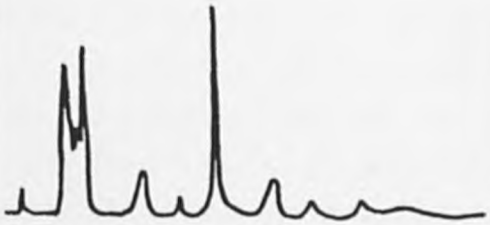
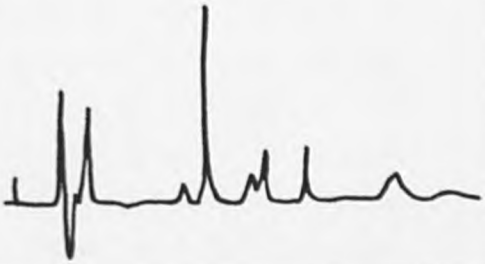
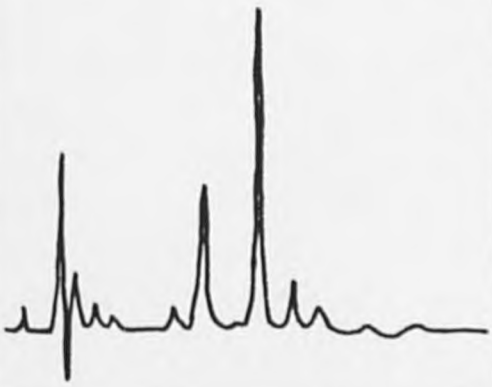
increase in stability was observed following treatment in Blankit D solution. For this purpose N-acetyl tryptophan (1.5g dm^{-3}) was dissolved in water, 2% Blankit D and 2% Rongalit C solutions respectively. Using quartz photol -ysis tubes, 60ml of each of these solutions was irradiated simultaneously in the 300nm reactor for 42 hours. In the 350nm reactor, a duplicate set of tubes was irradiated for 168 hours.

As before the Blankit D solution became only slightly discoloured under 300nm irradiation, with the grey metallic coating formed on the inside of the quartz tube. A very different result was observed for the Rongalit C solution under these conditions. A high degree of discolouration occurred and a whitish grey sediment was formed at the bottom of the tube. No metallic deposit was seen but this was as expected, since Rongalit C is the sodium equivalent of Blankit D. HPLC analyses showed that the Rongalit had reduced the extent of the degradation slightly (as compared with N-acetyl tryptophan above), but no major product formation was found. Thus from the above result it would appear that the product formed in the Blankit D solution is directly related to the stabilizing effect conferred by that treatment, and its lack of formation in the presence of Rongalit C is a possible explanation for the poor protective effect which follows. The chromatograms for the above experiments are shown together in Figure 2.19, together with those for the solutions irradiated at 350nm for 168 hours. The latter show that whereas virtually no degradation occurs in the presence of Blankit D, an appreciable amount was observed in the Rongalit C solution. This in turn was less than that observed for N-acetyl tryptophan alone. So although some protection is seen with the sodium formaldehyde sulphoxylate, it is again very much less than with the zinc salt.

2.11.6 Photodegradation Under Oxygen & Under Argon

The photodegradation of wool is known to be a photooxidation process, and has been shown (see Chapter 1) to be greatly retarded by the exclusion of oxygen. If the reaction of N-acetyl tryptophan in the presence of Blankit D is also a photooxidation process, the same decrease in rate might be expected under anaerobic conditions.

Fig 2.19 Comparison of the Photodegradation of N-acetyl Tryptophan under Different Conditions, Followed by HPLC

	300nm Irradiation	350nm Irradiation
N-ac-trp		
Rongalit C		
Blankit D		

It was therefore required to know the effect of oxygen on the rate of this reaction under 300nm light, and also, for comparison on any "dark reaction" which might occur between the tryptophan and the Blankit D. Virtually no reaction had been detected by U.V. spectroscopy for solutions standing in the dark, but this was now to be studied by HPLC.

A solution containing 5g dm^{-3} N-Acetyl tryptophan (0.05% w/v) and 20g dm^{-3} Blankit D (2% w/v) was made up in distilled water, and used throughout this experiment. From this stock solution, 60ml was placed in each of four quartz photolysis tubes. Two were flushed with oxygen for five minutes and two were flushed with argon for the same time period. Flushing was effected using a pasteur pipette, drawn out to a fine point in a microburner flame, through which small bubbles of gas could pass at a constant flow rate from a gas cylinder. In this way, each tube was degassed or aerated to the same extent.

One oxygen-flushed and one argon-flushed tube were placed in the 300nm photochemical reactor and irradiated for three days continuously. It was found that pressure in the degassed tube had built up and the stopper blown off, thus allowing the ingress of air, so this part of the experiment was repeated using a rubber suba septum instead of a glass stopper. This allowed the pressure to be relieved each day without admitting air to the tube. The other two tubes were placed in a dark cupboard at room temperature, also for three days.

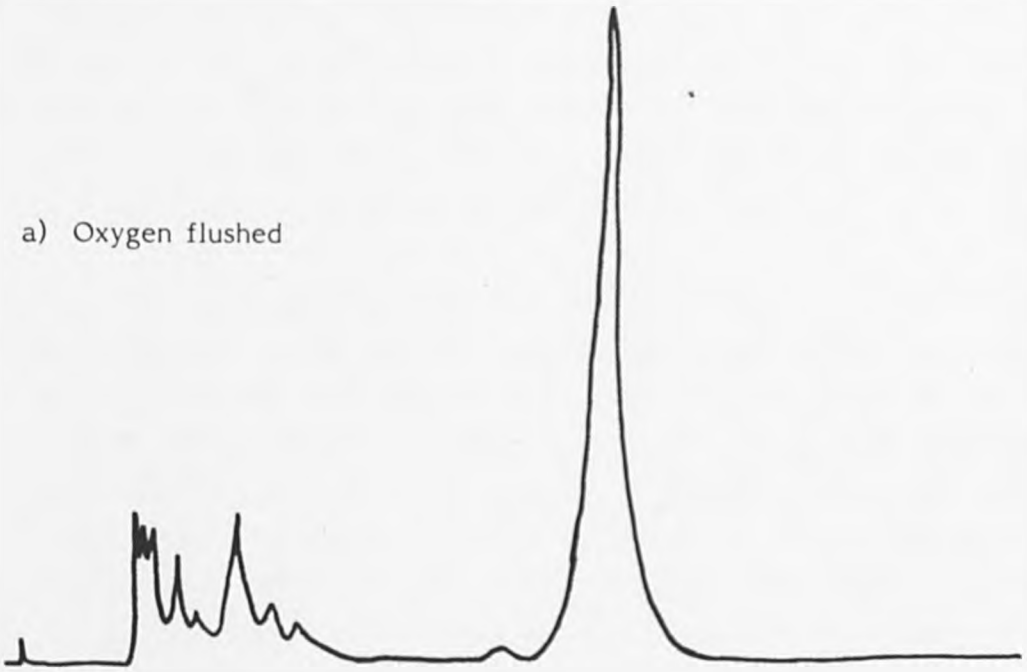
ie: Tube 1 : Oxygen flushed for 5 mins. Irradiated in 300nm reactor for 3 days.
Tube 2 : Argon flushed for 5 mins. Irradiated in 300nm reactor for 3 days.
Tube 3 : Oxygen flushed for 5 mins. Stored in dark for 3 days.
Tube 4 : Argon flushed for 5 mins. Stored in dark for 3 days.

These solutions were then analysed by HPLC.

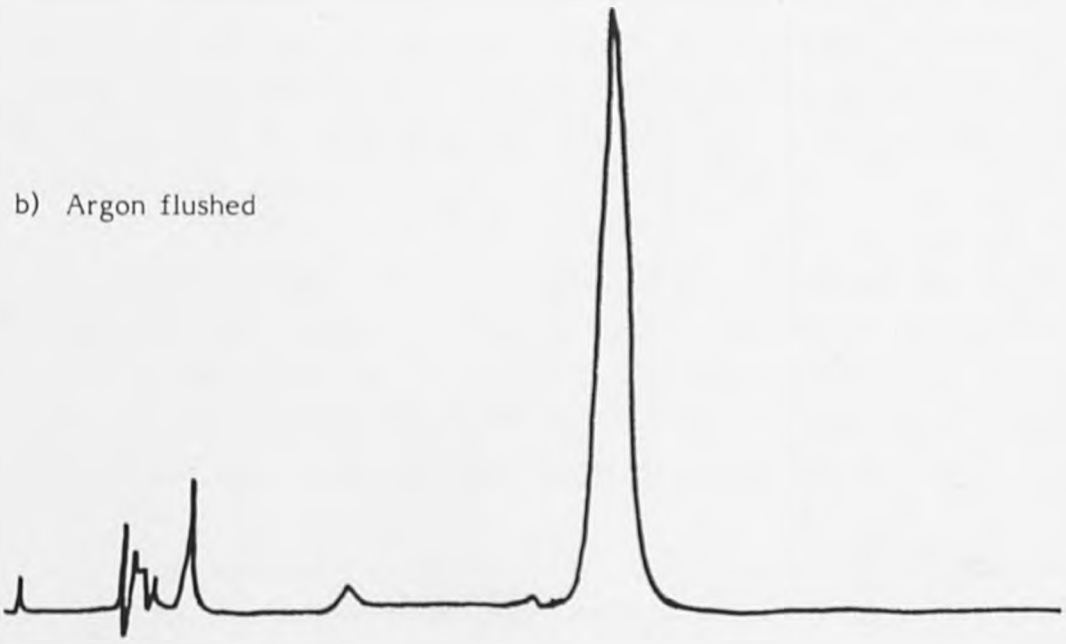
The chromatograms for the solutions stored in the dark are shown in Figure 2.20. It is clear from these that in the presence of oxygen, a small percentage of the N-acetyl tryptophan had reacted or decomposed to give a variety of products in low concentration, eluting shortly after

Fig 2.20 N-acetyl Tryptophan in Blankit D Solution Stored in the Dark for 3 days

a) Oxygen flushed



b) Argon flushed



the solvent front. However, in excess of 90% of the starting material remained unchanged. In contrast, under argon very little reaction had occurred, 99% of the original tryptophan remaining. Thus it appears that in the dark, a very slow reaction of N-acetyl tryptophan occurs, with both oxygen in the solution and the Blankit D, the presence of oxygen being required for the process to proceed.

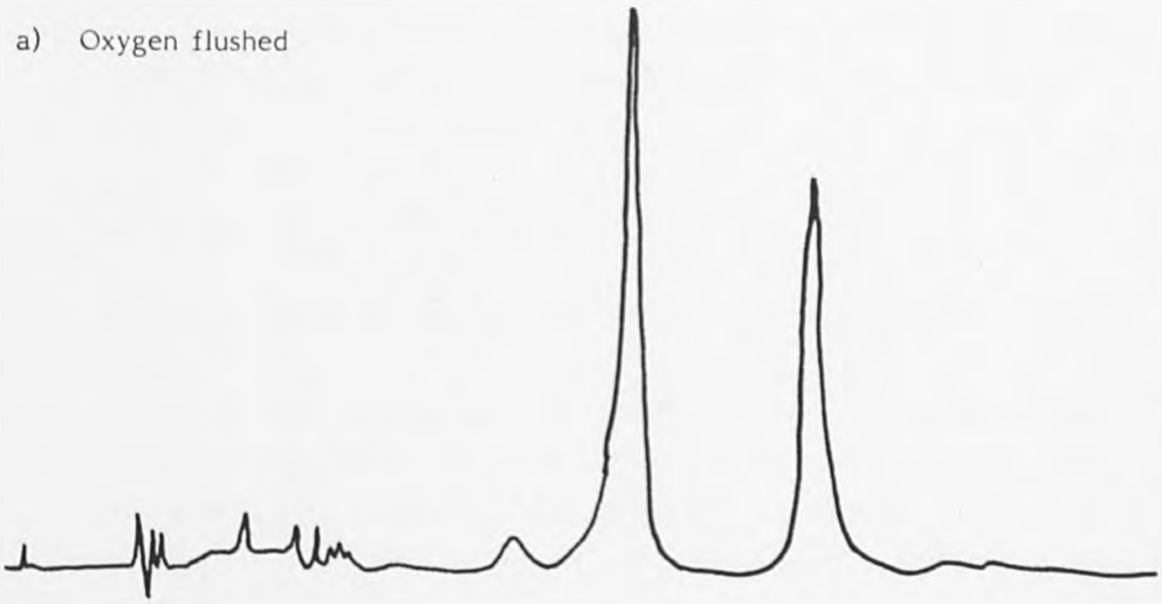
The irradiated solutions were also examined, the results being shown in Figure 2.21. The aerated solution had photodegraded to give similar products to those obtained previously under air (see Fig.2.18). The major product peak was still present, as expected, along with another small peak eluting just before the N-acetyl tryptophan which had also been observed before. Slight differences existed in the range of products eluting shortly after the solvent, but in general, the chromatogram showed little change from Figure 2.18. However, the solution irradiated after degassing with argon yielded a very different result. Virtually all of the original amino acid had apparently reacted, but very few product peaks were found. The only peak of any size corresponded to the major product found in the presence of oxygen. All other components of the solution were either of very low concentrations, or were retained on the column. The amount of the major degradation product detected (Fig 21.6) was only 20% less than the aerated sample, (Fig 21a), which therefore suggested that it's formation was not dependent upon the presence of oxygen. This is in contrast to the photooxidation of tryptophan, which shows a strong oxygen dependence. This indicated that the reaction in Blankit D solution under 300nm light to form the major product may not be a photooxidation process.

Having observed that the pressure had increased during irradiation of the degassed solution, it was decided to analyse the gas above the liquid in each tube. For this purpose, GC-Mass Spectrometry was chosen. This is a semi quantitative technique, but provides information regarding the relative quantities of each gas present in a given sample.

The gas chromatograph was a Pye 104, and was used only as a means to introduce the sample to the mass spectrometer. It performed no separation of the constituent gases so the oven temperature was set at 100°C,

Fig 2.21 N-acetyl tryptophan Irradiated in Blankit D for 3 days

a) Oxygen flushed



b) Argon flushed

using an OV225 column. Argon was used as carrier gas, at a flow rate of 20ml per minute. Possible components of the gas to be analysed were hydrogen, methane, nitrogen, carbon monoxide, oxygen, argon and carbon dioxide. The mass spectrometer was thus tuned down to mass 28, methane and hydrogen being of too low molecular weight for this instrument (a Kratos MS301) to detect accurately.

Solutions were prepared as follows:-

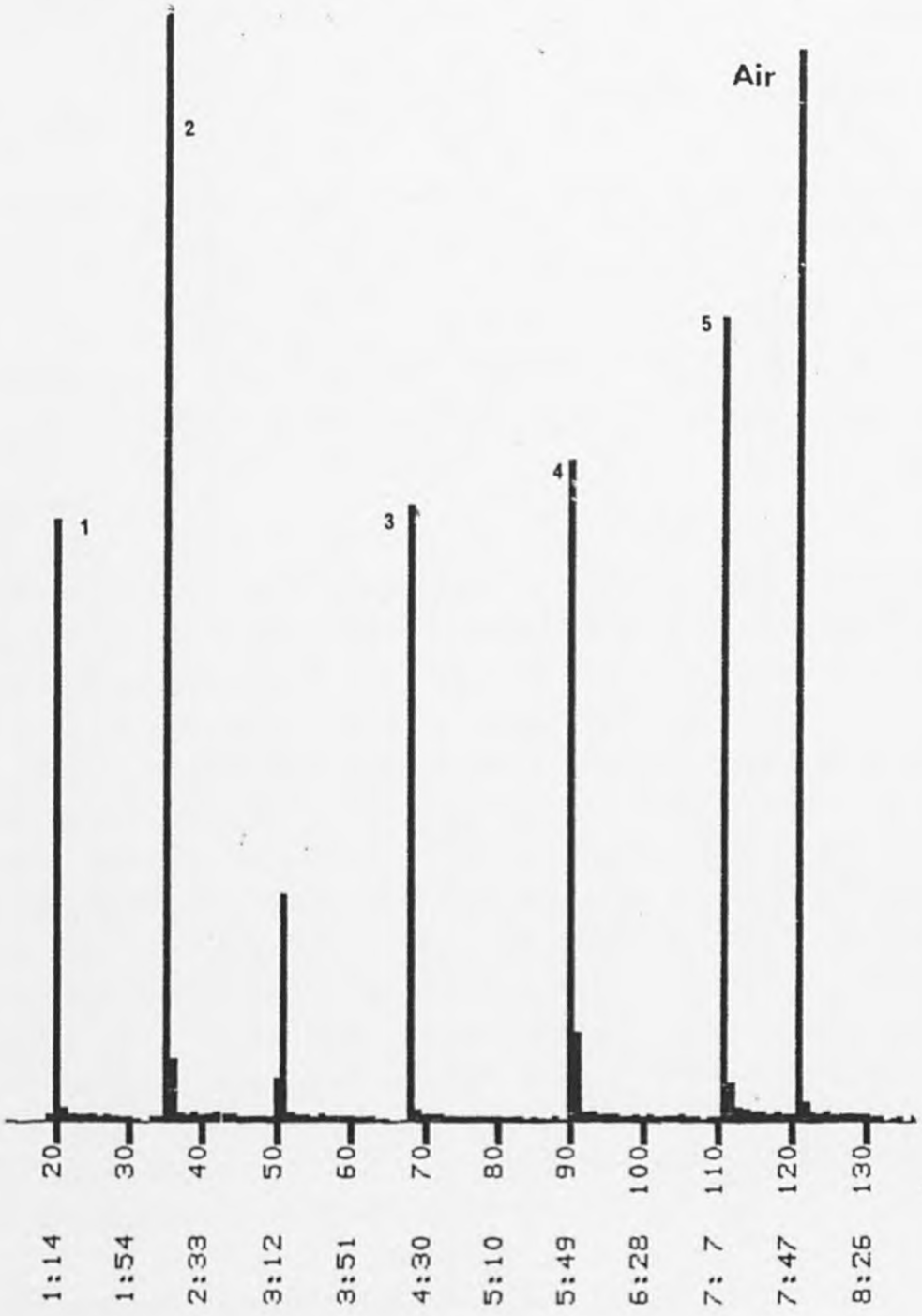
1) 0.05% N-acetyl tryptophan, 2% Blankit D, Oxygen flushed, quartz tube.
2) 0.05% N-acetyl tryptophan, 2% Blankit D, not flushed, glass tube.
3) 0.05% N-acetyl tryptophan, 2% Blankit D, not flushed, quartz tube.
4) 0.15% N-acetyl tryptophan, 2% Blankit D, not flushed, quartz tube.
5) 0.05% N-acetyl tryptophan, 2% Blankit D, argon flushed, quartz tube.

These were irradiated simultaneously at 300nm for 3 days.

A 500µl sample of the gas in each tube was injected sequentially into the GC-MS at approximately 1 minute intervals, followed by a 500µl injection of air. The total ion count (TIC) plot is shown overleaf This is equivalent to the GC trace. The ion counts observed cannot be used for accurate quantitative calculations, but provide relative data, enabling comparisons to be made. To determine the reproducibility of the injection technique, five consecutive injections of air were run. The T.I.C. values were the same, ± 10%.

Results

The ion counts for masses 28, 32, 40 and 44 were recorded for each of the samples, and are listed below in Table 2.19. No other ions were detected in this region.



Total Ion Count

Table 2.19 Ion Counts for Gas Samples from N-Acetyl Tryptophan Decomposition

Sample / Gas	1	2	3	4	5	Air
N_2 or CO	0	119 858	81 164	105 701	11 986	107 589
O_2	54 641	18 055	1 425	2 376	0	60 343
Ar	0	4 759	4 759	0	118 970	7 138
CO_2	20 496	45 221	13 339	4 880	826	1 653

Discussion

Tubes 1 and 5 were flushed with oxygen and argon respectively, and hence it is not surprising that they have very low ion counts for mass 28 (probably nitrogen). For the same reason, it was expected that tube 1 should have a high value for oxygen, and tube 5 for argon. It is significant, however, that tubes 2,3 and 4 have very much lower oxygen ion counts than observed for the control air sample, indicating that oxygen has been consumed during the irradiation period. This observation is supported by the considerably higher proportion of oxygen found in tube 2 (the glass tube) than in 3 or 4. Glass absorbs all wavelengths below 330nm, thus limiting the energy input to the photodegradation reaction inside the tube. In the case of carbon dioxide, a wide variation in ion counts was found, high values generally being observed in solutions with high oxygen concentration and vice versa. The lowest value was for tube 5, which had been flushed with argon, this count being of the same order as that observed for the control air sample.

It appeared, therefore, that apart from the presence of argon, tube 5 contained lower proportions of nitrogen, oxygen and carbon dioxide than the other tubes. It was concluded from this that the increase in pressure observed must have been due to some other gaseous product, such as hydrogen or methane, not detectable on the mass spectrometer.

To confirm this conclusion, the gaseous mixtures were analysed by gas chromatography using another Pye 104 instrument. The column was packed with a molecular sieve, and a katharometer was used as the detector. The column temperature was 100°C, the injection volume 500µl and the katharometer set at 95mA. Argon was again used as the carrier gas.

The retention times of hydrogen, oxygen and nitrogen were determined using known samples from gas cylinders. Under the conditions used, these were:-

Hydrogen : 1.13 minutes
Oxygen : 1.80 minutes
Nitrogen : 2.45 minutes

Argon could not be determined, since it was used as the mobile phase. Carbon dioxide was not seen because at this temperature it was irreversibly adsorbed onto the column. Other workers at the City University using similar conditions with this instrument had noted that methane and carbon monoxide eluted after nitrogen from this column. Retention times were not specified, but as the results show, these values were not required in this work.

Samples of the gas in each of the five tubes were injected onto the G.C., and the peak heights used to indicate the approximate relative concentrations of each constituent gas. No peaks were observed after the nitrogen had eluted (2.45 minutes) in any chromatogram. It was therefore concluded that methane was absent, and carbon monoxide likewise. The peak heights are listed below in Table 2.20, the attenuation changes required being given in parentheses.

Table 2.20 Hydrogen, Oxygen & Nitrogen Compositions of the Gas Samples from N-acetyl Tryptophan Photodecomposition

	Peak Heights (mm) (x Attenuation)				
GAS	Sample Tube No. 1	2	3	4	5
Hydrogen	112(x1)	112(x1)	193(x10)	206(x10)	188(x5)
Oxygen	194(x2)	15(x1)	5(x1)	Lost under H_2 Peak tail	4(x1)
Nitrogen	34(x1)	196(x1)	122(x1)	108(x1)	30(x1)

The values obtained for oxygen and nitrogen generally correspond to the trend observed in the mass spectrometry results. Where differences exist, they serve to hightlight the limitations of the GC-MS technique to provide quantitative results. The hydrogen concentrations in these samples are very significant, since it may be a product of the photodegradations of N-acetyl tryptophan. Alternatively, it may be formed as a result of the reaction of metallic zinc with protons from the acidic Blankit D solution:

$$Zn + 2H^{+} \longrightarrow Zn^{2+} + H_2$$

It was probably the formation of hydrogen that caused the pressure in the tubes containing the degassed solutions.

2.11.7 The Effect of Blankit D Upon Carbon Dioxide Formation

The formation of carbon dioxide during photodegradation of N-acetyl tryptophan was also followed by the GC-Mass spectrometry technique, to determine whether the presence of Blankit D influenced this aspect of the reaction. The amino acid solution was photolysed as usual with 300nm light, without degassing, using a suba septum to prevent the escape of any carbon dioxide formed. A parallel reaction was carried out in the presence of 2% Blankit D, and the gases in both tubes analysed on the mass spectrometer. The results are listed in Table 2.21 and show clearly that approximately seven times as much carbon dioxide was formed in the absence of Blankit D as when it is present. The results for a sample of air are included as an indication of the background level in the tubes at the start of the experiment. This table also shows that the amount of oxygen consumed (Mass 32) is approximately the same in the presence or absence of Blankit D.

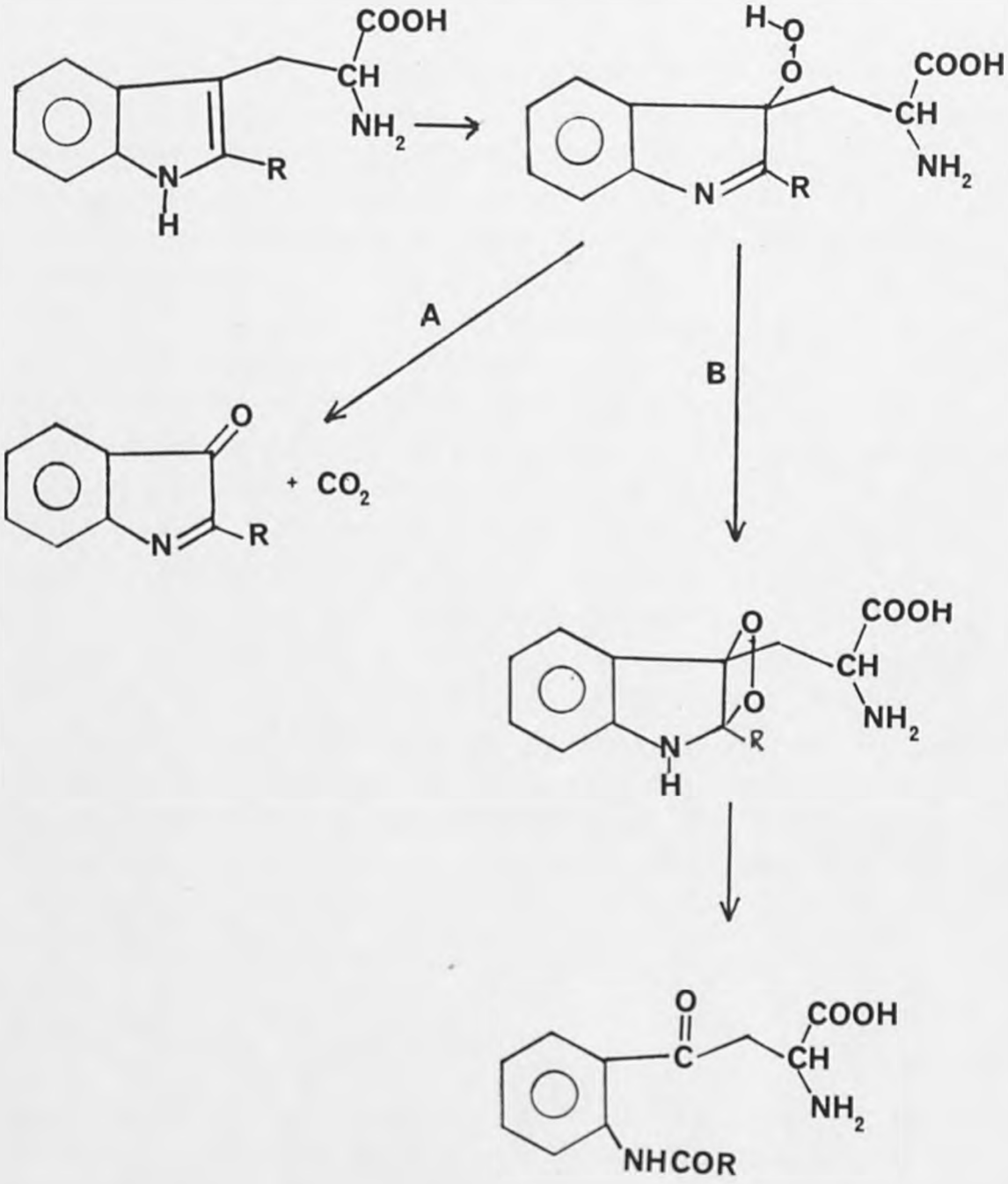
Table 2.21 Comparison of CO_2 Formation in Presence and Absence of Blankit D

SAMPLE	% Intensity *		CO_2 ION COUNT
	Mass 32	Mass 44	
N Ac Trp	45.1	58.4	72937
N Ac TRP + D	44.3	8.8	10352
Air	61.6	3.5	2117

* All peaks normalised, largest peak being set at 100%.

NOTE : All injections carried out in triplicate. Mean values shown.

To account for the observed reduction in CO_2 formation, it is necessary to refer back to the mechanism for tryptophan oxidation by singlet oxygen, as described in chapter one:



In the absence of Blankit D, R=H and pathway A is probably favoured. When Blankit D is present, if R is an alkyl substituent, then the dioxetane cleavage would occur more readily and pathway B would be favoured. The 2-position on the indole ring is thus a likely site of reaction with Blankit D.

2.11.8 HPLC Column Subsidence - its Manifestation & Cure

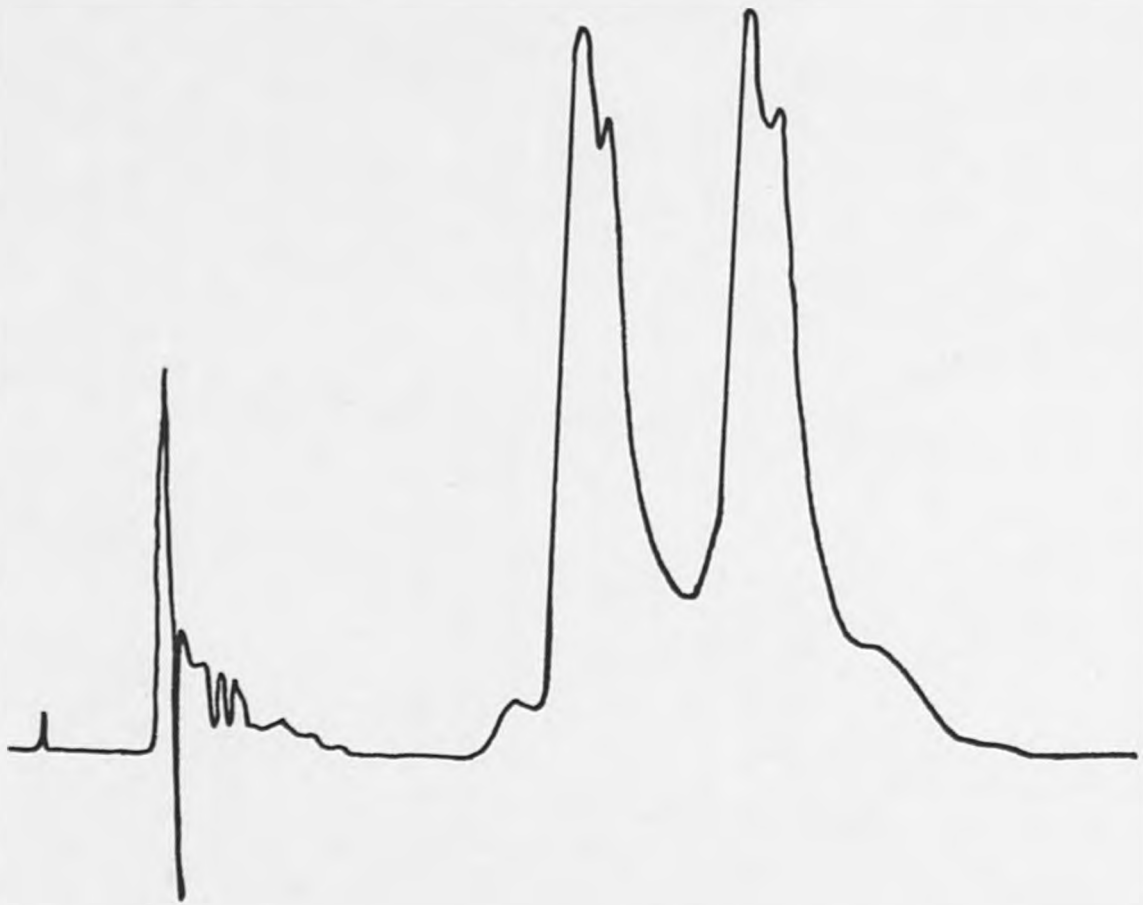
A problem which was repeatedly encountered during the course of this work was the deterioration in efficiency of chromatographic separations on the HPLC. Initially the peaks were observed to be broader than usual, but with time, double peaks appeared for each component of the sample, and separation was incomplete. These symptoms are typical of a void volume being formed in the column, usually as a result of column subsidence. This arises after prolonged use with an aqueous mobile phase, which attacks the residual silanol sites on the surface of the silica support media. Under such conditions, the stationary phase is slowly eroded, and subsidence occurs as a result of the high operating pressures used. A typical chromatogram illustrating this problem is shown in Figure 2.22. This problem was solved each time by removing the top end fitting of the column to expose the void, and filling the gap with a small quantity of dry Hypersil ODS. The addition of a drop of methanol assisted the settling of the new packing material, which was then smoothed level and the end fitting replaced.

Another problem associated with column subsidence is a gradual increase in the back pressure, for a given flow rate. This is caused by very fine particulate matter blocking the eluent flow path through the column, and there is no solution but to repack the column. The problem can also arise if the samples are not filtered prior to injection, so a 0.45μ membrane filter was always used for this work.

2.11.9 The Use of a Fluorescence Detector

A convenient means to obtain further information about the nature of the photodegradation products was to use a fluorescence detector in-line with the U.V. detector. An obvious feature is that only fluorescent compounds can be detected, which is often a disadvantage, but for this reaction made it an ideal probe to establish the presence of the indole chromophore.

Fig 2.22 The Effect of a Void upon the HPLC Separation Efficiency



An aerated solution of N-acetyl tryptophan (0.15% w/v), was irradiated in the presence of 2% Blankit D, as before, at 300nm for three days. The resultant solution was filtered, and run on the HPLC. As can be seen from the chromatogram in Figure 2.23, the major product and several others are all fluorescent. However, the response of the fluorescence detector was poor, because the lowest wavelength excitation filter available was 310nm, where tryptophan has only a minimal UV absorption. The noise level obtained was therefore quite high, the instrument sensitivity being set at its maximum. Thus although fluorescence peaks were observed for the major components of the sample, others may have been lost in the noise signals for this reason. The technique was not used extensively in this study.

2.11.10 The Collection of the Major Product Fraction

All the HPLC separations so far had been carried out on the 12.5 x 0.46cm analytical column. However, it was then decided to collect the eluent fractions containing the major product, with a view to isolating and characterizing it. To achieve this, the separation process was scaled up to use a semi-preparative column with a higher sample loading capacity. The column chosen was 30.0x0.94cm, packed with ODS hypersil, as before. Sample injections were increased from 20µl to 200µl, and the flow rate from 2ml min^{-1} to 5ml min^{-1}. The column achieved a maximum efficiency of 18500N for this separation using a flow rate of 2.0ml min^{-1}, but this separation dropped to 8500N at 5ml min^{-1}. The separation was slightly better than that obtained with the analytical column.

The sample having been prepared and filtered, a trial injection was made to confirm that only one peak would be collected. The product was eluted into a spotlessly clean flask between the points shown in Figure 2.24. The first and last sections of the peak were discarded to avoid possible contamination with other species which might elute immediately before or after this major product. The collected eluent was re-injected, using a higher detector sensitivity, and only one peak was obtained.

It was found that good separation could still be achieved with injection volumes as high as 500µl. This volume was therefore used to increase the sample concentration in the collected eluent fractions. Thirty injections

Fig 2.23 The use of a Fluorescence Detector

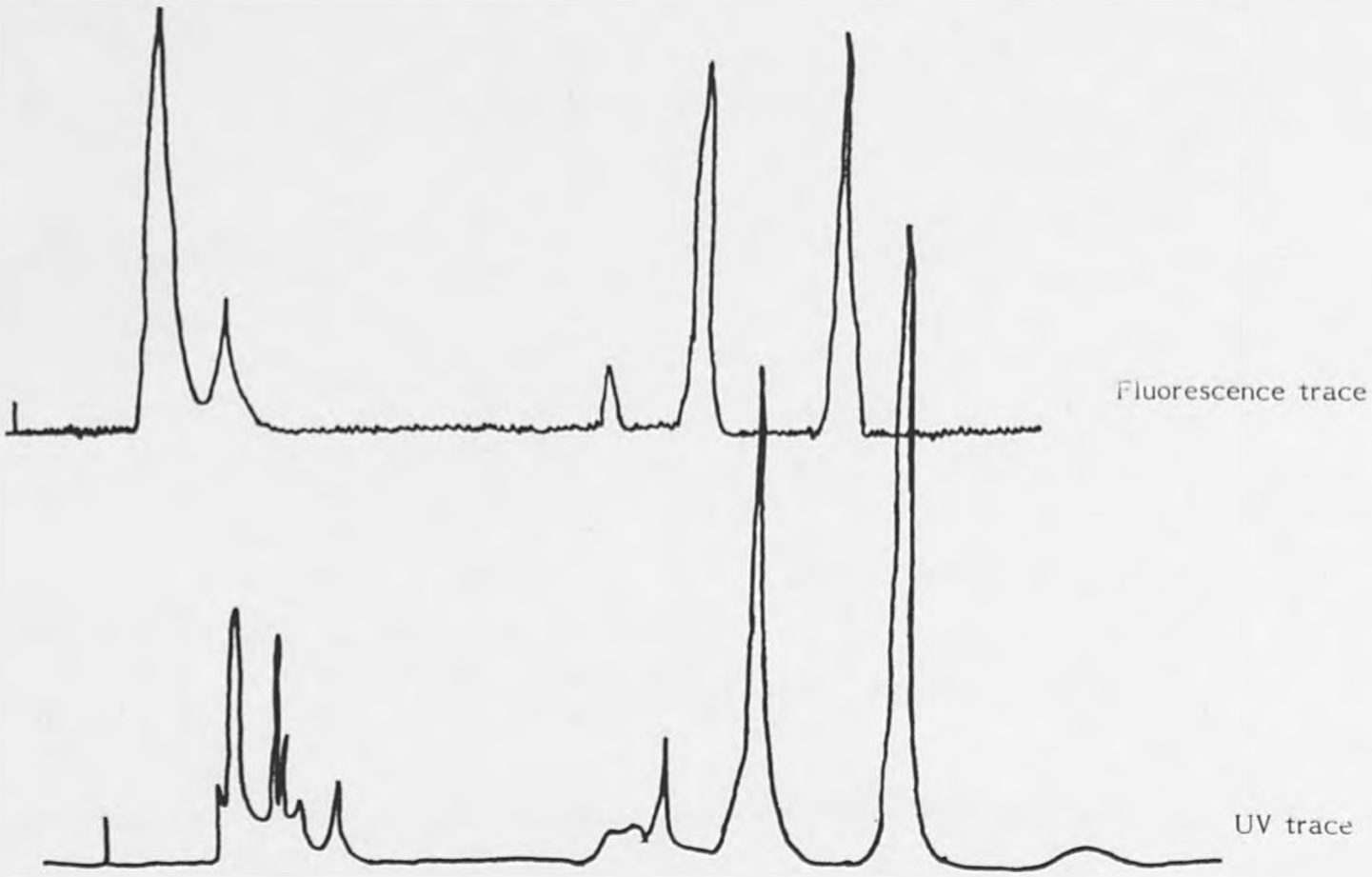
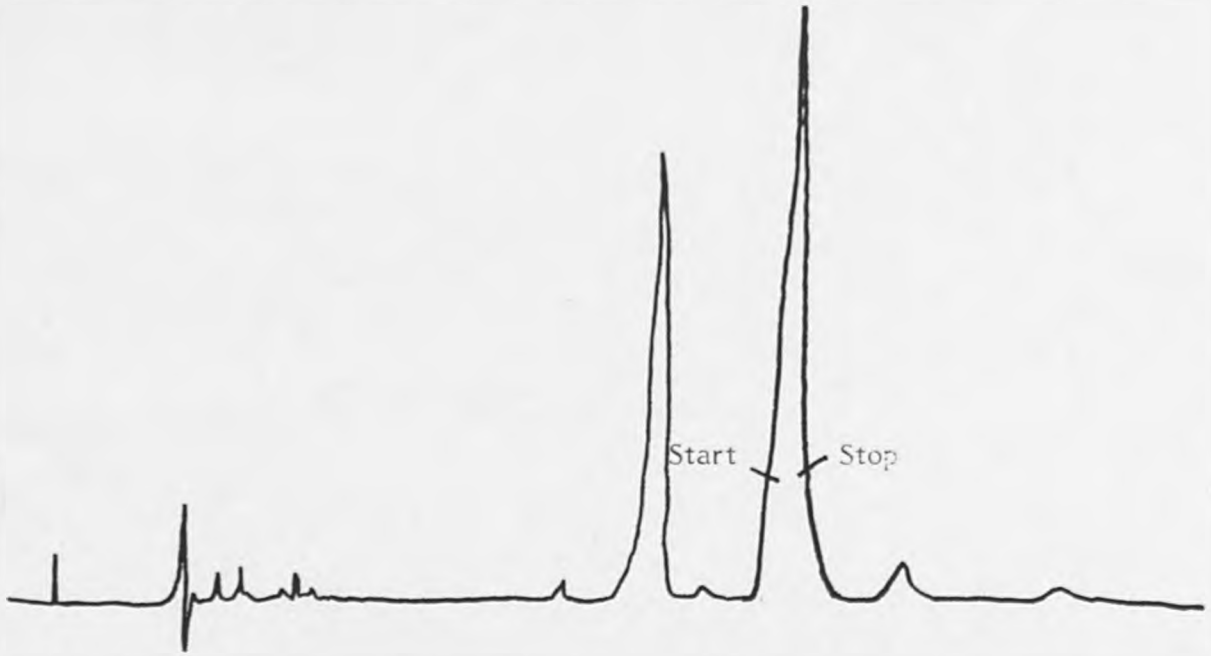


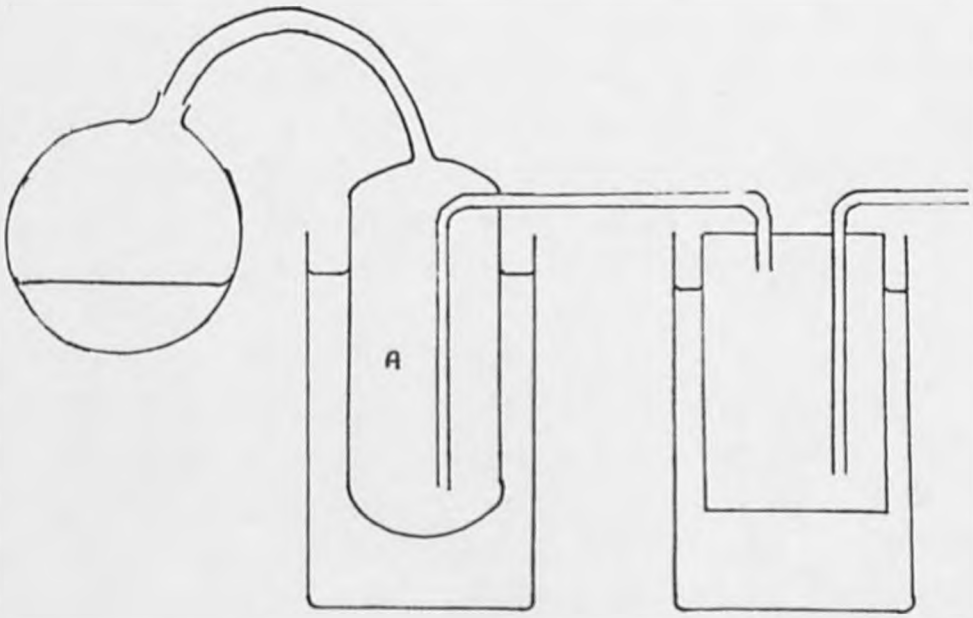
Fig 2.24 Fraction Co llection of Major Product



were carried out, the product fraction being collected each time, and the resultant liquid freeze dried to remove the water, methanol and acetic acid from the eluent. No Blankit D was present, this having been separated on the column.

The freeze drier used was custom built at the City University and is represented diagramatically in Figure 2.25. The sample solvent, maintained at room temperature, established a certain vapour pressure, and consequently diffuses into the vessel A. The vessel is immersed in liquid nitrogen, which causes the vapour to condense. This in turn causes the vapour pressure to drop, more of the sample solvent evaporates and diffuses into vessel A, where it condenses out. This process is extremely slow, and is accelerated by the application of a vacuum. Even so, it took in excess of 24 hours to obtain a dry sample. When initially applying the vacuum, the sample was cooled with liquid nitrogen to prevent the solvent boiling due to the sudden drop in pressure. From that point on, it was left at room temperature. Once all visible traces of solvent had gone, the vacuum was maintained for a further two hours to complete the drying operation. The product was a fluffy white solid, weighing 0.8mg.

Figure 2.25 The Freeze-Drying Apparatus



This collection/freeze drying process was repeated with a view to obtaining a larger quantity of product material for subsequent analysis. Injection volumes were increased to 800µl, with minimal loss in resolution or separation efficiency, and the product fraction was collected over 55 HPLC runs. This time the dried product weighed 3.1mg, again a white fluffy solid. The material was then analysed and characterized, as detailed in the following sections.

2.12 Characterization of Major Product

2.12.1 Atomic Absorption Spectroscopy

At this stage, the information known about the major product of N-acetyl tryptophan photodegradation in Blankit D solution may be summarized as follows:-

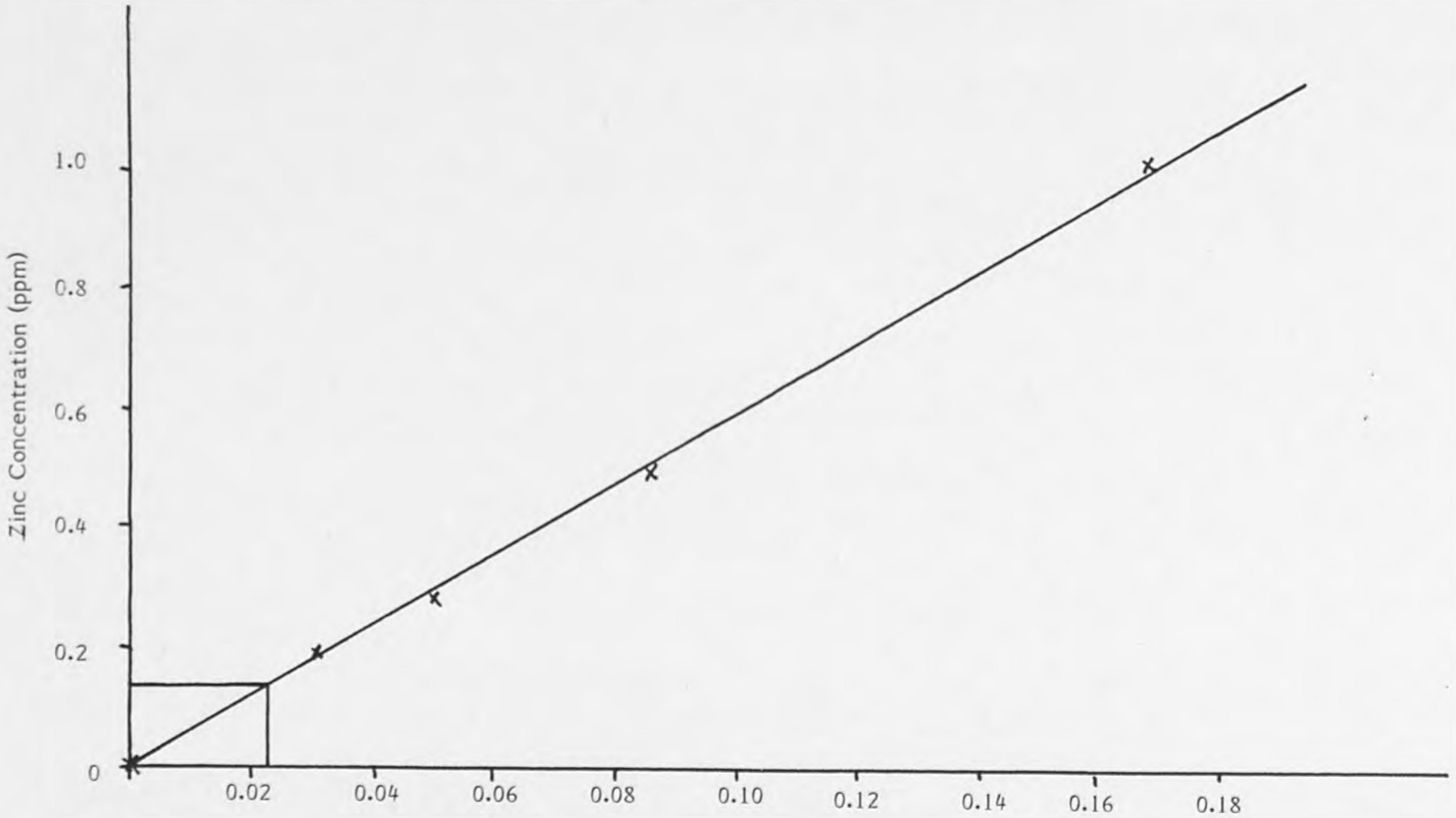
1) Soluble in aqueous methanol.
2) Fluorescent.
3) U.V. absorption similar to that of N-acetyl tryptophan.
4) Probably slightly less polar than N-acetyl tryptophan since it is retained longer on an ODS column.
5) A reaction may well have occurred at the 2-position on the indole ring.
6) Oxygen not required for its formation.

Atomic absorption spectroscopy was used to determine whether a zinc salt had been formed in the reaction. A sample of pure N-acetyl tryptophan was run as a control. The reference solutions for the calibration curve were made up by diluting a BDH standard 1000ppm zinc sulphate solution to give 1ppm, 0.5ppm, 0.3ppm and 0.2ppm respectively. The 'unknown" solutions were then made up in deionized water:-

(a)	N-acetyl tryptophan	1.982mg in 50ml
(b)	Major Product	1.015mg in 25ml
(c)	Blankit D	1.822mg in 50ml. Diluted 1:25

The four reference and three "unknown" solutions were then analysed using a Perkin Elmer atomic absorption spectrophotometer. The results

Fig 2.26 Atomic Absorption Calibration Graph



are listed below in Table 2.22 from which the calibration graph was drawn. This is shown as Figure 2.26.

It is clear that no zinc is found in the pure sample of N-acetyl tryptophan, which is as expected. The zinc content of Blankit D and the major product were calculated from the equation:-

$$\%Zn = \frac{(ppm \times 10^{-6}) \times \text{Volume of sample (ml)} \times 100\%}{\text{Mass of sample in solution (g)}}$$

Table 2.22 Atomic Absorption Values for Zn in N-Acetyl Tryptophan and its Major Product

SOLUTION	AA READING	AVERAGE
1.0ppm	0.168	0.167
	0.168	
	0.167	
0.5ppm	0.087	0.086
	0.086	
	0.085	
0.3ppm	0.054	0.054
	0.055	
	0.053	
0.2ppm	0.033	0.032
	0.032	
	0.032	
N-acetyl Trp.	0.000	0.000
	0.000	
	0.000	
Major Product	0.024	0.024
	0.024	
	0.024	
Blankit D	0.023	0.024
	0.025	
	0.024	

From the graph it was found that for the major product, the zinc concentration was 0.14ppm. Hence:-

$$\% \text{ Zn in Major Product} = \frac{0.14 \times 10^{-6} \times 25 \times 100}{1.015 \times 10^{-3}} = 0.34\%$$

In the Blankit D solution (1.822 mgs in 50µl, diluted to 1250ml) the concentration was also 0.14ppm.

$$\% \text{ Zn in Blankit D} = \frac{0.14 \times 10^{-6} \times 1250 \times 100}{1.822 \times 10^{-3}} = 9.6\%$$

Thus a very small amount of zinc was found in the major product. The percentage composition of 0.34% is far lower than would be expected if the compound was a zinc salt, so this was concluded to be an impurity, possibly indicating that the product was contaminated with a trace of Blankit D. The molecular weight of N-acetyl tryptophan is 246, so the presence of one atom of zinc (atomic mass 65) would lead to a percentage composition of approximately 21%! It had therefore been shown that the compound was not a zinc salt.

2.12.2 U.V. Absorption & Fluorescence

The U.V. absorption of N-acetyl tryptophan and of its major product were recorded in aqueous solution using a Perkin Elmer Lambda 5 scanning UV-Vis spectrophotometer. The spectra were very similar both having a λmax at 280nm, with a shoulder at 288nm. This clearly suggested that the indole chromophore had not been changed during the course of the reaction.

The fluorescence of the above two solutions was then measured on a Perkin Elmer MPF-4 spectrofluorimeter. The excitation wavelength was set at 285nm, and the emission scanned from 295nm to 450nm. In the case of N-acetyl tryptophan, a fluorescence maximum was obtained at 358nm, whilst the major product exhibited an emission maximum at 363nm. This difference of 5nm was verified by running fresh solutions under identical conditions, when the above results were repeated exactly. The major product solution was scanned through excitation wavelengths, monitoring the fluorescence emission at 363nm. This gave a maximum at 290nm.

The UV and fluorescence studies thus showed that the structure of the product is very similar to that of the starting material, with probably little or no modification to the indole nucleus.

2.12.3 Mass Spectrometry

The mass spectra were run on a Kratos MS30 mass spectrometer, controlled by a DS50S data station. This was a high resolution instrument, and was used for all mass spectra quoted in this thesis.

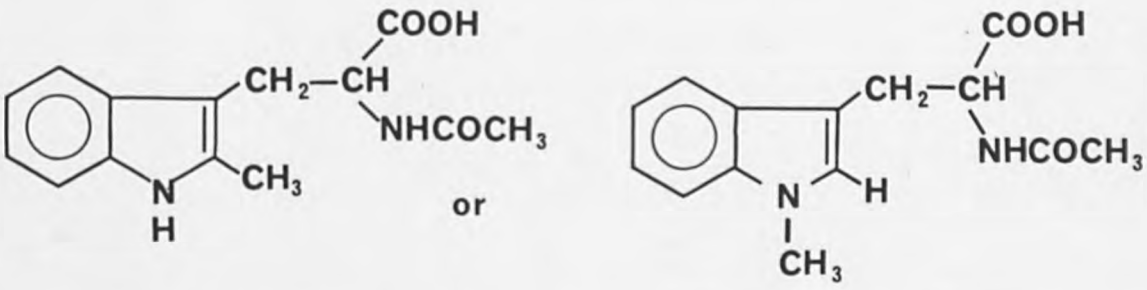
The major product was analysed, followed by N-acetyl tryptophan A marked correlation was found to exist between the fragmentation patterns obtained, the major product peaks being 14 mass units higher than those for the N-acetyl tryptophan. The relationship is shown below in Table 2.23.

Table 2.23 Mass Spectral Fragments of N-acetyl Tryptophan & its Major Product

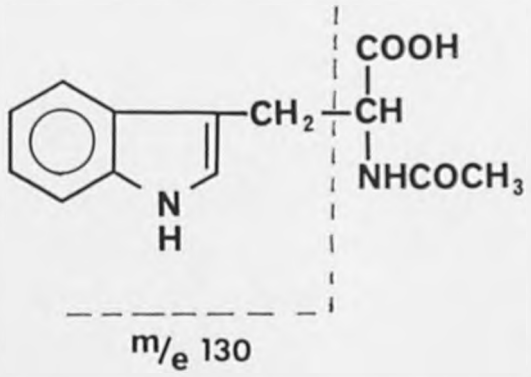
N-Acetyl Tryptophan		Major Product	
Measured Mass	% Intensity	Measured Mass	% Intensity
247 (M+1)	0.5	261 (M+1)	0.5
246	4.1	260	2.6
228	0.4	242	2.8
187	18.6	201	3.1
131	10.6	145	10.8
130	100.0	144	100.0

The existence of this correlation suggested that the tryptophan molecule had been modified at a position such that the substituent was not readily cleaved in the mass spectrometer. This would support the suggestion that the 2-position of the indole was likely site of reaction. The molecular ion was observed to be of mass 260, suggesting the addition of a unit of mass 14 to the N-acetyl tryptophan. This was unlikely to be the addition

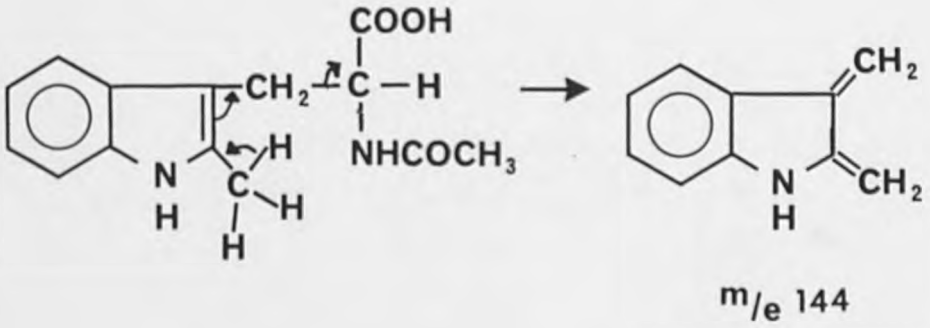
of a nitrogen atom, and was assumed to indicate the substitution of a methyl group for a proton. Two possible structures for this molecule could therefore be:



The base peak for N-acetyl tryptophan is at mass 130, which may be due to a cleavage process as follows:-



This would also apply to the major product base peak at 144, if the proposed structures (given above) are correct. An alternative mechanism, which does not apply to the starting material, is a Maclafferty rearrangement, as shown below:-



2.12.4 Elemental Analysis

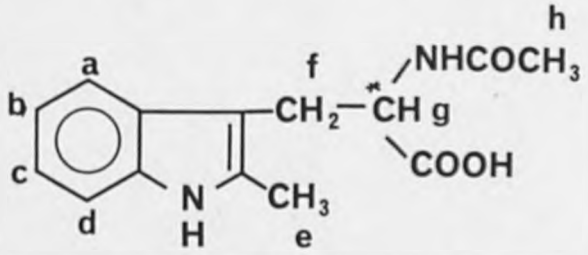
In an attempt to confirm the proposed structures incorporating the added methyl group, the elemental analysis (C,H,N and S) of the major product was carried out. The results obtained were rather lower than the theoretical values. This meant that either the structure was wrong, or the trace of zinc found earlier was depressing the percentage composition of other elements. The theoretical and observed values are listed below:-

ANALYSIS	C	H	N	S	O
Theoretical Values	64.60	6.20	10.76	0	18.44
% Found	55.21	5.64	8.69	<0.5%	-

These results were therefore inconclusive.

2.12.5 Nuclear Magnetic Resonance

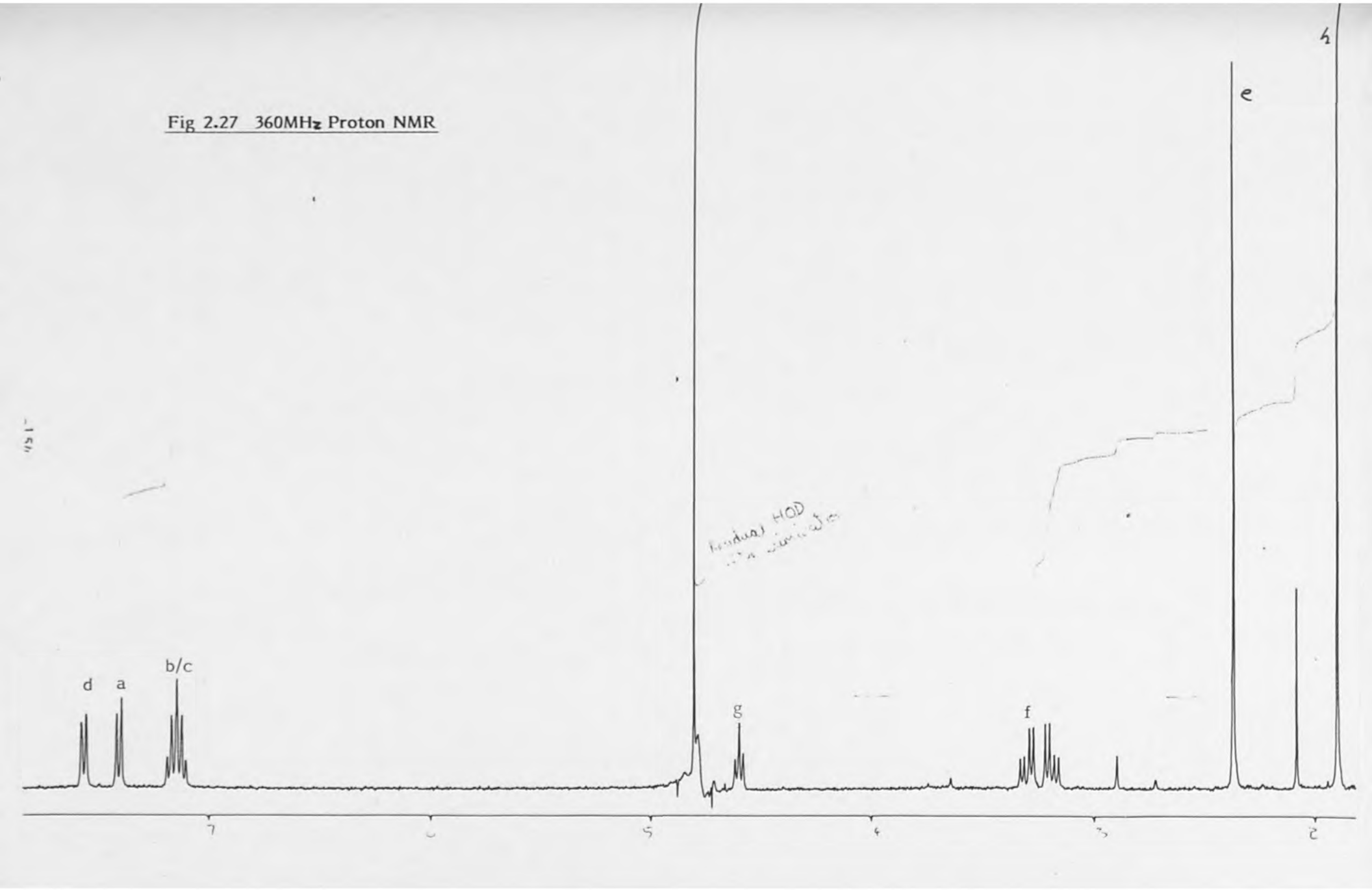
As a consequence of the small amount of sample available, the major product was sent to Edinburgh University for proton n.m.r. studies at 360.13MHZ. A very elegant spectrum was obtained from a solution in D_2O, accumulating data from 160 scans. The spectrum is shown in Figure 2.27, and clearly defines the structure of the product as:-



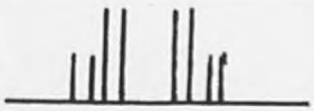
The spectrum is assigned as follows:-

The two methyl groups, e and h, are expected to give singlet peaks of integral 3. The group on the indole ring (e) is more deshielded than that in the N-acetyl group (h), and will hence resonate at lower frequency. The peak at 1.90δ is thus assigned to the 'h' protons, while that at 2.37δ corresponds to the 'e' protons.

Fig 2.27 360MHz Proton NMR



The methylene protons are potentially different because the adjacent carbon atom is chiral. They therefore not only couple to proton g individually, but also each to each other:-



Proton g couples to both the methylene protons individually, and hence gives a triplet. It is deshielded by the carbonyl group and the nitrogen, thus resonating downfield at 4.60δ. The two protons attached to the nitrogen atoms, and the carboxyllic acid protons are not observed in D_2O.

The aromatic protons are assigned on the basis that a and d will only couple to b and c respectively, and hence will give two doublets, while b and c can each couple to each other, and to a and d respectively. Proton d is deshielded slightly more than a by the indolic nitrogen, and is therefore assigned to the signal at 7.57δ, while proton a corresponds to the "doublet" at 7.40δ. The quintet/multiplet at 7.14δ represents protons b and c.

The spectrum certainly eliminates the possibility of the methyl group e being bonded to the indolic nitrogen, since its resonance would then be between 4-5δ, instead of the observed 2.37δ. High field nmr was thus used to definitively determine the structure of the major product, the result being in accord with inferences from other analytical methods used.

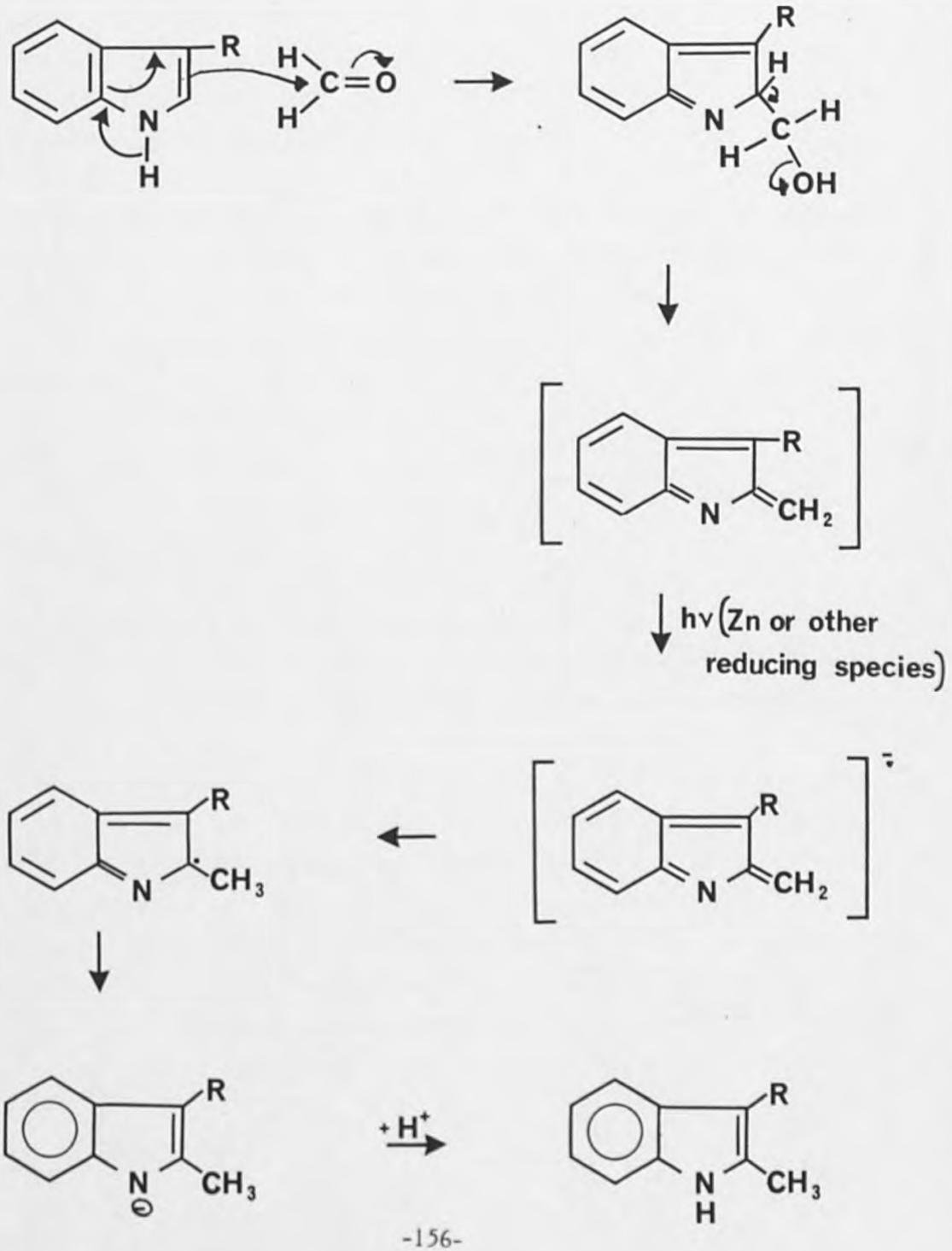
2.12.6 Infrared Spectroscopy

The infrared spectrum of the major product was run as a KBr disc but contributed little to the structural assignment The peaks observed were weak, but could all be assigned in accord with the proposed structure.

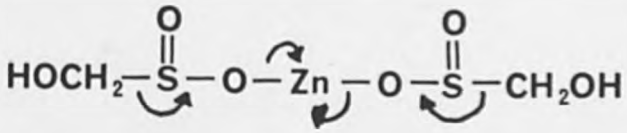
2.13 The Mechanism of Methylation

The process is unlikely to be a standard methylation reaction, because the indolic nitrogen would be a more likely site of attack under such conditions. The most probable mechanism is therefore the addition of the aldehyde group, followed by reduction, as shown below in Figure 2.28:

Fig 2.28



Thus the components of the Blankit D molecule are all necessary, but acting in different ways. The formaldehyde fragment is used to react with the 2-position of the indole ring, preventing further degradation reactions occurring at this site. The zinc hydroxymethane sulphinate molecule then may dissociate further to give zinc and SO_2 in solution,



which act as photoreducing agents for the final steps of the reaction mechanism in Fig.2.28. The reason for Blankit D causing the effect while only a slight improvement was seen with Rongalit C may be due to the reducing properties of zinc, as compared to sodium.

One interesting feature of the N-acetyl tryptophan major degradation product is that it was found to be much more readily soluble in water than the parent amino acid. The reason for this is not clear, since the product structure is not apparently modified in a way which would increase its affinity for water.

2.14 Dityrosine

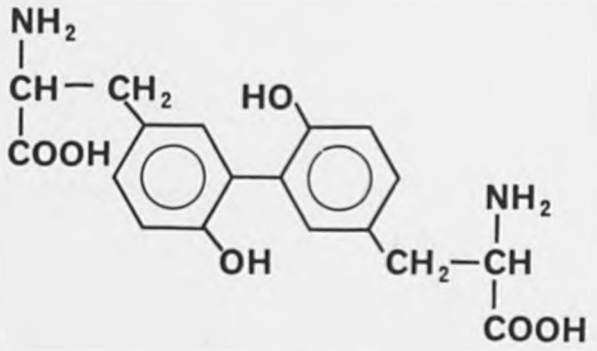
2.14.1 Introduction

Having observed the relevance of tryptophan to the photoyellowing process, and established the nature of the photodegradation pathway in the presence of Blankit D, attention was then centred on dityrosine.

Tyrosine has long been known to be involved in wool photoyellowing, [52,53], but does not give rise to highly coloured products to any appreciable extent. Dityrosine has never been reported to be associated with the yellowing of wool, and has probably not been studied in this context. It was only recently that dityrosine was found to be present in the cuticle of wool in much higher concentrations than in the bulk of the fibre, [54]. This aroused interest because wool photoyellowing is essentially a surface phenomenon. When amino acid analyses had been carried out on wool

samples in the past, dityrosine values were rarely measured because the overall concentration was extremely low. Using the technique of Otterburn et.al., [55], the amino acid composition of the cuticle cells alone could be determined and a comparison made with the values obtained for the cortex.

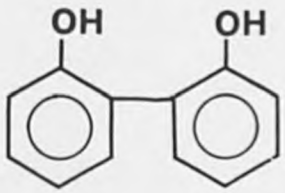
The dityrosine structure is shown below:



Hair is similar in structure to wool, and it is interesting to note that the amino acid analyses of the cuticle and cortex of human hair also show differences in composition [56-59]. Dityrosine content has not yet been determined in hair.

It may be formed in vitro by the action of horse-radish peroxidase and hydrogen peroxide on tyrosine [60]. Heidemann et.al. [61] have also generated dityrosine in collagen by the reaction of peracetic acid and hydrogen peroxide. Its absorption spectrum is pH dependent, varying from λmax 280nm at pH 3.5 to λmax 325nm at pH 10.0 [61], while its fluorescence emission is constant at 410nm.

Treatment of tyrosine with horse-radish peroxidase and hydrogen peroxide were reported [60] to yield a number of oxidation products, requiring column chromatography on cellulose phosphate to separate and purify the dityrosine. The yield of this method was low, and it was therefore decided to use o,o'-dihydroxybiphenyl as a model for studying the photoyellowing process, and the effect of Blankit D. This was obtained from Sigma, having the structure shown overleaf.



To determine whether the photoyellowing properties of dityrosine and biphenol were likely to be similar, solutions of N-acetyl tyrosine and phenol were irradiated in parallel with 300nm and 350nm light in quartz photolysis tubes. The colour changes observed for N-acetyl tyrosine were very similar to those seen in the phenol solutions, so it was concluded that the phenol was a reasonable model compound in this respect.

2.14.2 Experimental Procedures Used

A 3% solution of o,o'-biphenol was prepared in dilute aqueous ammonia and padded onto a 20g sample of salts serge, achieving 100% pick up. This was dried in air, leaving a fine coating over the wool fabric. A strip of this sample was then irradiated for 24 hours under 5-10mm of water with 350nm light, as described at the beginning of this chapter, with aluminium foil covering half of the surface.

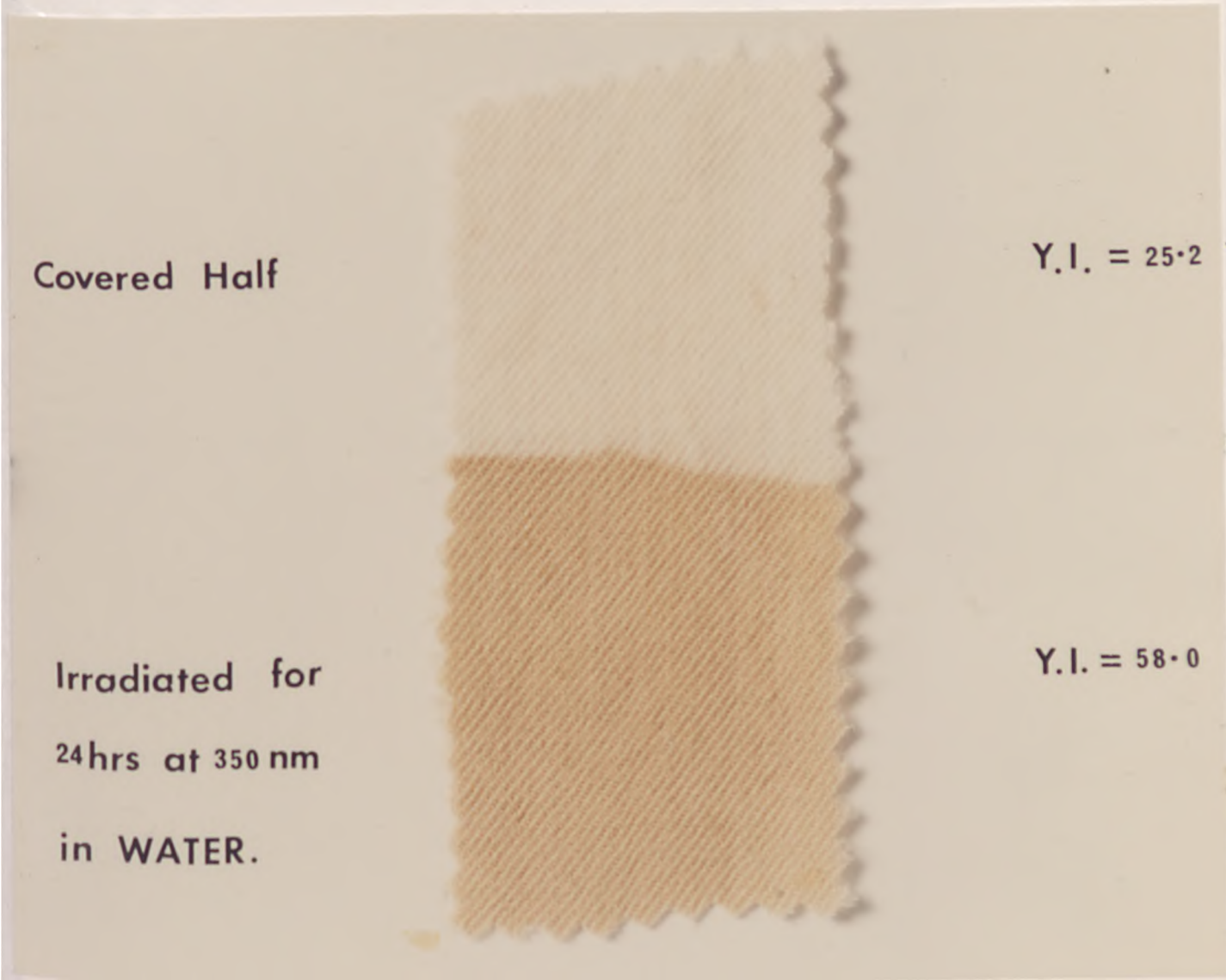
2.14.3 Results & Discussion

The irradiated half was strongly discoloured, as can be seen from the samples in Figure 2.29. A similar strip from the coated fabric was irradiated for the same period under 2% Blankit D solution. This sample was not discoloured at all, but was actually bleached by the treatment. Subsequent irradiation in water only resulted in a small degree of yellowing. The yellowness index values are shown in Table 2.24.

Table 2.24

	Y.I. Before Irradiation	Y.I. After Irradiation
Wool coated with o,o'-biphenol	25.2	58.0
Coated wool, irradiated in Blankit D Solution	18.9	10.9
Blankit D treated wool, irradiated in water	11.0	24.6

FIGURE 2.29


Covered Half
Y.I. = 25·2
Irradiated for
24 hrs at 350 nm
in WATER.
Y.I. = 58·0

Covered Half

Irradiated for 24 hrs at 350nm in BLANKIT D.

Y.I. = 10·9

Covered Half

Y.I. = 18·9

Following the Blankit D treatment, irradiated for 24 hrs at 350 nm in WATER.

Y.I. = 24·7

Y.I. = 18·8

These results showed that the dihydroxybiphenyl had either been photo stabilized by the treatment, or been extracted into the Blankit D solution.

To ascertain which of the above was true, the fluorescence of the wool samples were measured at each stage, using a Perkin Elmer MPF-4 spectrofluorimeter. The excitation wavelength used was 300nm, scanning emmission wavlengths between 310nm-500nm at low sensitivity.

Results

The biphenol-coated wool, prior to any irradiation, showed a strong fluorescence at λmax 387nm. Following irradiation for 24 hours in water, this peak almost totally disappeared. When the coated fabric was irradiated in Blankit D solution the λmax of the original peak shifted to shorter wavelength at 350nm. Further irradiation in water did not totally remove this peak, but resulted in a 70% decrease in intensity.

The reaction in the Blankit D solution was concluded to have caused a modification to the biphenol structure, which resulted in the blue shift in fluorescence emmission, rather than extracting the biphenol from the fabric. Had the dihydroxybiphenyl been extracted by the Blankit D solution, a marked decrease in peak intensity would have been expected after the initial treatment, and little change following subsequent irradiation.

It had thus been established that Blankit D stabilized biphenol to photodegrdation, and it was considered highly probable that this would also apply to dityrosine. No information had been obtained, however, regarding the nature of the chemical modification to the biphenol structure which had achieved this stabilization. To this end, biphenol degradation was studied in solution, and followed using HPLC.

2.15 HPLC Studies on Biphenol Photodegradation

Using the eluent found to be suitable for N-acetyl tryptophan separations (70% water:20% methanol:10% glacial acetic acid), aqueous biphenol solution (0.15% w/v) had a retention time of nearly one hour. This was obviously far too long, so the eluent strength was increased by raising the proportion of methanol present. It was found that using methanol and water in equal proportions, the biphenol eluted with a k' of 1.67. This was a broad peak, however, and so glacial acetic acid was added, with good effect. This final eluent was used throughout the following biphenol separations.

The semi-preparative ODS column was also used throughout, partly because it gave a better separation efficiency than the 12.5cm analytical column, and partly because fraction collection would ultimately be required.

Solutions of biphenol (0.15% w/v) were prepared in water and in 2% Blankit D solution. From each of these, 50ml samples were placed into two quartz photolysis tubes, four in total. One tube of each solution was placed in a photochemical reactor equipped with 350nm black light fluorescent tubes, and one tube of each in a similar reactor fitted with 300nm lamps. Biphenol has a U.V. absorption maximum at 280nm with a very low absorption at 350nm. The reaction at this wavelength was therefore expected to be slow, and the solutions were irradiated for 4 days. In the 300nm reactor, irradiation for 21 hours was found to be sufficient to cause substantial discolouration to the solution in water alone.

After four days irradiation with 350nm light, the biphenol solution without Blankit D present was found to be dark brown with some sediment in the bottom of the photolysis tubes. In contrast, the Blankit D solution remained clear and colourless. These two solutions were analysed on the reversed phase HPLC column, and the chromatograms are shown below in Figure 2.30.

Fig 2.30 Photooxidation of Biphenol

(i) Biphenol Solution (aq) irradiated at 350nm

(ii) Biphenol irradiated in 2% Blankit D solution at 350nm

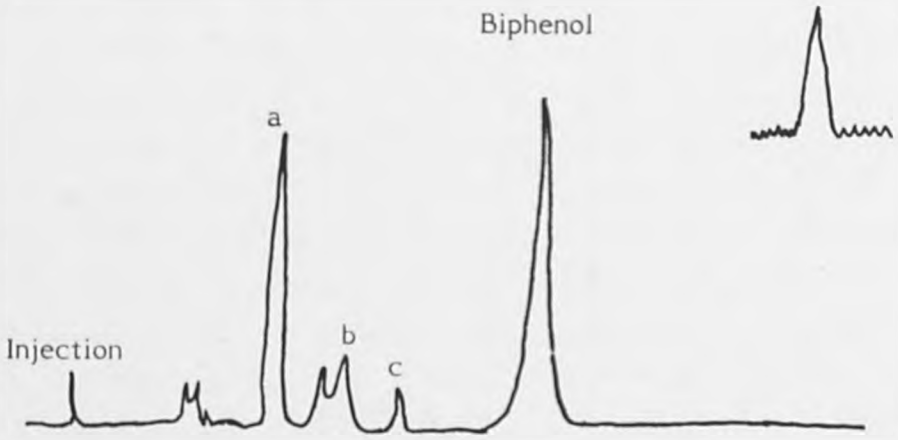
It can be seen from (i) that much of the biphenol has reacted, there being many products eluting shortly after the solvent. In (ii) however, much of the original material remains unchanged, with only three small product peaks seen on the chromatogram. It appears that in this case the Blankit D has served to retard the rate of reaction, and also to change its course, since new product peaks are observed. The most obviously new peak is that which elutes after the biphenol. This was not observed for the solution irradiated in the absence of Blankit D, even though extensive decomposition had occurred.

In contrast to the above, irradiation at 300nm predictably caused a much more rapid reaction. The lamps were switched off after 21 hours,. by which time the solution in the absence of Blankit D had become intensely brown. The other solution had again remained colourless, but had produced a metallic deposit on the walls of the photolysis tube. This was clearly a very similar result to that obtained from the photolysis of N-acetyl tryptophan under these conditions, and the metallic deposit was believed to be zinc, formed by the decomposition of the zinc formaldehyde sulphoxylate.

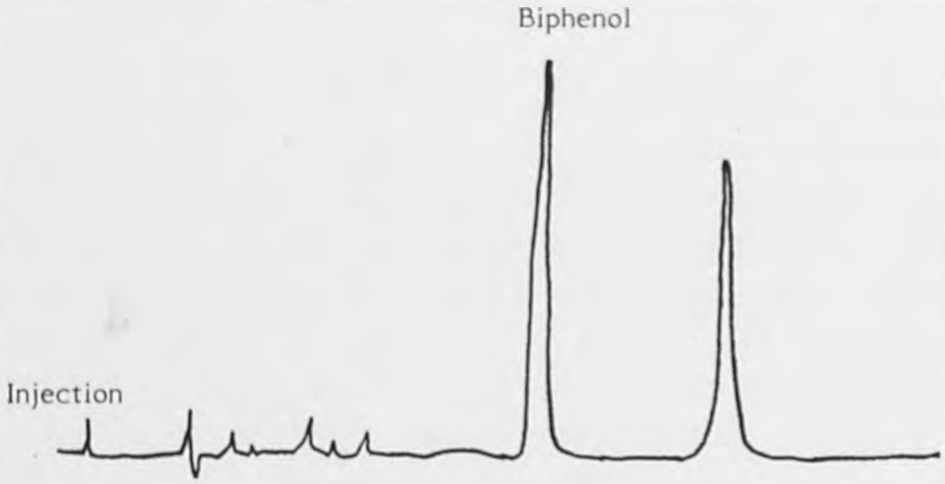
The two solutions were analysed by HPLC, and the results are shown in Figure 2.31

Fig 2.31

(i) Biphenol solution irradiated at 300nm



(ii)Biphenol solution in Blankit D.



Using the Perkin Elmer M1000 fluorescence detector in line with the U.V. detector showed that the new product, observed for irradiation in Blankit D solution (ii), was fluorescent. The excitation filter had a cut-off at 310nm, being opaque to wavelengths below this value, and the emission filter at 340nm. As a consequence of this, biphenol excitation occurred at wavelengths where the UV absorption was low, and the detector was used close to its maximum sensitivity. Even so, its use revealed the existence of a product peak in (i) eluting several minutes after the biphenol, not equivalent to the major product in (ii). The capacity factors of the peaks in all four chromatograms were calculated and listed for comparison in Table 2.25. From this it can be seen that with the exception of the starting material, the products formed are completely different when Blankit D is present. Certain extra peaks are seen for the solutions irradiated at 300nm, as would be expected because of the higher energy of the radiation absorbed. But apart from this, the change in reaction pathway caused by the Blankit D is consistent for irradiation at both 350nm and 300nm.

Having established that in Blankit D solution, the reaction pathway is different and the products are colourless, attention was again focussed upon the major product formed under these conditions. This corresponded to peak II with $k' = 3.59$. The procedure of running repeated injections and collecting the fractions for subsequent analysis was adopted in the same way as used for the tryptophan product. In this instance, however, the concentrations of the product was lower in the sample (the peak was much smaller, under the same detection conditions). Far more injections were therefore required to obtain even a small volume of the collected fraction.

In total, 78 injections were made of volume 1000µl each, and the fractions for the major biphenol product collected. The fractions were collected and combined, and then freeze dried as before. This yielded a total of 2.1mg of product which was stored under nitrogen prior to analysis.

Table 2.25

Comparison of Products Detected after Photolysis of Biphenol Solutions

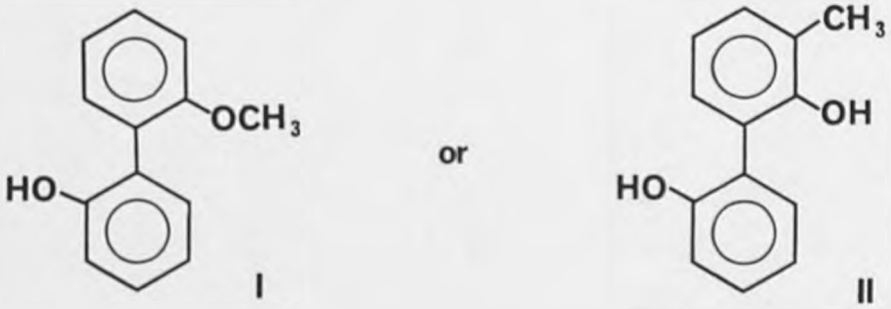
SOLUTION	Peak k'											
	0.46	0.52	0.71	0.82	0.92	0.97	1.27	1.36	1.65	2.05	3.59	4.74
Biphenol hν 350nm	✓			✓	✓		✓			✓		✓
Biphenol hν 300nm	✓			✓	✓		✓		✓	✓		✓
Biphenol/D20 hν 350nm		✓				✓				✓	✓	
Biphenol/D20 hν 300nm		✓	✓			✓		✓		✓	✓	

2.16 Characterization of the Biphenol Product

2.16.1 Mass Spectrometry

The molecular ion for biphenol is seen at 186 on the mass spectrometer. However, for the major degradation product, a molecular ion was observed at m/e=200, with the M+1 peak at 201. The spectrum appeared full of tiny peaks at almost every mass number, with intensities of around 1% or less, which suggested that the sample was somewhat impure.

Based upon the above information, two possible structures were proposed. Since the Blankit D irradiation of tryptophan had been found to increase the molecular weight by 14 units, and this had been found to be due to the addition of a methyl group, it was considered likely that the same could be true with biphenol, the photochemical treatment and the molecular weight increase being the same. The two suggested molecular structures for the product are shown below:



If methylation had occurred, structure I was considered to be the most likely site for attack, although structure II would fit the mechanism proposed for the reaction with tryptophan. This is discussed later.

The major peaks from the mass spectrum are listed below in Table 2.26, together with possible assignments, where known.

Table 2.26

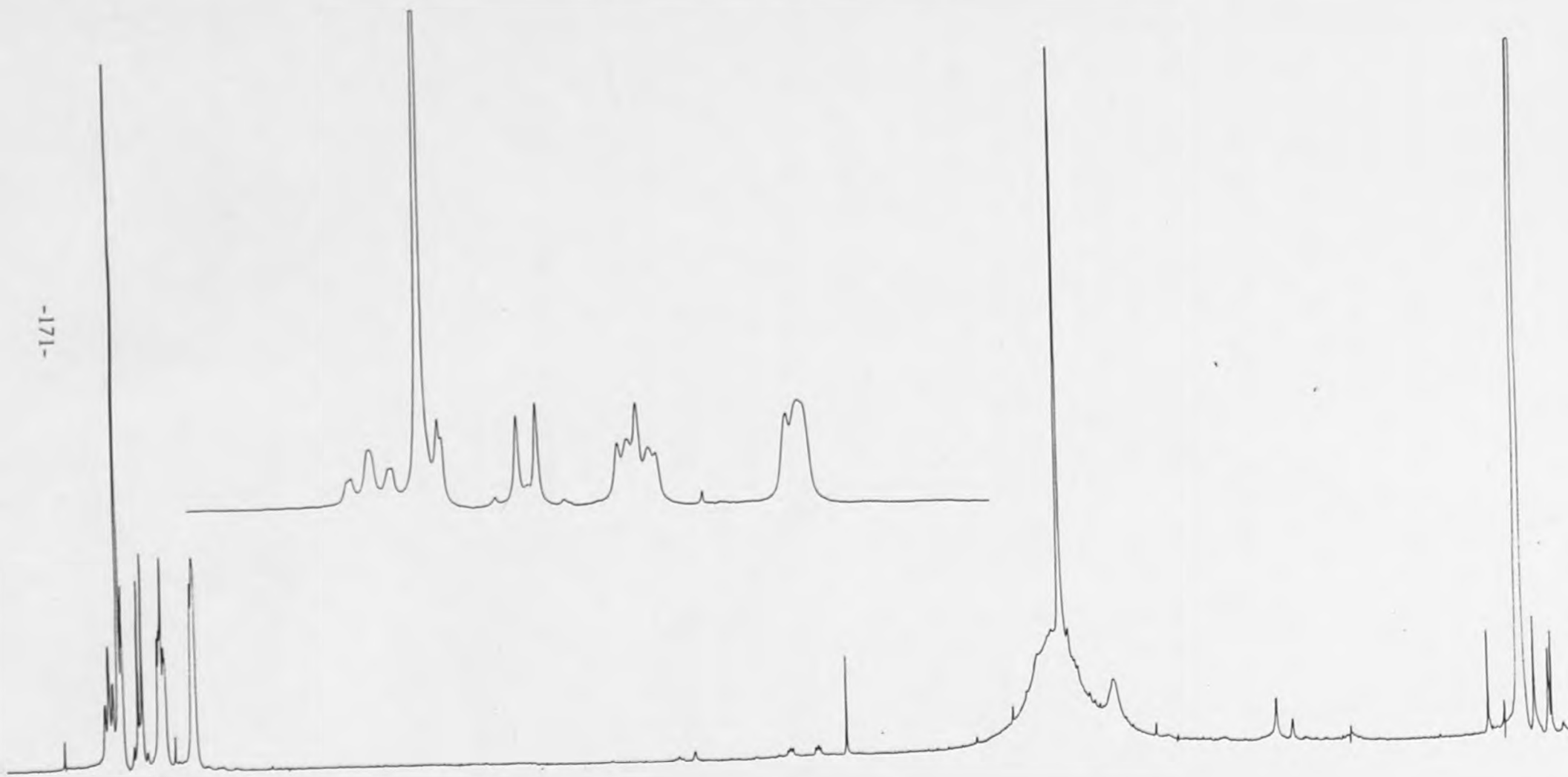
Mass Spectrum of Biphenol Major Product

m/e	Intensity (%)	Assignment
201	13.2	M+1
200	85.2	$M^{\oplus}$
199	7.4	M-H
185	11.8	$M-CH_3$
183	11.7	
182	5.6	$M-H_2O$
181	13.7	
172	17.8	M-CO
171	18.6	M-CHO
157	13.0	
149	28.4	
129	13.6	
111	12.5	
97	23.1	
95	20.3	
55	100.00	

2.16.2 Nuclear Magnetic Rosonance

. A very small sample of the major product was submitted to the University of Edinburgh for 360MHz proton nmr analysis. The resultant output was the cumulation of 1068 scans, and in consequence the impurities present manifested themselves a cross the spectrum. This is shown as Figure 2.32.

Fig 2.32 360MH$_2$ Proton NMR Spectrum of the Biphenol Product



The interpretation of this spectrum is unclear, because of the high percentage of contaminant peaks.

2.16.3 Other Analytical Methods

Very little of the material was available for these tests. The infrared spectrum was extremely weak, but consistent with either structure. UV spectroscopy showed no detectable difference between the product and the parent biphenol. Elemental analysis was not carried out because the material was already known to be impure.

To distinguish between the two possible structures for the product, a technique was required which would be sensitive to the difference between the hydroxy and methoxy substituents at very low concentrations. Attention was thus again turned to chromatography, as described in the next section.

2.17 Reaction of Biphenol with Dimethyl Sulphate

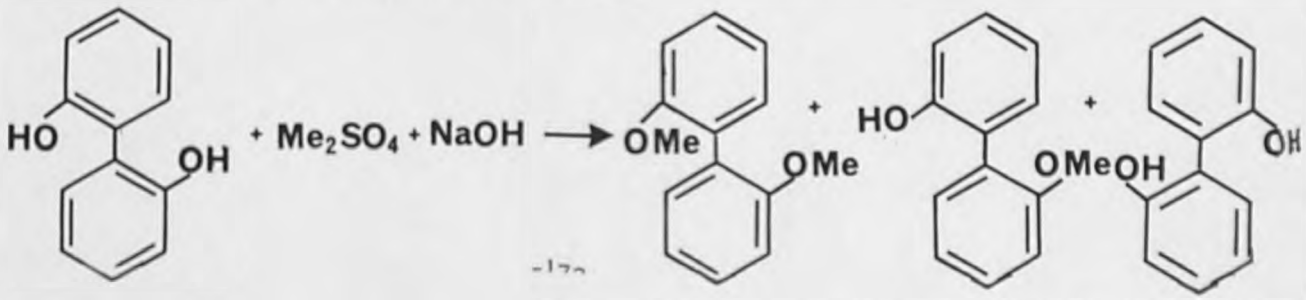
In order to determine which of the two proposed structures corresponded to the unknown product, it was considered reasonable to methylate o,o'-biphenol with dimethyl sulphate, which was known to methylate the hydroxyl groups. The products of this reaction could then be analysed by GLC and HPLC, and the peak retention times compared with that obtained for the unknown material.

2.17.1 Methylation of o,o'-biphenol with Dimethyl Sulphate

Dimethyl sulphate is a methylation reagent, known to react with alcohol functionalities as follows:

$$R-OH + (CH_3)_2SO_4 + NaOH \longrightarrow ROCH_3 + CH_3NaSO_4 + H_2O$$

In the case of biphenol, the reaction is:-



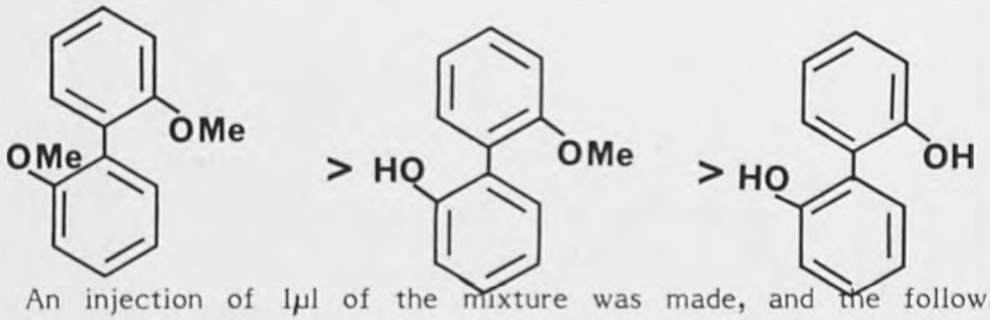
The biphenol (0.93g, 0.005M) was dissolved in 150ml of 0.21M sodium hydroxide. To this was added 1.00g (0.008M) dimethyl sulphate (ie. in slight excess). The reaction was stirred at 100°C on an oil bath overnight.

The reaction mixture was allowed to cool, and extracted with 3x30ml dichloromethane. The extracts were combined, and concentrated down to 10ml on a rotary evaporator. This extract was then analysed by GLC and HPLC.

2.17.2 GLC Analysis

The gas chromatograph used was a Perkin Elmer Sigma 3 instrument, fitted with an OV225 column. The mobile phase was nitrogen, with a flow rate of 30ml/minute. The oven temperature was set to 200°C, and the injection zone to 250°C. The recorder scale was set at 0-1mV, and the instrument attenuation to 10/128.

Using a polar column, components elute in order of increasing polarity, so the three components of the synthesized mixture were expected to elute in the order:

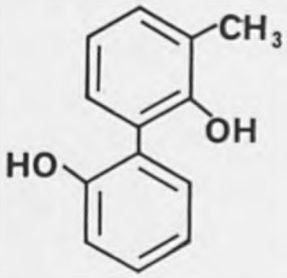


An injection of 1μl of the mixture was made, and the following retention times were observed (measured in mm):

Dihydroxybiphenyl	120mm
Hydroxymethoxybiphenyl	58.5mm
Dimethoxybiphenyl	42mm

The unknown product was then injected, dissolved in Ethanol, giving a peak at 94mm, together with two other very small peaks, one at 60mm and the other at 113mm.

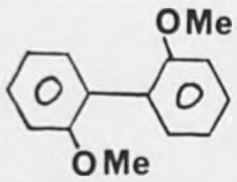
This result was interpreted to mean that the biphenol photodegrdation product was not the methoxyhydroxy biphenyl, although a small trace of this material was probably present, causing the peak eluting at 60mm. The degradation product had eluted between the mono-methoxy- and dihydroxybiphenyl peaks, suggesting that its polarity was intermediate between the two. This was consistent with the second postulated structure:



The assignment of the GLC peaks was confirmed by GC-Mass spectrometry. The methylated product mixture was injected into the same column, but mounted in a Pye 104 gas chromatograph under the same conditions. The peaks eluted were scanned by the mass spectrometer, and the molecular ions determined. The results were as follows:-

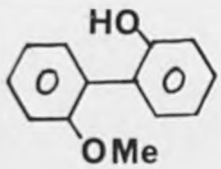
Scan 69 - first peak.

M^+ 214 3.88% $C_{14}H_4O_2$



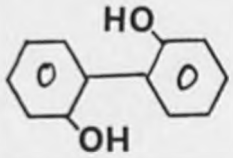
Scan 84 - second peak.

M^+ 200 93.05% $C_{13}H_{12}O_2$



Scan 178 - third peak.

M^+ 186 51.55% $C_{12}H_{10}O_2$



The assignment of the methylation mixture peaks was thus shown to be correct.

2.17.3 HPLC Analysis

The HPLC column used previously for biphenol separations was used, since suitable elution conditions were already known. The column was packed with ODS hypersil, so the elution order was the reverse of that observed for the polar GLC column. For the synthetic mixture, the retention figures are listed below, quoted as the column capacity factors, k'.

$$k' = \frac{tr-to}{to}$$

tr = retention time of peak

to = retention time of solvent

Dihydroxy biphenyl	k' = 2.2
Hydroxymethoxybiphenyl	k' = 5.85
Dimethoxybiphenyl	k' = 9.4

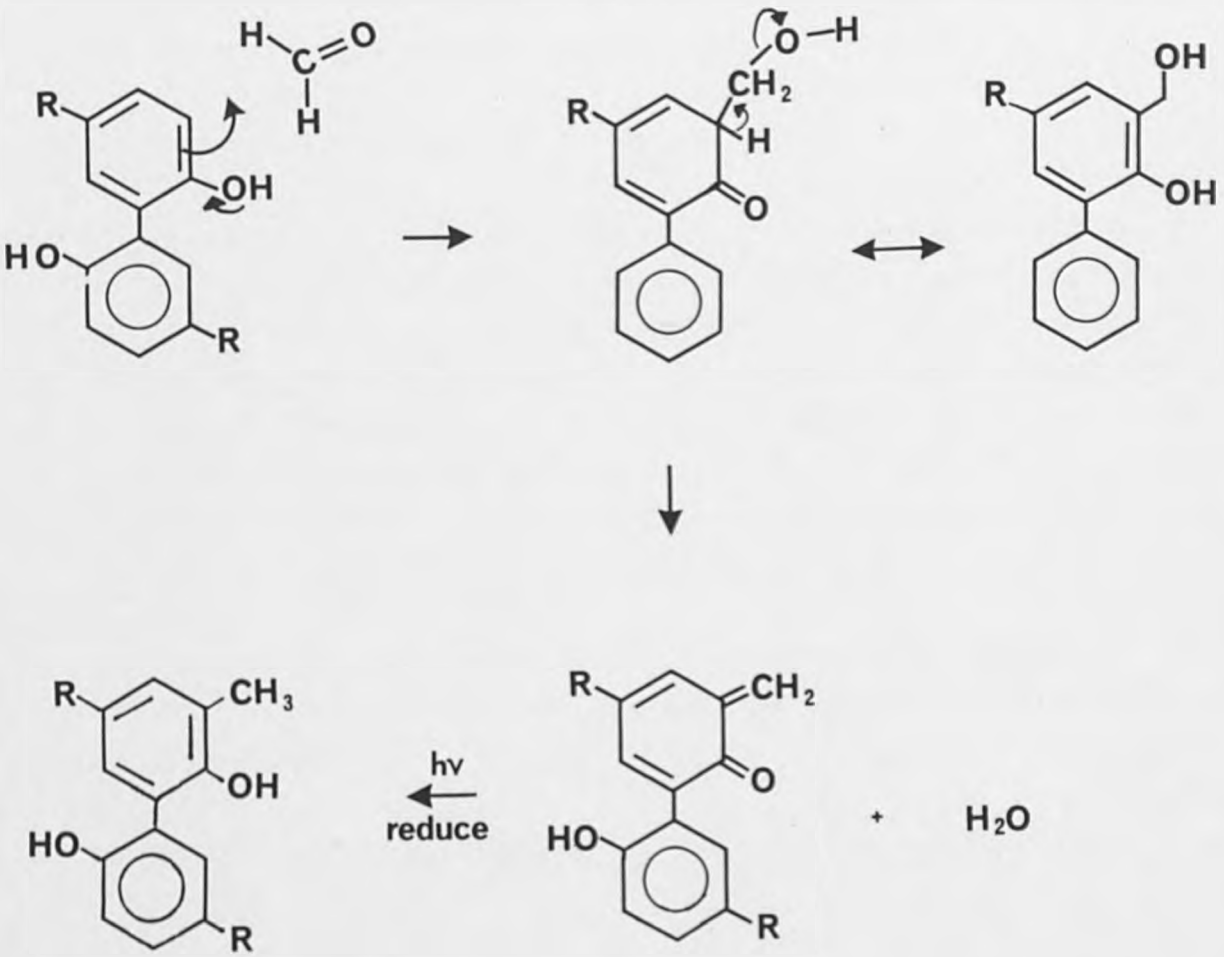
The photodegradation product was then injected and found to have a retention value k' = 3.7. This was known from previous experiments.

Again, it was thus found that the unknown product eluted between the dihydroxy- and methoxyhydroxy- biphenyl peaks. This was taken as confirmation that the assignment was correct.

2.18 Mechanism of Dityrosine Methylation

The mechanism proposed for the reaction with tryptophan is believed to apply to the dityrosine reaction also. This involved the addition of the formaldehyde molecule as a methylol group to the site adjacent to the hydroxyl function, followed by reduction to yield the methyl substituent. This is shown below in Figure 2.33.

Fig 2.33 Reaction Mechanism for Dityrosine Methylation



2.19 Treatment of Wool with Dimethyl Sulphate

Having established that the methylation of wool by irradiation in Blankit D solution results in such a marked increase in photostability, it was necessary to derive a chemical treatment which would achieve the same effect if the process was to be commercially useful. Wool was thus methylated with dimethyl sulphate and the effect on photostability determined.

2.19.1 Methylation of Wool

Dilute sodium hydroxide (0.2M) was placed in two 300ml tubes in the Jeffreys Dyemaster. To these were added 2g (0.016M) dimethyl sulphate. In one tube was placed 10g of untreated salts serge, and in the other, 10g of wool whitened with Uvitex NFW. The resultant solutions were adjusted to pH8 with sulphuric acid to minimize wool yellowing due to presence of alkali, and the solutions heated to 70°C with constant agitating for 5 hours. At the end of this time, the wool samples were rinsed to remove any unreacted material and dried in air at room temperature.

Both samples were found to be yellowed by this treatment, and no improvement in photostability was observed. The yellowness index measurements are listed in Table 2.27.

Table 2.27 The Effect of Methylation of Wool with Dimethyl Sulphate

SAMPLE	Y.I.	Y.I. After Photoyellowing	Δ Y.I.
Untreated Wool	14.2	19.2	5.0
Untreated Wool After Methylation	18.5	22.7	4.2
Wool Whitened with Uvitex NFW	-5.1	17.4	22.5
NFW Wool, After Methylation	2.4	18.6	21.0

Thus methylation with dimethyl sulphate was found to be unsuccessful.

2.19.2 Irradiation of Wool in the Presence of Dimethyl Sulphate

Before making the assumption that dimethyl sulphate was totally unsuitable for stabilizing wool, a sample of wool was irradiated in a solution of the methylating agent overnight. In this way it was planned to confirm whether it was a photodegradation intermediate on wool which should be methylated, rather than the untreated fibre.

Two litres of 0.2M sodium hydroxide were placed in the steel irradiation tray, and 2g dimethyl sulphate was added. When thoroughly mixed, 10g of prewetted serge and 10g of fluorescently whitened serge were placed in the tray 10mm under the surface of the solution and irradiated with 350nm light for 24 hours. The two wool samples were then thoroughly rinsed and dried in air. Following photoyellowing tests, the yellowness index values were measured, as listed below in Table 2.28.

Table 2.28 The Effect of Irradiation of Wool in Dimethyl Sulphate

SAMPLE	Y.I.	Y.I.After Photoyellowing	Δ Y.I.
Untreated Wool	14.4	19.3	4.9
Untreated Wool in Dimethyl Sulphate	18.7	23.4	4.7
NFW Wool	-4.7	17.0	21.7
NFW Wool, Irradiated in Dimethyl Sulphate	11.3	23.4	12.1

It is clear from the above results that irradiation in the presence of dimethyl sulphate solution still causes yellowing, and no photostabilization is achieved.

A possible reason why the dimethyl sulphate failed to stabilize the wool, where Blankit D has succeeded, is that the site of reaction would be different. Tryptophan would be methylated on the indolic nitrogen atom, and biphenol or dityrosine on the hydroxyl groups. Reaction and modification at these sites is thus concluded to be ineffective in stabilizing wool against photoyellowing.

2.20 Methylation of Wool with Iodomethane

Iodomethane is another methylation reagent capable of methylating wool. It is highly carcinogenic and would therefore be of little use commercially. But the attempt was made to methylate wool with this reagent to determine whether any improvement in photostability could be achieved.

For solubility reasons, dimethylformamide (DMF) was used as solvent. Two 300ml dyemaster tubes were filled with DMF, and 1g of sodium bicarbonate was added to each. To both tubes was then added 2ml of iodomethane. In one was placed 10g of untreated salts serge, and in the other, 10g of wool fluorescently whitened with Uvitex NFW. The two tubes were maintained at room temperature and agitated at 30rpm for 5 hours. The wool samples were then removed, washed thoroughly in water and dried in air at room temperature. Both samples were found to be quite markedly yellowed and the FWA had been extracted almost completely. This latter was probably due in part to the choice of solvent, but the level of yellowing observed rendered this process useless in the stabilization of wool.

2.21 Amino Acid Analysis of Wool Irradiated in Blankit D Solution

The analyses described in this section were run at the Queen's University, Belfast, to whom thanks are due.

Having established that irradiation in Blankit D stabilizes not only tryptophan but dityrosine against photoyellowing, a sample of treated wool was subjected to amino acid analysis to determine which, if any, other amino acids were affected by the process.

The amino acid analyses were carried out on an LKB 4101 Amino Acid Analyser. Five wool samples were tested. The first was untreated salts serge, pH7 buffered, which was run as a standard for comparison. Two samples of wool irradiated in Blankit D solution were examined, the first irradiated with 300nm light, the second at 350nm. The latter was markedly whiter than the former. Finally, two peroxide bleached wool samples were run. One had been bleached using the alkaline long-liquour process described earlier, and the other had been treated with acid peroxide under pad/batch conditions. The results of these analyses are shown in Table 2.29.

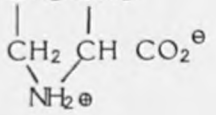
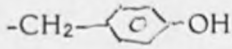
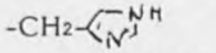
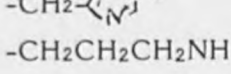
Table 2.29 Amino Acid Analyses of Wool

Amino Acid Content (μ mol/gram)	SAMPLE *				
	A	B	C	D	E
Aspartic Acid	521.63	172.91	151.12	272.66	333.18
Threonine	508.24	443.77	305.28	430.85	332.47
Serine	826.33	555.01	691.47	760.74	583.42
Proline	645.45	250.32	219.48	306.62	204.12
Glycine	608.41	459.49	500.66	607.32	435.26
Alanine	374.60	308.34	326.65	390.31	277.18
Valine	386.77	326.48	315.97	426.08	297.02
Cystine	712.70	473.98	549.52	691.58	517.48
Methionine	20.86	24.18	25.95	27.82	21.98
Isoleucine	244.23	198.30	209.12	275.84	231.09
Leucine	530.18	476.41	460.98	542.14	418.24
Tyrosine	207.73	56.83	85.48	223.37	162.33
Phenylalanine	214.68	255.14	158.75	189.99	136.11
Lysine	194.69	175.33	183.17	200.32	146.74
Histidine	46.93	42.32	42.74	46.90	38.28
Arginine	459.78	435.30	431.98	452.31	350.19
Cysteic Acid	-	-	-	51.67	82.23
Glutamic Acid	891.46	671.09	679.26	878.39	584.12

* A = Untreated salts serge, pH 7 buffered.
B = Blankit D 2% /hν 300nm.
C = Blankit D 2% /hν 350nm.
D = Acid Peroxide Bleach, pad/batch.
E = Alkaline Peroxide Bleach.

NB. Tryptophan is degraded by the hydrolysis conditions used, and hence cannot be determined by this method.

Table 2.30

	A	C	% Decrease	Amino Acid Structure $R\text{-}CH(NH_2)CO_2H$
Aspartic Acid	521.63	151.12	81%	$-CH_2\ CO_2H$
Threonine	508.24	305.28	40%	$-CH(CH_3)OH$
Serine	826.33	691.47	16%	$-CH_2OH$
Glutamic Acid	891.46	679.26	24%	$-CH_2CH_2CO_2H$
Proline	645.45	219.48	66%	
Glycine	608.41	500.66	18%	$-H$
Alanine	374.60	326.65	13%	$-CH_3$
Valine	386.77	315.97	18%	$-CH\text{-}(CH_3)_2$
Cystine	712.70	549.52	23%	$-CH_2SS\ CH_2-$
Methionine	20.86	25.95	20%	$-CH_2CH_2\text{-}S\text{-}CH_3$
Isoleucine	244.23	209.12	14%	$-CH(CH_3)CH_2CH_3$
Leucine	530.18	460.98	13%	$-CH_2CH(CH_3)_2$
Tyrosine	207.73	85.48	59%	
Phenylalanine	214.68	158.75	26%	
Lysine	194.69	183.17	6%	$-CH_2CH_2CH_2CH_2NH_2$
Histidine	46.93	42.74	9%	
Arginine	459.78	431.98	6%	

Of particular interest are the values in column C, relating to wool which had been irradiated in the presence of Blankit D solution under 350nm light. These figures are listed again in Table 2.30, where the percentage decrease in concentrations of each amino acid are calculated. It is clear from this table that all amino acids (except methionine) are in some measure modified by the treatment. Those which are of particular interest are those which have in the past been claimed to be involved with photoyellowing and those which are now seen to be markedly affected. Those believed to be involved with yellowing are:

Tryptophan
Tyrosine
Histidine
Cystine
Methionine
Phenylalanine

Tryptophan has been shown colorimetrically to be 65% degraded by a 24 hour irradiation in Blankit D, and tyrosine is now observed to be 59% modified by the process. Cystine and Phenylalanine appear to have undergone 25% decomposition, whilst histidine showed little change. Methionine appears to have been enriched during the process, but this was thought unlikely to be due to the Blankit D reaction, since increases were also observed from the other bleaching treatments.

Substantial changes were also observed for aspartic acid, threonine and proline, but since these amino acids do not produce marked quantities of coloured products upon irradiation, it was considered unlikely that these modifications were contributing significantly to the photostabilizing effect.

2.22 Conclusions

It has thus been shown that the treatment of wool with Blankit D in the presence of UV light achieves a significant increase in photostability. The chemical reactions primarily responsible have been elucidated and mechanisms proposed. It has been shown that the treatment has widespread effect on the amino acids present in wool protein, and this is possibly

the reason why the process is successful. For this method to become commercially viable, it now remains for a purely chemical treatment to be developed which achieves the amino acid modifications described in this chapter, but this is not expected to be easy!

REFERENCES

CHAPTER TWO

1) D.R.Graham & K.W.Statham, J.Soc.Dyer.Col., 1956, 72, 434

2) L.A.Holt, B.Milligan & L.J.Wolfram, Text.Res.J., 1974, 44, 846

3) ASTM Test for Yellowness of plastics, D1925-73

4) Jacquemart, Teintex, 1962, 27, 79 & 163

5) J.Cegarra, J.Gacen & M.Caro, J.Soc.Dyer.Col., 1978, 94, 85

6) M.J.Palin, D.C.Teasdale & L.Benisek, J.Soc.Dyer.Col., 1983, 99, 261

7) J.M.Gillespie, Nature, 1959, 183, 322

8) J.M.Gillespie, I.J.O'Donnell, E.O.P.Thompson & E.F.Woods, J.Text. Inst., 1960, 51, T703

9) R.Consden, A.H.Gordon & A.J.P.Martin, Biochem.J., 1946, 40, 580

10) W.R.Middelbrook & H.Phillips, Biochem.J., 1947, 41, 218

11) BASF Process Data Sheet : "Processes of Bleaching Wool, alone and with other Fibres".

12) Private Communication from Herr.K.Reincke, BASF to Dr.D.M.Lewis, I.W.S. 25/4/84

13) N.H.Leon, Textile Progress, 1975, 7, 1

14) Wool Science Review, 1970, No.39, 27

15) M.G.King, J.Text.Inst., 1971, 62, 251

16) C.H.Nicholls & M.T.Pailthorpe, J.Text.Inst., 1976, 67, 397

17) C.M.Bhatnagar & L.C.Gruen, Aust.J.Biol.Sci., 1968, 21, 1325

18) I.H.Leaver & G.C.Ramsay, Photochem.Photobiol., 1969, 9, 531

19) A.G.Kempton, M.Greenberger & A.M.Kaplan, Text.Res.J., 1962, 32, 128

20) S.Kokot, M.Feughelman & R.M.Golding, Text.Res.J., 1972, 42, 704

21) S.Kokot, M.Feughelman & R.M.Golding, Text.Res.J., 1973, 43, 146

22) S.Kokot, M.Feughelman & R.M.Golding, Text.Res.J., 1974, 44, 523

23) G.J.Smith, Text.Res.J., 1975, 45, 483

24) J.R.McPhee, Text.Res.J., 1965, 35, 382

24a) C.H.Nicholls, Developments in Polymer Photochemistry, 1980, P129 Ed.N.S.Allen

25) C.H.Nicholls, Private Communication re. University of New South Wales Ph.D.thesis, 1980.

26) A.S.Inglis & R.J.Simpson, Text.Res.J., 1968, 38, 109

27) I.C.Watt & R.Morris, Text.Res.J., 1970, 40, 952

28) E.H.Hinton, Text.Res.J., 1974, 44, 233

29) J.B.Speakman & P.L.D.Peill, J.Text.Inst., 1943, 34, T70

30) R.N.Reddie & C.H.Nicholls, Text.Res.J., 1971, 41, 841

31) H.Fraenkel-Conrat & H.S.Olcott, J.Biol.Chem., 1948, 174, 827

32) J.Lewis, Text.Res.J., 1974, 44, 707

33) L.A.Holt & B.Milligan, 1984, 54, 521

34) B.I.O.S. Report 422

35) B.I.O.S. Report 271

36) G.Brearley & J.Starkie, J.Soc.Dyer.Col., 1948, 64, 278

37) E.Herzfeld, Biochem.Z., 1913, 56, 258

38) I.Kraus, J.Biol.Chem., 1925, 63, 157

39) C.E.May & E.R.Rose, J.Biol.Chem., 1922, 54, 213

40) J.R.Spies & D.C.Chambers, Anal.Chem., 1948, 20, 30

41) J.R.Spies & D.C.Chambers, Anal.Chem., 1949, 21, 1249

42) D.Graham & K.W.Statham, Text.Res.J., 1960, 30, 136

43) D. Graham & K.W.Statham, Text.Res.J., 1962, 32, 908

44) G.Mazingue, G.Decroix, & M.van Overbeke. Bull.Inst.Text.France, 1956, 47, 37

45) J.Opienska-Blanth, M.Charezinski & H.Berbec, Anal.Biochem., 1963, 6, 69

46) P.Miro, Investigacion e Informacion Text., 1961, 4, 25

47) K.K.Juneja, A.D.Sule & V.B.Chipalkatti, Text.Res.J., 1968, 38, 461

48) J.Cegarra & J.Gacen, J.Soc.Dyer.Col., 1968, 84, 216

49) M.Tswett, Proc.Warsaw Soc.Nat.Sci.(Biol). 1903, 14, No.6

50) J.C.Kraak & J.F.K.Huber, J.Chromatogr., 1974, 102, 333

51) J.H.Knox & J.F.Parcher, Anal.Chem., 1969, 41, 1599

52) R.S.Asquith & D.E.Rivett, Text.Res.J., 1969, 39, 633

53) R.S.Asquith, L.Hirst & D.E.Rivett, Text.Res.J., 1970, 40, 285

54) M.S.Otterburn, Queens University, Belfast, Private Communication

55) M.S.Otterburn, P.E.Gargan, J.Chrom., 1984, 303, 429

56) J.A.Swift, Hair Research, 1981, 65-72

57) J.A.Swift & B.Bews, J.Soc.Cosmet.Chem., 1974, 25, 13

58) J.A.Swift & B.Bews, J.Soc.Cosmet.Chem., 1974, 25, 355

59) J.A.Swift & B.Bews, J.Soc.Cosmet.Chem., 1976, 27, 289

60) G.S.Bayse, A.W.Michaelis & M.Morrison, Biochim.Biophys.Acta., 1972 284, 34

61) E.Heidemann, Ch.Keller & P.Waykole, Proc.5th Int. Text.Res.Conf. Aachen, 1975, III, 253

CHAPTER 3

A Study of the Effects of Wool Yellowing & FWA Decomposition using U.V. Diffuse Reflectance Techniques

CHAPTER 3

A STUDY OF THE EFFECTS OF WOOL YELLOWING & F.W.A. DECOMPOSITION USING U.V. DIFFUSE REFLECTANCE TECHNIQUES

CHAPTER 3

A Study of the Effects of Wool Yellowing & F.W.A. Decomposition Using U.V. Diffuse Reflectance Techniques

3.1 Introduction

Wool treated with a fluorescent whitening agent is considerably whiter than untreated wool, and photoyellows much more rapidly. Practically all white wool marketed today is treated with a fluorescent whitening agent, and considerable effort has been made to understand the reasons for this accelerated photoyellowing. [1-3]. This subject has been discussed in section 1.4.4., and the conclusion drawn was that FWA's promote yellowing by acting as sensitizers, probably to the processes occurring in natural wool [2,4,5].

In this chapter, the stability of several commercial FWA's is examined, both in solution and on wool. The rates of photoyellowing are compared, and two methods of monitoring the degradation process are described.

There are many types of fluorescent whitening agents [6], but for wool, the most commonly used species are stilbenes, pyrazolines and coumarins. It has become apparent that wool treated with these compounds undergoes photoyellowing, and this is caused by the wool and the FWA undergoing degradation [2,7]. There have been numerous studies on the photodegrdation of pyrazolines [2,8-11], but fewer on the breakdown of stilbenes [1,3,12]. In many cases, these degradation studies have focussed upon the photostabilities of the FWA's in solution, and in some cases the stability related to structure [1,3,8-11]. It has been reported that some pyrazoline FWA's which show varying degrees of photo-stability in aqueous solution, show a similar structure -reactivity relationship when applied to wool [2]. Surprisingly, wool treated with pyrazolines which exhibit differing photostabilities photoyellows at a rate which is independent of the type of pyrazoline used.

The common photodegrdative route for pyrazolines is oxidation to pyrazoles (both in solution and on wool) [10]. However, for stilbenes, oxidative degradation occurs in solution, [1,3,12], but on wool the FWA's

appear to be photoreduced rather than photooxidized [1]. This particular study [1] showed that many of the degradation products became firmly attached to the wool, and perhaps, based on earlier studies of the photoreactions of pyrazolines with proteins, [13], this attachment is via covalent bonds.

3.2 Experimental Procedures

3.2.1 FWA Stability in Solution

These results were obtained by Dr.D.King, at the City University, and are included for comparison. The various FWA's were dissolved in solvents to give and OD of 1.0. They were then flushed with oxygen for 1 minute and irradiated in quartz cuvettes. The light sources were fluorescent tubes, with a λmax of 300nm or 350nm, arranged as a circular array of 16x8W (RPR 3000A) Rayonet tubes and 16x8W (FT8TS/BLB) Sylvania black light lamps respectively. The decrease in OD was measured at frequent intervals to ascertain the time taken for 50% degradation. The results are expressed as relative stabilities compared with Uvitex NFW, which was taken as 1.0 in water illuminated with 350nm lamps.

As most of the solutions discoloured on irradiation, the stabilities must be regarded as apparent only.

3.2.2. Application of FWA's to Wool & Photoyellowing

Wool serge ($200gm^{-2}$) was bleached with hydrogen peroxide as described in chapter two, and whitened as follows:-

Blankit IN	6g/l
Lissapol N	0.6ml/l
FWA	1% owf

pH4, Liquor:Wool ratio 60:1

The temperature was raised to 80°C at 1°C/min. and held at 80°C for 1 hour. The fabric was then rinsed in cold water and dried at room temperature. All the FWA's applied gave exhaustion values in excess of 90%.

The photoyellowing was carried out by exposing the wool to long wavelength U.V. tubes with a λmax at approximately 350nm. The samples were immersed under 5-10mm of water and irradiated at room temperature. Each sample was divided into two halves. One half was covered with foil whilst the other half was exposed to the light for 24 hours.

3.2.3. Extraction of FWA from Wool

The FWA residual on the wool after irradiation was extracted by the method of Evans et.al. [2]. Weighed fabric samples (approximately 200mg) were extracted on a boiling water bath with 0.5% aqueous ammonia solution. Extraction was complete after three treatments (1x15ml, 2x10ml) for 10 minutes each, after which the combined extracts were filtered and diluted to 50ml for spectrophotometric determination of the FWA.

3.2.4. Yellowness Index Measurements

A Zeiss RFC3 relectance spectrophotometer was used. Measurements were taken and 20nm intervals with barium sulphate as a reference. The light source was a Xenon lamp. This instrument was controlled by a computer, programmed to give Y.I. values (ASTM D 1925) calculated from the C.I.E. tristimulus values as follows:-

$$Y.I. = 100\ \frac{X-Z}{Y}$$

3.2.5 Diffuse Reflectance Spectroscopy

Diffuse relectance UV spectra were obtained using a Perkin Elmer Lambda 5 UV/Visible spectrophotometer. The Lambda 5 is double-beam recording instrument, with a filter-grating monochromator. It has a holographic grating of 1440 lines/mm, with deuterium and tungsten-halogen light sources. The optical system in the absorption mode is represented diagramatically in Figure 3.1. For measurements of turbid or solid samples, and integrating sphere attachment was used, with magnesium carbonate as the reference. Solid samples were clamped into a special holder, mounted normally to the incident radiation beam. The instrument had the facility

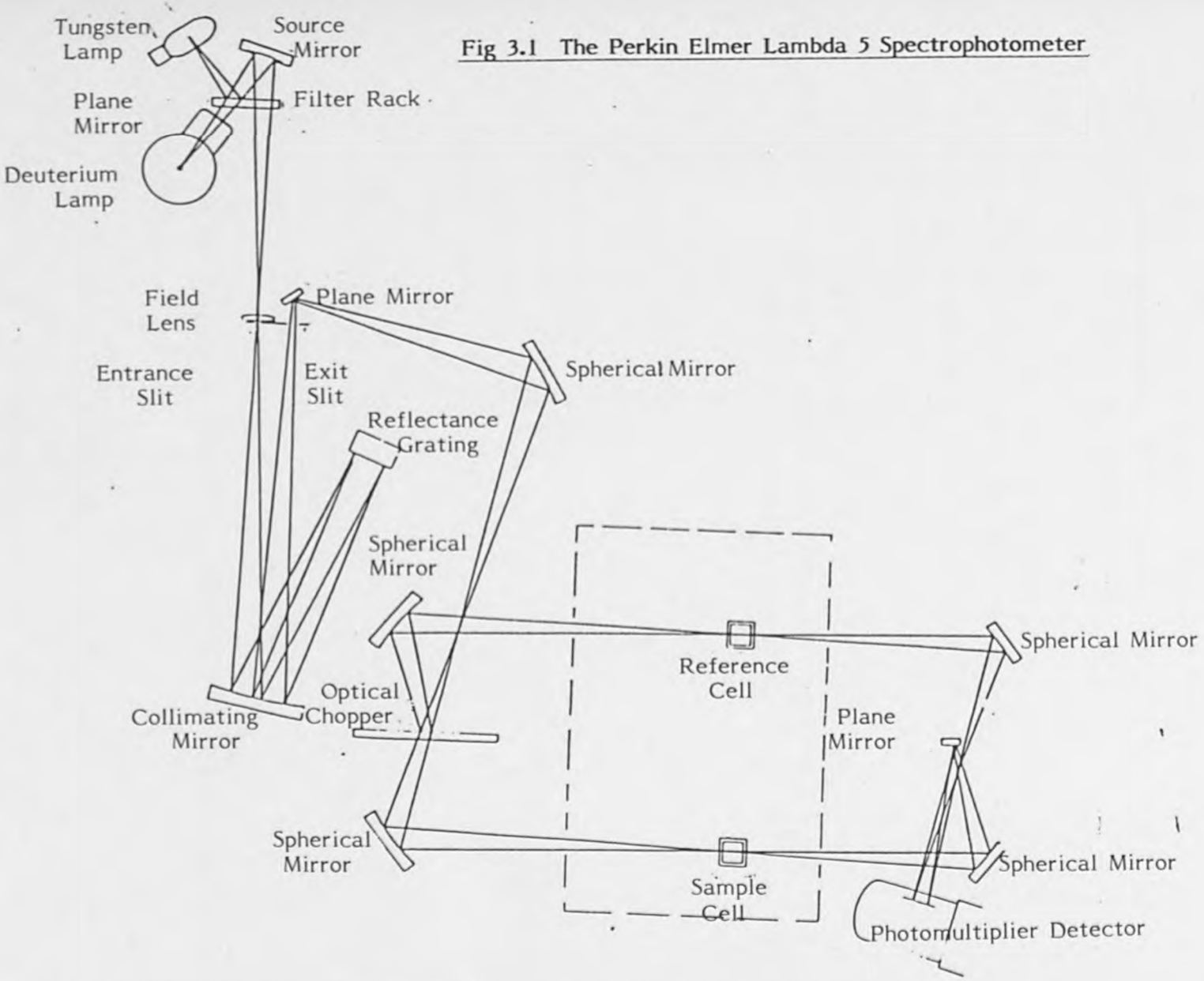


Fig 3.1 The Perkin Elmer Lambda 5 Spectrophotometer

to position highly reflecting samples at an angle of 8° to the plane normal to the incident light, so that reflected light would fall into the integrating sphere, and not back along the optical path.

The spectrum observed for a given sample was the sum of the reflected light and the fluorescence. Thus absorption appeared weaker as fluorescence increased. This presented a number of problems when interpreting spectra, and is discussed later.

The instrument was used primarily in the transmission mode. This was because absorption, being a log scale, was unsuitable for subsequent subtraction of digitized spectra. The general operating conditions and parameters are listed below:

Transmission Mode
Slit : 2nm
Scan Speed : 60nm/min
Response : 0.5 sec
Lamp : 332.8nm
Peak Threshold : 2% T
Ordinate : 0-100%T
Abscissa : 190 - 800nm
Abscissa Format : 20nm/cm

3.3. Results & Discussion

3.3.1 Diffuse Reflectance Spectra of Untreated, Bleached & Fluorescently Whitened Wool

The diffuse relectance spectrum of untreated salts serge is shown in Figure 3.2, directly compared with bleached wool. The main absorption maximum at 287nm probably arises from the tryptophyl residues in wool. It is observed that the peroxide bleaching process yielded a general decrease in absorption across the entire spectrum. However, the most significant change is the decrease in absorption in the blue region (400-430nm), which causes the whitening effect of the bleach.

The effect of photoyellowing is shown in Fig. 3.3. Untreated serge

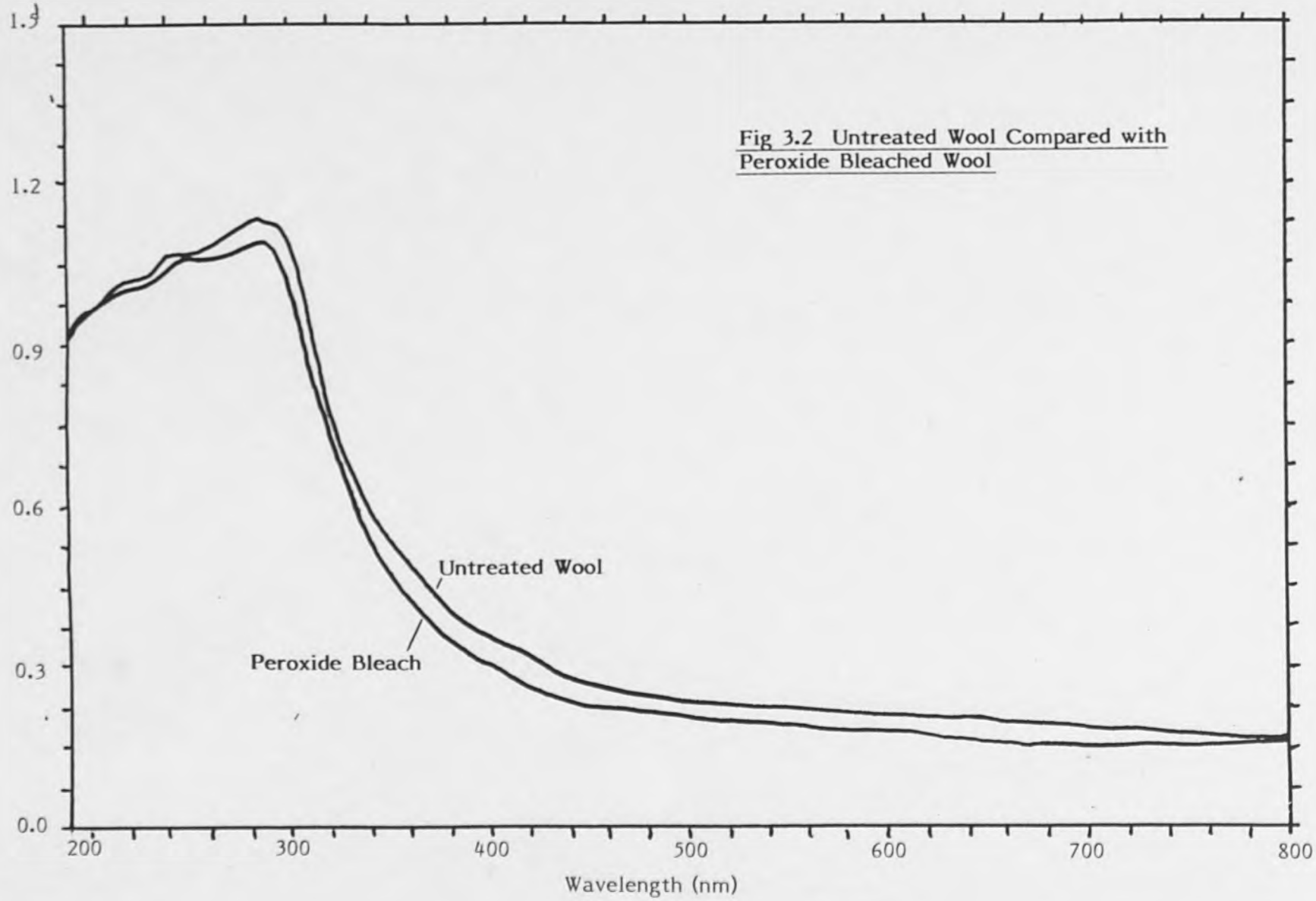


Fig 3.2 Untreated Wool Compared with Peroxide Bleached Wool

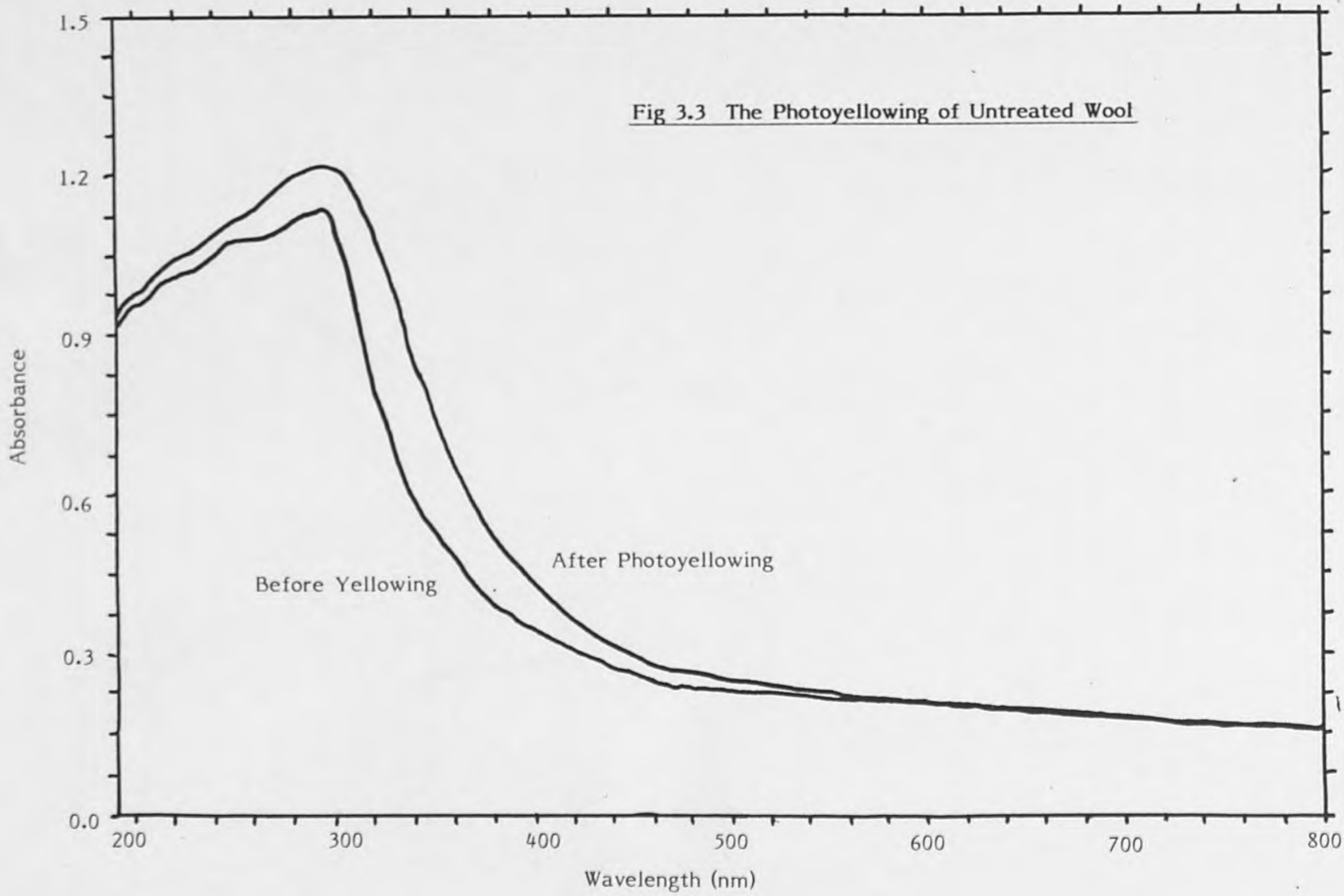


Fig 3.3 The Photoyellowing of Untreated Wool

was studied before and after irradiation for 24 hours at 350nm. The increase in yellowness is observed to arise from a marked increase in absorption in the near UV and blue region of the spectrum.

When an optical brightener was applied to wool, the reflectance spectra showed the presence of the FWA. The absorption spectrum obtained for Uvitex NFW is shown in Fig 3.4. Also shown on figure 3.4 is the spectrum for Uvitex NFW in solution. It can be seen that the absorption maxima from the solution spectrum show a marked correlation with the minima obtained from the treated wool sample. This effect is discussed later.

During the course of this work, five commercial fluorescent whitening agents were used:

FWA	MANUFACTURER	STRUCTURAL DETAILS (if known)
Uvitex NFW	Ciba Geigy	bis-styryl biphenyl
Uvitex CF	Ciba Geigy	heterocyclic stilbene derivative
Uvitex BHT	Ciba Geigy	Not Known
Hostalux PR	Hoechst	Not Known
Blankophor BA	Bayer	Stilbene disulphonic acid

The diffuse relectance spectra for these FWA's, before and after yellowing, are given together with their solution spectra as Figs 3.4-3.8. The correlation between the maxima of the solution absorption spectra and the minima of the corresponding diffuse relectance spectra is remarkably consistent. It is thus possible to differentiate between one FWA and another, and to identify an unknown FWA, provided a file of absorption spectra of commonly used FWA's are available.

This correlation of absorption minima and maxima was not expected, because the background (effectively the spectrum of untreated wool) was far from flat. However, when the spectrum of blank wool was digitized and subtracted from that of the fluorescently whitened wool, the resultant difference spectrum bore little resemblance to that of the FWA in solution. It was thus concluded that in whitening the wool, the profile of the wool

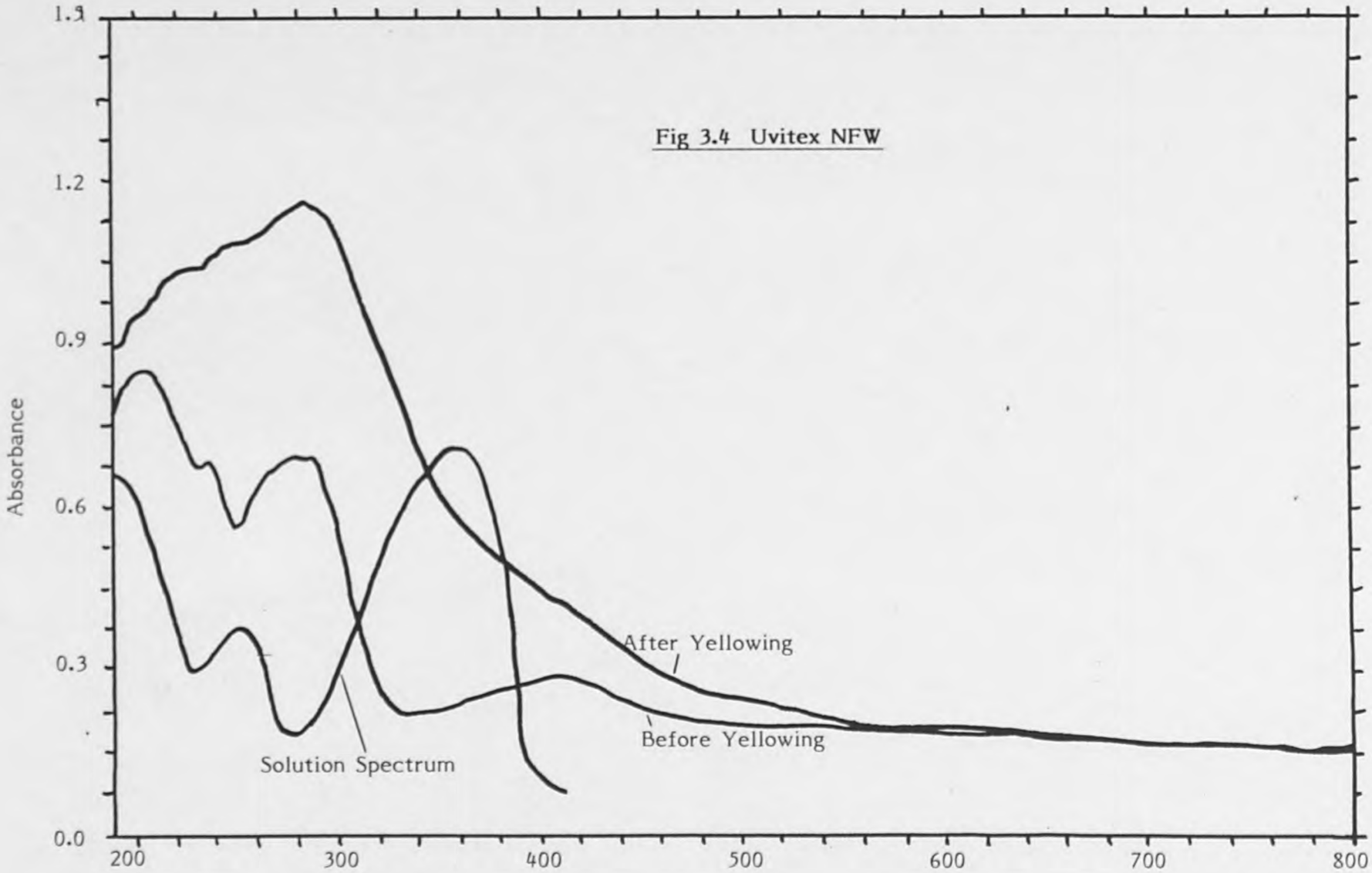


Fig 3.4 Uvitex NFW
Absorbance
1.5
1.2
0.9
0.6
0.3
0.0
200
300
400
500
600
700
800
After Yellowing
Before Yellowing
Solution Spectrum

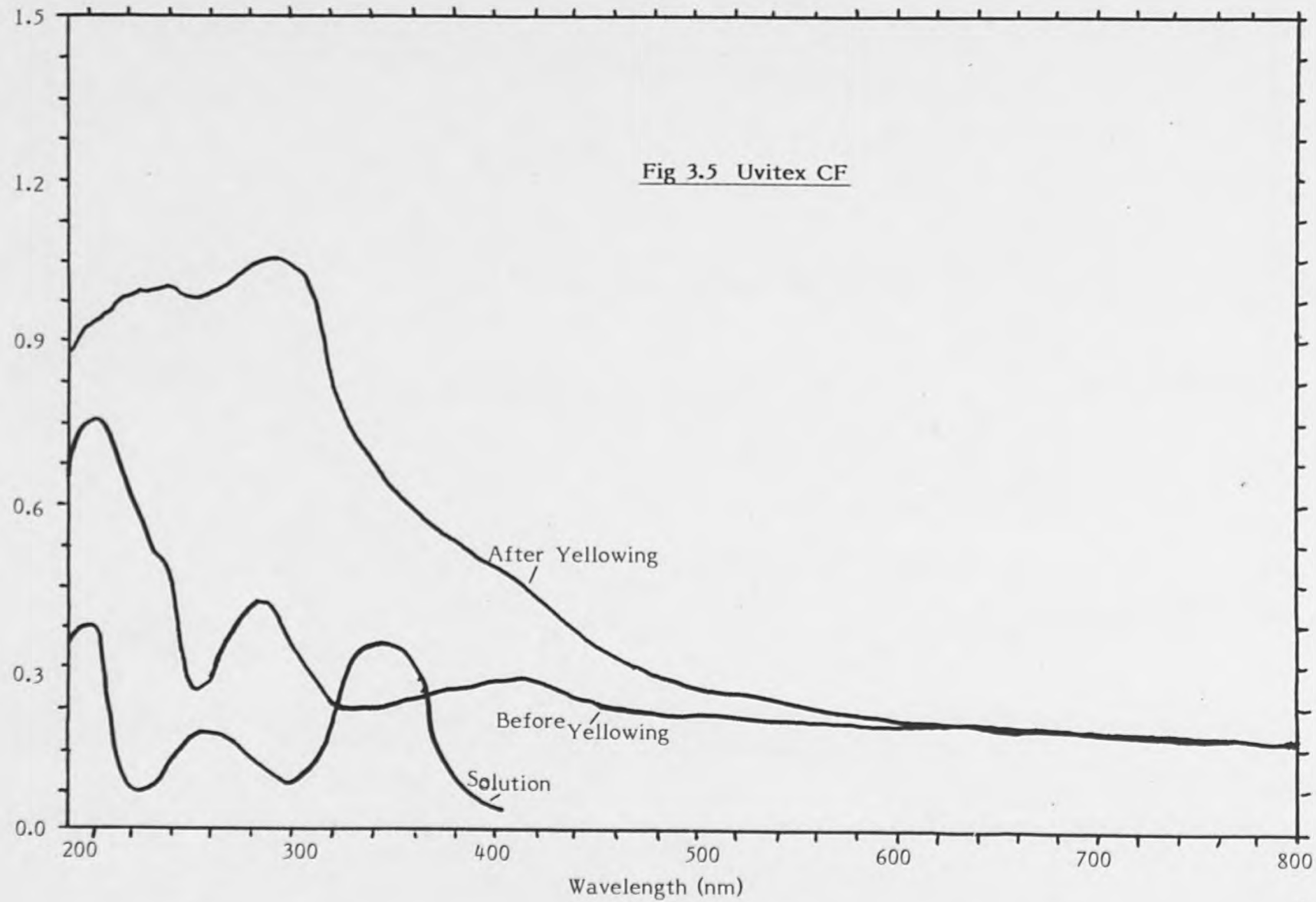


Fig 3.5 Uvitex CF

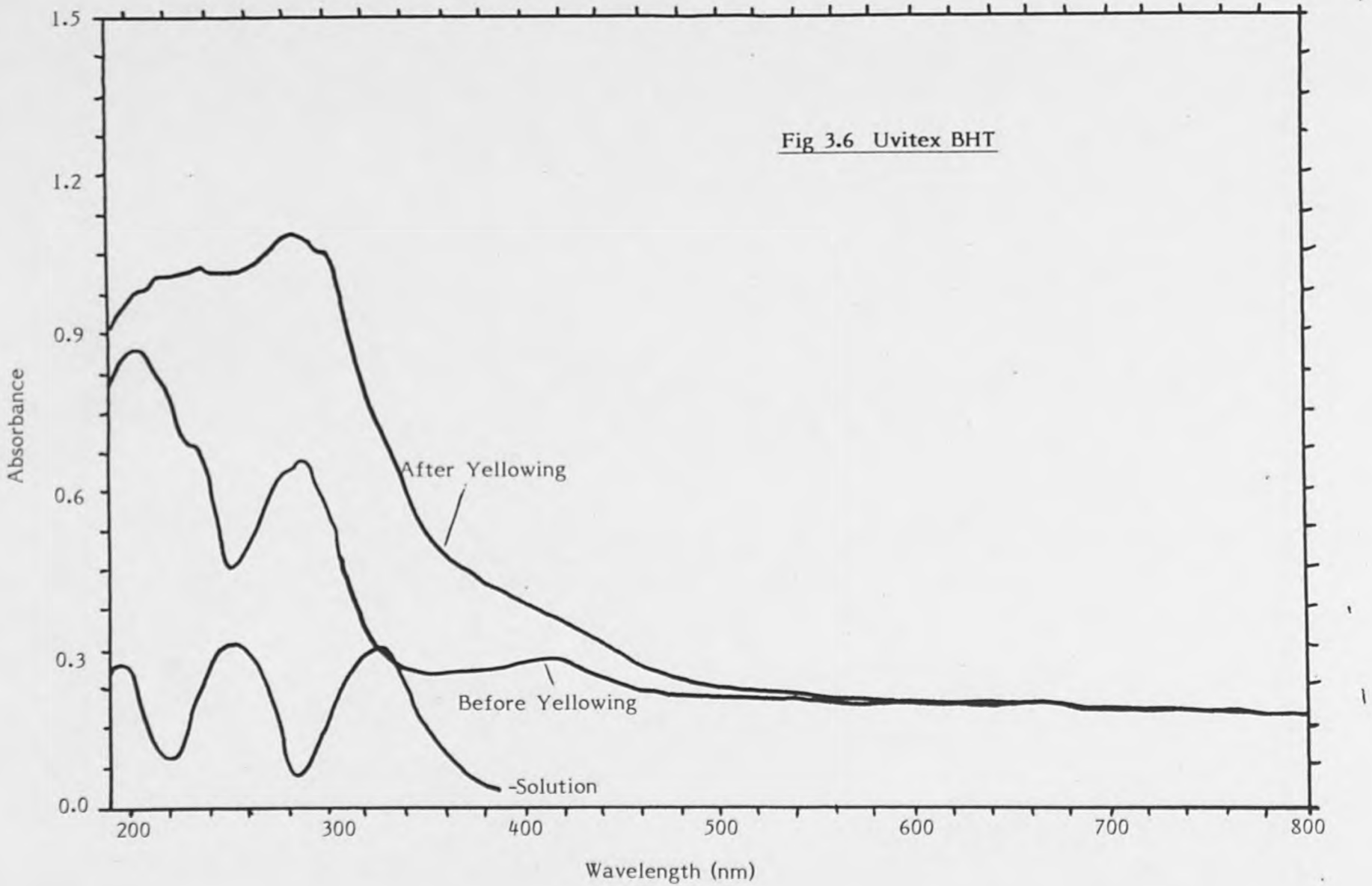


Fig 3.6 Uvitex BHT

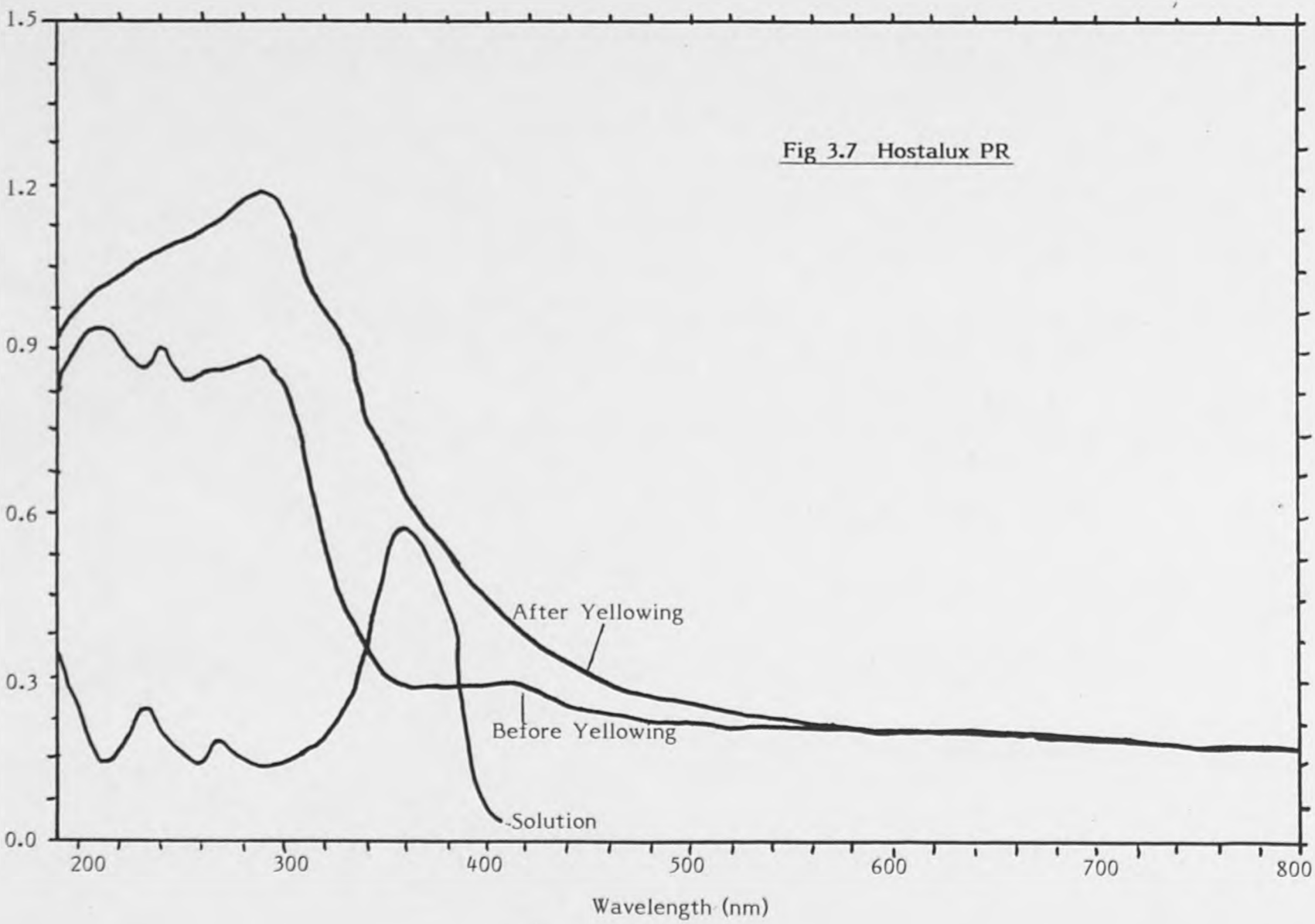


Fig 3.7 Hostalux PR

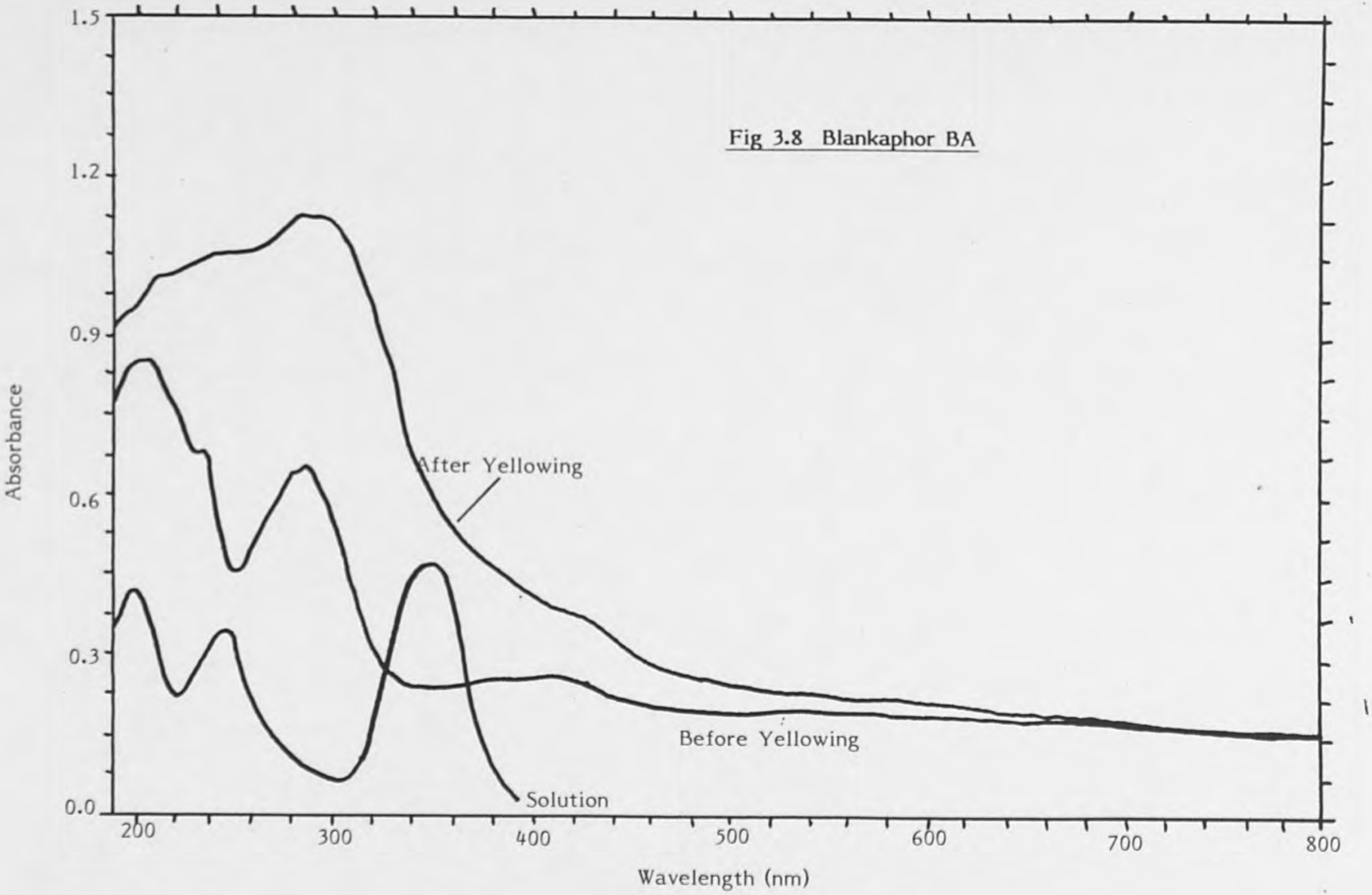
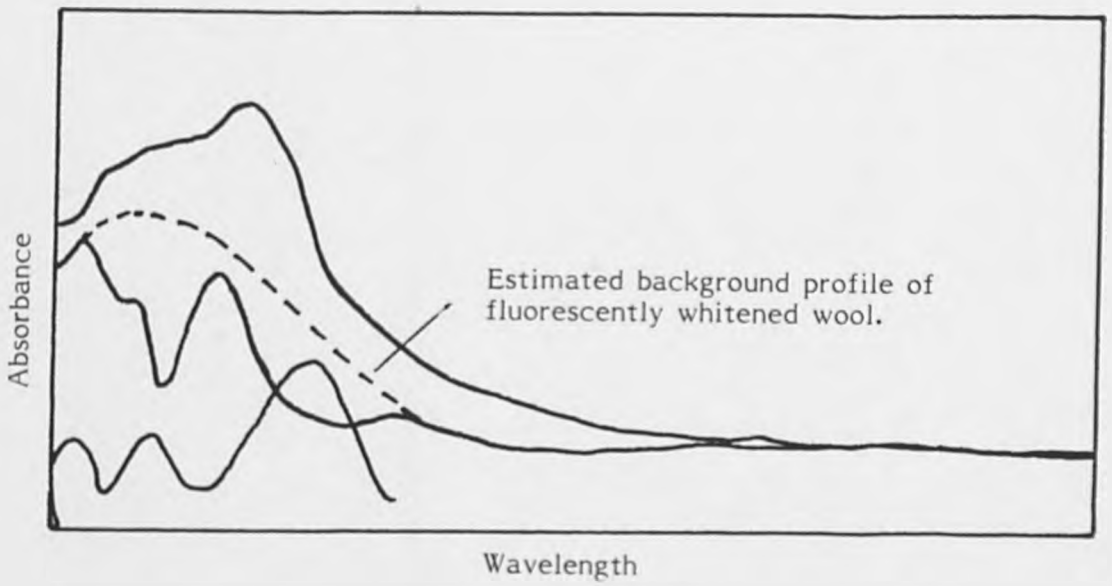


Fig 3.8 Blankaphor BA

spectrum was changed, and the FWA absorption maxima are observed against that background:
ie.



3.3.2 FWA Degradation in Solution

The results are shown in Table 3.1. It is clear that structural features, solvent, and the wavelength distribution of the illumination source affect the photostabilities of the FWA's. The commercial FWA's show a range of reactivities, and due to a lack of information concerning their structure, it is not clear whether stability is affected by the presence or absence of particular groups. For most of the compounds studied, photodegradation occurs more rapidly in methanol than water (exceptions being Uvitex CF and Blankophor BA). The fact that the solubility of oxygen in methanol is eight times greater than that in water [14] may account, at least in part, for this greater photoreactivity in methanol. Another feature which may be of some importance is the greater reducing ability of methanol.

TABLE 3.1 RELATIVE PHOTOSTABILITIES OF SOME FWA'S AND THE EXTENT TO WHICH THEY PHOTOYELLOW WOOL

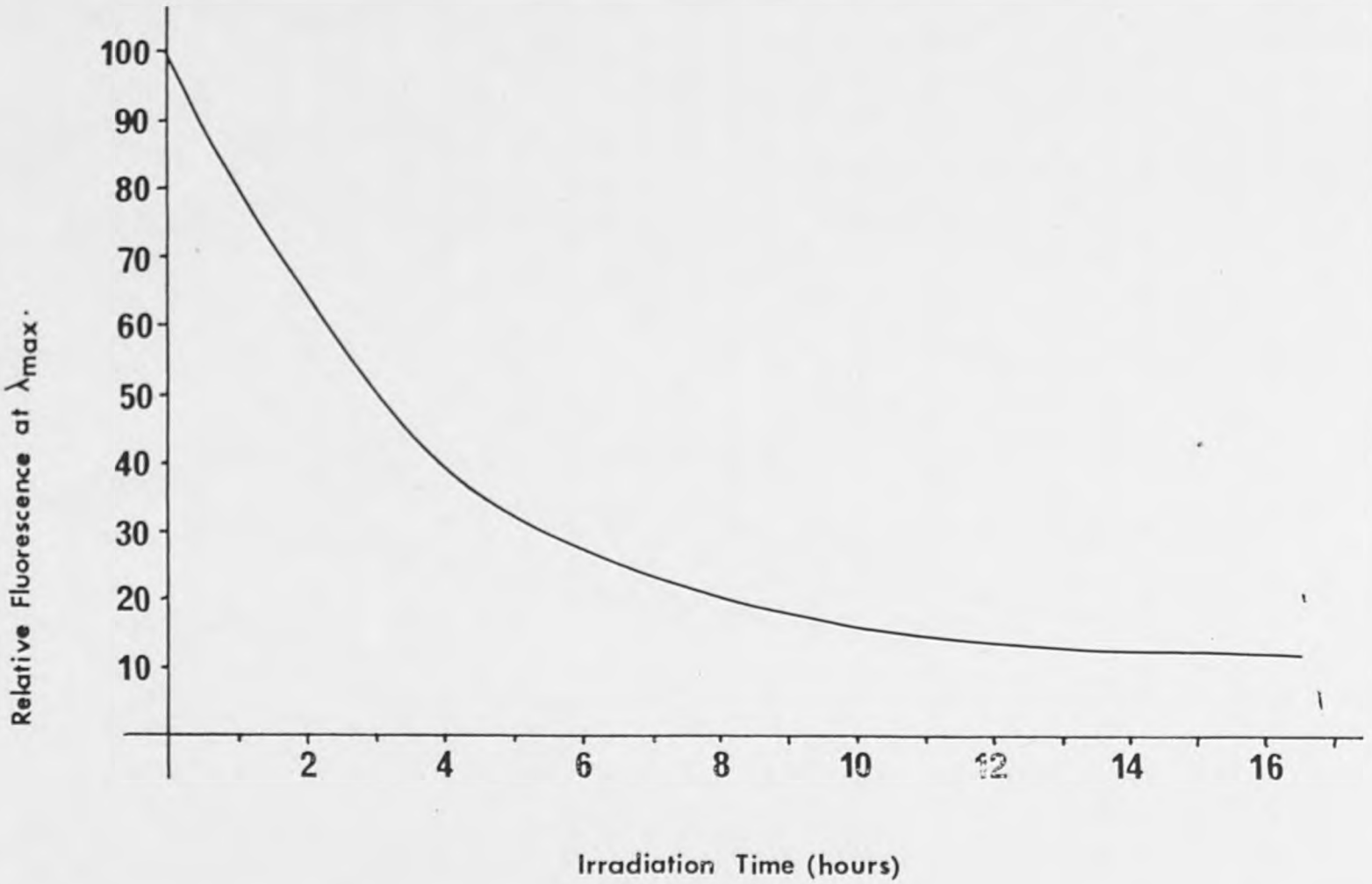
F.W.A.	Relative Stability in Solution				Amount of FWA Remaining Following Irradiation of Treated Wool				Change in Yellowness Index after Irradiation of Treated Wool Δ Y.I.	
	300nm hν		350nm hν							
	Water	Methanol	Water	Methanol	2 hours	4 hours	6 hours	24 hours	6 hours	24 hours
UVITEX CF	<0.10	<0.10	<0.01	<0.05	95%	85%	78%	55%	8.2	35.5
UVITEX NFW	0.60	<0.15	1.0	0.29	95%	8'%	69%	28%	7.3	28.2
BLANKOPHOR PR	0.10	<0.10	<0.01	0.03	99%	90%	85%	54%	5.4	26.8
HOSTALUX PR	0.70	0.25	1.08	0.38	90%	74%	55%	17%	6.8	30.9

The effect of the wavelength distribution of the illumination source upon the relative stabilites is difficult to rationalize. The fact that the commercial FWA's have prominent absorption maxima close to 350nm would suggest that the use of 350nm lamps should lead to more degradation than when 300nm lamps were used, (given that the incident flux was the same for both types of lamp), since the number of photons absorbed per unit time would be greater. Inspection of Table 3.1 shows that although this was observed in some cases, eg. with Unitex CF and Blankophor BA, this is hardly the general trend. The results obtained with 350nm lamps should resemble normal daylight illumination more closely than the 300nm lamps, and therefore the order of reactivity of the FWA's obtained with these lamps should give some indication of the stability of the FWA's when applied to wool and exposed to daylight.

3.3.3 FWA Photodegradation on Wool Monitored by Y.I. & Extraction

The Y.I. (Yellowness Index) values shown in Table 3.1 indicate that irradiation leads to extensive discolouration, and furthermore the extent of discolouration does not appear to be markedly affected by the type of FWA. This is in acord with previous results obtained for pyrazolines [2]. It is inveresting to note that the extent of yellowing of the wool treated with commercial FWA's is similar, even after such a short irradiation time as 6 hours. If there is any differentiation to be made between the FWA's, one would have expected the differences to show up when short irradiation times were used. From fluorescence measurements (Fig. 3.9) and studies using photoacoustic spectroscopy [7], it appears that the FWA's photodegrade on the irradiated surface of the wool very quickly (in excess of 80% in 8 hours). However, as can be seen from Table 3.1, relatively small amounts of the FWA's are destroyed by 6 hour irradiation, and in some cases, substantial amounts of undegraded FWA's are present after 24 hours irradiation. It therefore appears that most of the degradation that occurs accrues from FWA molecules sited at the surface facing the source of illumination.

Figure 3-9 The Effect of Photoyellowing on the Fluorescence of Uvitex CF 200 on wool.



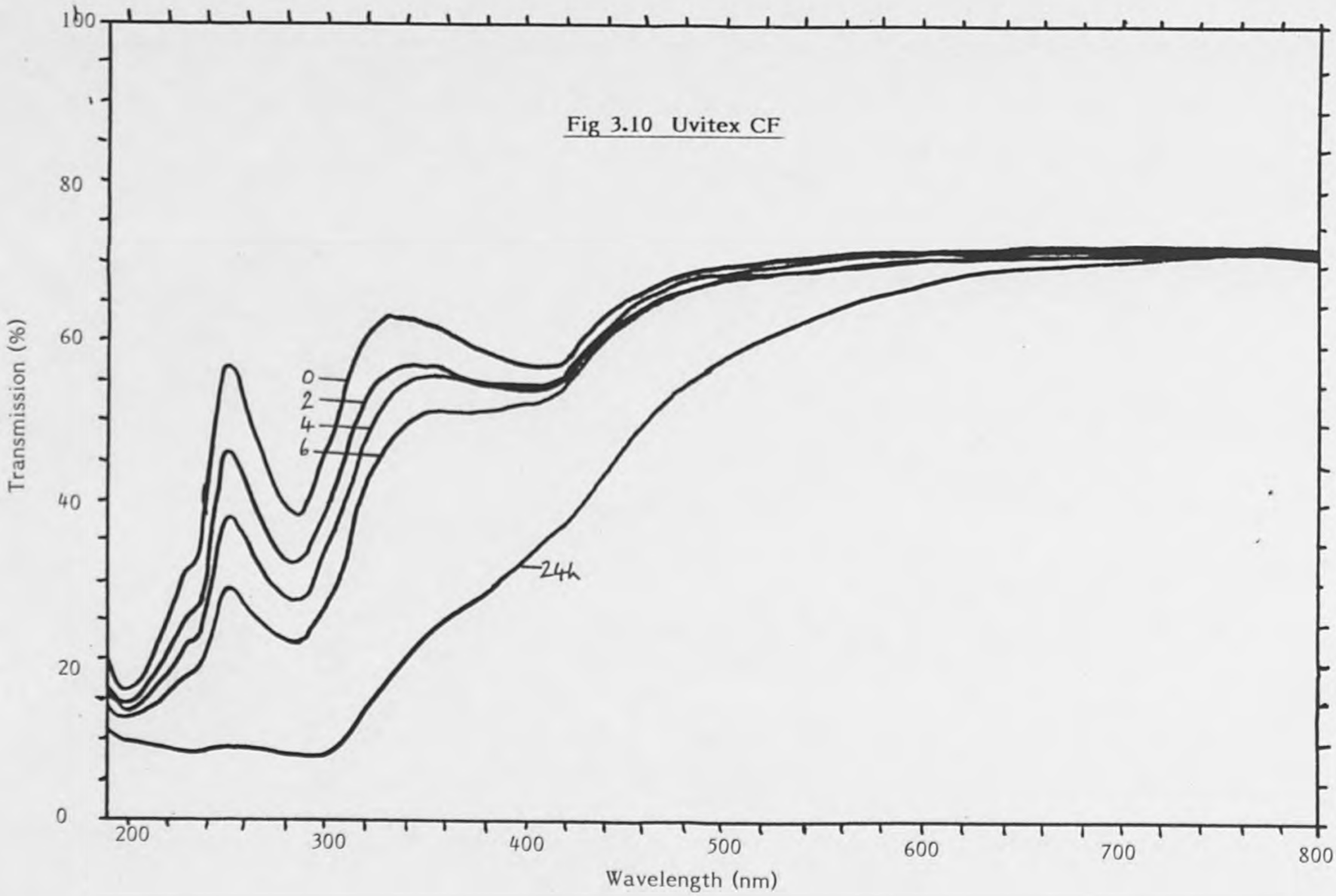
Another surprising result is that some of the FWA's which photodegrade rapidly in solution are relatively stable on wool (eg. Uvitex CF), whereas others which are relatively stable in aqueous solution (eg. Uvitex NFW and Hostalux PR) are relatively unstable on wool. This effect is most likely due to the rapid surface formation of a yellow screening layer in the case of solution unstable FWA's, thus protecting the FWA within the fibre, giving rise to the spurious indication of greater overall stability when assessing by ammonia extraction.

3.3.4 Diffuse Reflectance U.V. Spectroscopy Results

Reflectance spectroscopy was also used to study the degradation processes. As outlined previously, the optical arrangement of the Lambda 5 instrument employed takes light from the source, passes it through a monochromator, and the monochromatic light so obtained is allowed to impinge on the sample which is contained within an integrating sphere. The intensity of the reflected light, plus any fluorescence, is measured relative to magnesium carbonate using a photomultiplier tube. As a result of this particular optical configuration, the reflectance spectrum of wool carrying an FWA shows the absorptive bands of the FWA as apparent increases in transmission (see figures 3.4-3.8). This phenomenon is discussed later in the chapter. The diffuse reflectance method allows the presence of FWA's on wool to be readily detected, and with more spectroscopic detail than previously obtained by photoacoustic spectroscopy [7]. The use of the technique in this way enables one to determine the level of FWA applied to the wool and is thus suitable for following their photodegradation processes.

There are several features of this technique which give it a distinct advantage over others available. The first is that when wool becomes yellow, it absorbs the blue fluorescence from an FWA by an inner filter effect. This means that when wool which has been degraded is examined and the Yellowness Index calculated, the residual FWA appears to be absent because its fluorescence emission is reabsorbed before it reaches the photomultiplier tube. Residual FWA still absorbs, however, and hence this absorption can be observed by diffuse reflectance U.V. spectroscopy. This is exemplified in figures 3.10 - 3.14.

Fig 3.10 Uvitex CF



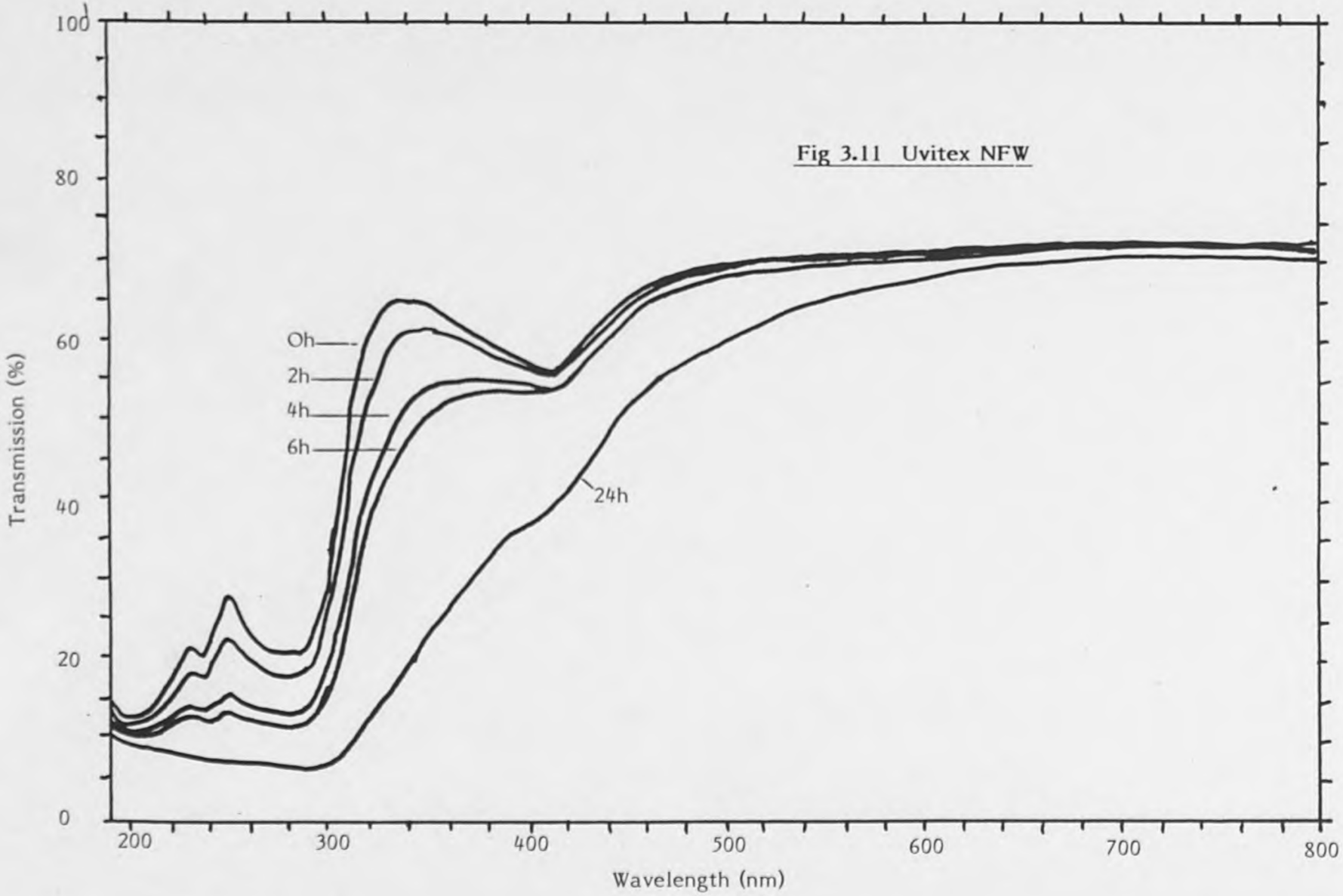


Fig 3.11 Uvitex NFW

Fig 3.12 Blankaphar BA

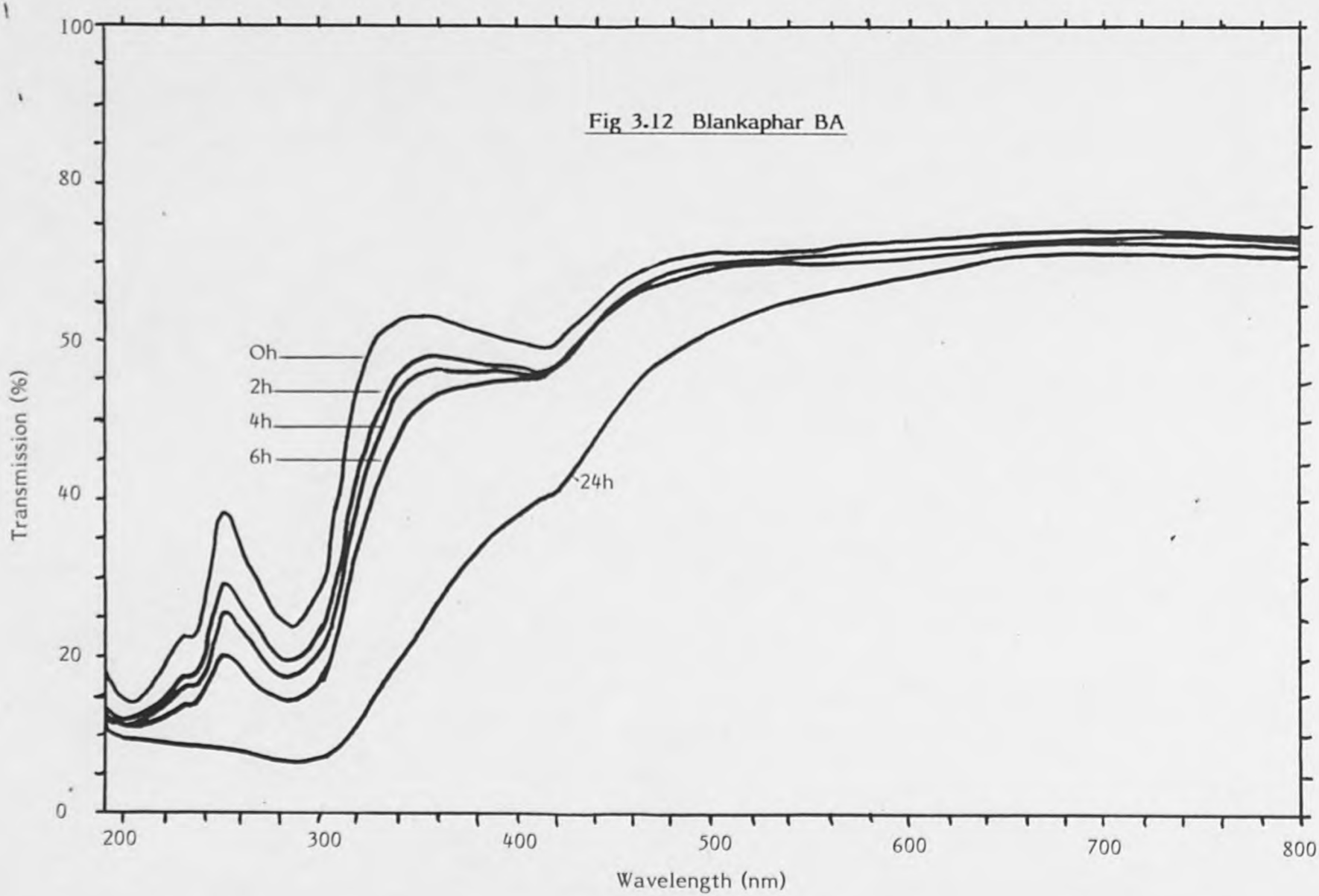
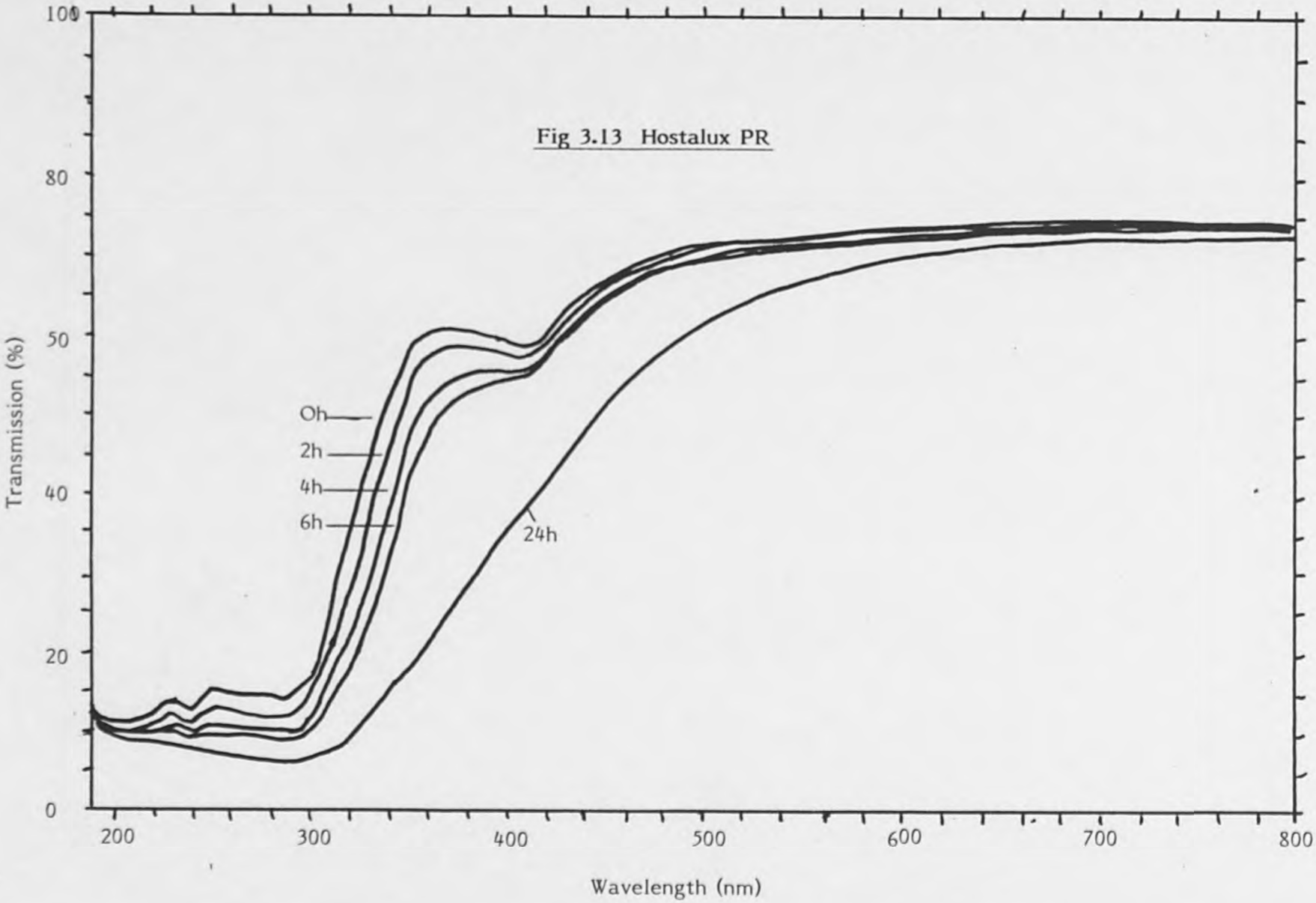


Fig 3.13 Hostalux PR



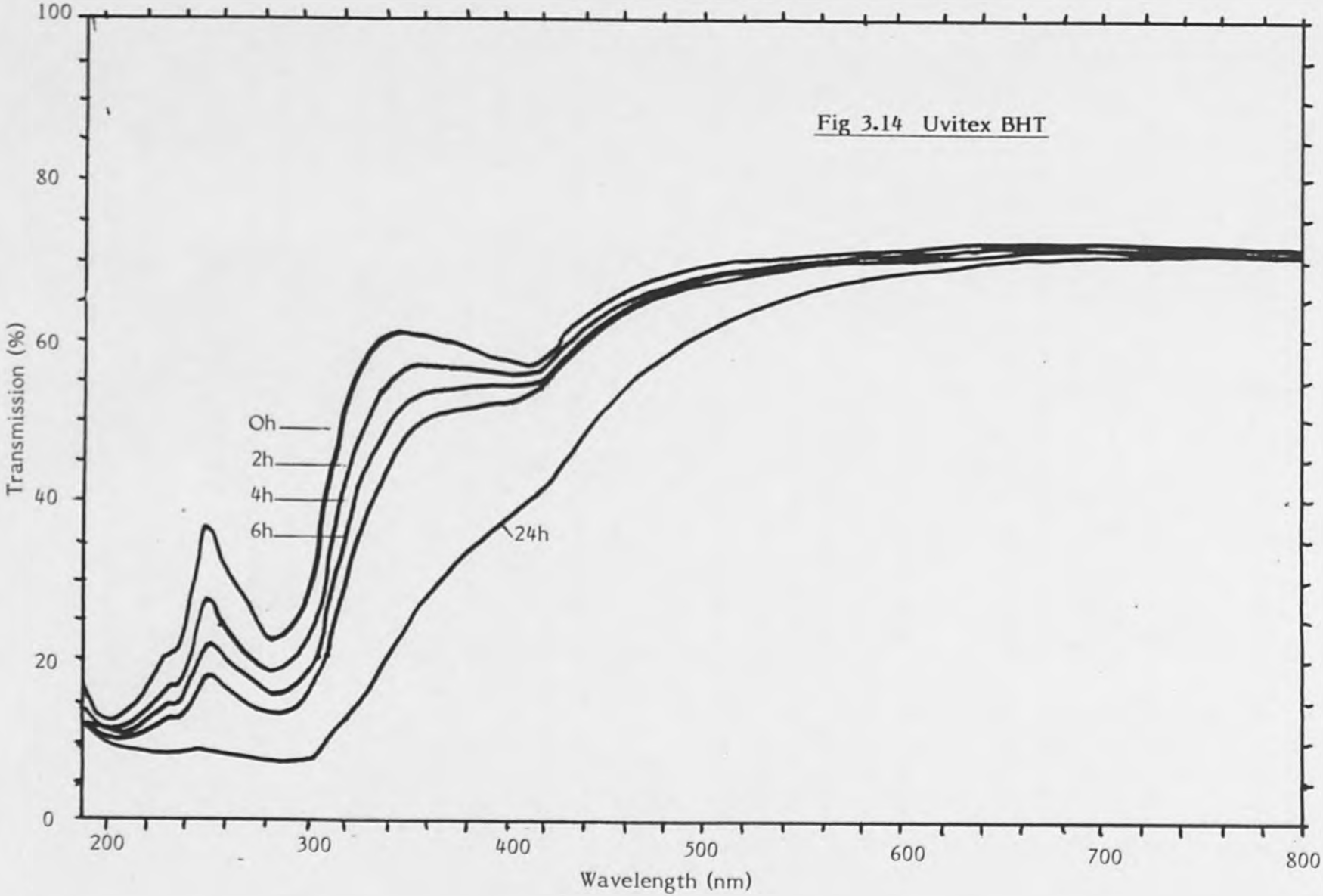


Fig 3.14 Uvitex BHT

The second advantage is that being a reflectance technique, it is possible to specifically study the surface of wool. Wool yellowing is essentially a surface phenomenon, and therefore methods such as ammonia extraction of FWA's tend to give misleading results. By monitoring only the changes occurring on the surface, a higher degree of sensitivity and selectivity is also achieved.

A further advantage arises from the use of the integrating sphere. The wool surface is most uneven, and consequently light scattering is a serious problem. This usually leads to very poor reproducibility, but with this optical arrangement, scattered light is not lost and the spectra are of very high quality. Reproducibility was so good that spectra run from one day to the next were almost identical.

Wool samples, treated with the five commercial FWA's previously described, had been irradiated for 2,4,6, and 24 hours under 350nm light. The Yellowness Index of each sample had been measured, and residual FWA extracted from representative portions. These results are shown in Table 3.1. The diffuse reflectance spectra were run subsequently, the five traces for each FWA being recorded on the same spectrum. These are shown in figures 3.10 - 3.14.

The background "baseline" for these spectra of the FWA decompositions is the spectrum of the wool; not of untreated wool, but of white wool. In other words, these spectra differ from that of untreated wool in that (i) they have a different profile, resulting from the whitening of the wool and (ii) they show the absorption peaks of the FWA in the U.V. region. When viewing the wool in daylight with the human eye, the colour of the wool (which controls the spectral profile) appears very brightly white because it includes the FWA fluorescence. However, diffuse reflectance uses monochromatic light, so when scanning the blue region, fluorescence is not excited, and changes observed are real colour changes - clearly only slight in each case. Variations in Yellowness Indices are a combination of colour change and decrease in FWA fluorescence.

It was believed that during the first few hours of irradiation any changes observed would arise from the FWA, since little colour change

is detectable from the spectra, relative to the brightener decomposition. To a very basic first approximation, it could therefore be said that the background wool spectrum remains fairly constant, and so by drawing in an estimated wool profile, the FWA decomposition may be followed. To this end, a line was drawn on each of the five spectra at the position estimated for the background wool fibre, and the FWA absorption for each irradiation time was measured (in % transmittance units). These results are listed in Table 3.2. Close examination of Figures 3.10-3.14 shows, however, that some slight variation in the background does occur over the 6 hour period, and so individual baselines were estimated for each curve, and the absorption peaks remeasured. These results are also listed in Table 3.2. It can be clearly seen that these two sets of results follow each other very closely.

Before discussing the results in Table 3.2, it is significant to note that, although somewhat approximate, this is the only method whereby the brightener may be specifically observed on the surface of wool. Yellowness Index is limited because it is calculated on the basis of "white light" illumination, monitoring at specific wavelengths and thus including fluorescence contributions. Extraction procedures are specific for the FWA's ionically bound in the fibre, but give a representation of the situation on the surface and in the bulk of the fibre combined.

The measurements given in Table 3.2 were made for the absorption maxima at around 350nm, partly because it is this absorption which excites the blue fluorescence, and partly because it is the absorption most likely to contribute to the sunlight yellowing of wool.

It would appear to be reasonable to predict that if the wool yellowed rapidly, and most of the FWA was found to be degraded, then the FWA would probably be decomposing to form yellow products. On the other hand, if the wool yellowed rapidly, and most of the brightener could be extracted undegraded, it would seem that the FWA was stimulating the wool degradation to form yellow products.

Applying the above logic to the data in Table 3.1 alone, one would draw the conclusion that Uvitex NFW and Hostalux PR fall into the first

Table 3.2

FWA	Irradiation Time (hours)	λ (nm)	FWA Absorption At λ .(%T units)	% FWA Remaining	FWA Absorption At λ .(%T units)	% FWA Remaining	Yellowing Measured From Diffuse Reflectance Spectra Δ %T at 420nm
Uvitex CF	0		30	100%	29	100%	-
	2		24	80%	24	83%	-
	4	340	21	68%	21	72%	-
	6		16	52%	19	66%	3%
	24		(4)	13%	4	14%	21%
Uvitex NFW	0		35	100%	29	100%	-
	2		31	89%	28	97%	-
	4	340	21	60%	21	72%	-
	6		17	49%	19	66%	3%
	24		(2)	6%	2	7%	16%
Blankophor BA	0		33	100%	25	100%	-
	2		26	79%	22	88%	-
	4	340	23	70%	21	84%	-
	6		18	55%	18	72%	3%
	24		(2.5)	8%	2.5	10%	18%
Hostalux PR	0		22	100%	22	100%	-
	2		19	86%	20	91%	-
	4	360	13	59%	17	77%	-
	6		9	41%	13	59%	3%
	24		(0)	0%	0	0%	18%

category, whilst Uvitex CF and Blankophor BA identify with the second. This latter would also be supported by previous workers' published results for stilbene degradation [15]. They had observed that a stilbene FWA yielded virtually no yellow material even after extensive decomposition had occurred. When irradiated in the wet state, wool whitened with the stilbene becomes very yellow, even though 65% of the whitener remained undegraded. However, solution studies on Uvitex CF and Blankophor BA at the City University carried out by [redacted] showed that both of these FWA's yielded yellow products upon irradiation. Further examination of Tables 3.2 and 3.3 show that after only 2 hours irradiation, a higher proportion of these two stilbene FWA's had degraded than observed for either Uvitex NFW or Hostalux PR, and that wool treated with the stilbene materials had yellowed more in that initial period. It therefore seems that no general trend is apparent. The information obtained for the individual commercial brighteners is now discussed.

3.3.5 Uvitex CF

Solution studies have shown that this FWA is very unstable to U.V. light in aqueous solution. It has a strong absorption at 260-280nm, and hence would not be expected to be stable to 300nm irradiation, and for the purposes of this study, only the results for aqueous solutions irradiated at 350nm are to be considered, these being the closest to the corresponding treatment on wool.

The FWA was thus known to be unstable in aqueous solution, and it was considered unlikely that it should be stabilized by interaction with wool. During the initial irradiation period, yellowness index measurments have shown that the wool yellows slightly more rapidly than other. However, the diffuse reflectance spectra indicate that all the samples yellowed at approximately the same rate, so the increase in yellowness index may be due to the FWA degrading, with the inherent loss in fluorescence. Table 3.1 shows that after 24 hours irradiation, more than 50% of the FWA remained undegraded, as extracted with dilute ammonia. So it was concluded that on the surface the FWA was stimulating rapid wool yellowing, forming a layer which screened the bulk of the fabric and the residual FWA from further photooxidation. It is perhaps significant to note that the diffuse reflectance measurements (Table 3.2) show that after 24 hours

Table 3.3

Irradiation Time (hrs)	Hostalux PR		Uvitex CF		Uvitex NFW		Blankophor BA	
	Y.I.	ΔY.I.	Y.I.	ΔY.I.	Y.I.	ΔY.I.	Y.I.	ΔY.I.
0	-0.44	-	-2.37	-	-1.61	-	-2.43	-
2	1.04	1.48	-0.33	2.04	0.23	1.84	-0.12	2.31
4	5.89	6.33	0.80	3.17	3.66	5.27	1.09	3.52
6	6.37	6.81	5.84	8.21	5.69	7.30	2.97	5.40
24	30.43	30.87	33.15	35.52	26.61	28.22	24.32	26.75

irradiation, Uvitex CF still has a residual 13-14% on the wool surface, and that after this time period, the wool treated with this FWA showed the most yellowing.

3.3.6 Uvitex NFW

This bis-styryl biphenyl derivative was observed to be relatively stable in aqueous solution when irradiated at 350nm. However it was found to be quite unstable when applied to wool, rapid yellowing being observed over the first 6 hours. During the first two hours, the degree of yellowing appeared to be similar to the other three (Table 3.3) but the FWA degradation (from diffuse reflectance) was minimal. This suggested that the FWA may be stimulating wool degradation, and the products thus formed then sensitizing the Uvitex NFW photodecomposition. If true, then this may account for the fact that although the yellow surface layer was formed, (as in the case of Uvitex CF), the FWA did not in this case appear to be screened against photodegradation in the bulk of the fibre, only 28% being extracted with ammonia. This does, of course, assume that all the Uvitex NFW within the wool matrix is ionically bound and can be removed by the ammonia extraction. Without a knowledge of the functionality of the FWA molecule it is impossible to comment upon this further.

3.3.7 Blankophor BA

Like Uvitex CF, this stilbene derivative showed a very low stability in aqueous solution to 350nm UV light. Again, there was no reason to expect the brightener to be any more stable when on wool, and this was supported by a rapid initial loss in FWA (over the first two hours) as observed by diffuse relectance. As with Uvitex CF, the ammonia extraction showed that over 50% of the brightener remained after 24 hours irradiation, and the conclusion was drawn that the rapid degradation on the surface caused the formation of a screening layer, protecting the bulk of the fibre. The UV reflectance spectra show a similar change in absorbance at 420nm. to that observed for the others, so it was assumed that the smaller changes in yellowness index resulted from less fluorescence being lost on the surface. This may be accounted for if the FWA is less fluorescent than the others under study. However, it may simply be that although the brightener

degrades at approximately the same rate as Hostalux and the two Uvitex FWA's, it does not sensitze wool yellowing to quite the same extent.

3.3.8 Hostalux PR

This fluorescent whitening agent of unknown structure was found to be the most stable of the four tested when irradiated in aqueous solution. However, it was seen to yellow as fast and more than most, and to be completely destroyed within 24 hours of irradiation on the surface of wool. Ammonia extraction results suggested that this degradation occurred throughout the fibre, because only 17% was recovered after the 24 hour period. The possibility that not all residual brightener was being extracted was again suggested, but in this case it was considered unlikely, based on the high level of decomposition recorded on the surface by diffuse reflectance after 24 hours irradiation. It can be seen from Table 3.2 that initial FWA decomposition was relatively slow, as was yellowing during the first 2 hours (Table 3.3), and it may be that this allowed photooxidation to take place deeper within the fibre, thus accounting for the overall extensive yellowing and brightener loss observed.

3.4 Conclusions

It is noteworthy to observe that a very good correlation exists between amount of FWA observed to remain on the surface of the wool after 24 hours irradiation (table 3.2) and the amount of residual FWA in the whole of the fibre extracted with dilute ammonia after the same irradiation period (table 3.1):

	Ammonia Extraction	Diffuse Reflectance
Uvitex CF	55%	14%
Blankophor BA	54%	10%
Uvitex NFW	28%	7%
Hostalux PR	17%	0%

This suggests that although the kinetics and mecahnisms involved may well be different, these figures give an overall index of the general stability on wool. Although the figures may be obtained from either source, considerably more information is derived simultaneously when diffuse reflectance U.V. spectroscopy is used.

In the introductory chapter of this thesis it was stated that fluorescent whitening agents appear to sensitize wool yellowing by absorbing U.V. light at around 350nm and by some means transferring the energy to the wool. However, from this work, it seems likely that in addition to the above, the FWA degradation products also sensitize yellowing of wool, and the wool degradation products may sensitize FWA decomposition.

3.5 Calibrating the System for the Determination of Photoyellowing

The diffuse reflectance spectra already included in the chapter have shown the changes which occur as fluorescently whitened wool is photo-yellowed. Over the first 2 hours, virtually no change occurs in the visible region as very little yellowing occurs. However, in the UV, and especially at or near the absorption maxima of the FWA, changes may be detected after as little as 20 mintues irradiation. After 24 hours, there is a marked change in the spectral profile, resulting from the yellowing of the wool. Once the changes that occur are known, it is possible to run a series of experiments, irradiating for a range of time periods, and construct a "calibration spectrum". From this, the degree of photoyellowing of an unknown sample can be determined.

Wool samples, pretreated with Uvitex NFW, were irradiated in the wet state with 350nm light for a range of time intervals from 30 minutes to 100 hours. The diffuse reflectance spectrum of each sample was run, recording all the traces on the same sheet of chart paper. This is shown as Fig 3.15. From this it can be seen that detectable wool yellowing commences after approximately 1 hour. After 24 hours, virtually all of the profile from the FWA is lost, and the difference then is in the overall absorption. Of particular interest is that in the blue region (400-420nm) which corresponds to yellowing. A limiting value is reached after approximately 80 hours, when little further yellowing occurs.

The calibration spectrum was found to have limited usefulness, because differences in batches of Uvitex NFW treated wool were greater than the five differences observed between samples irradiated for short periods.

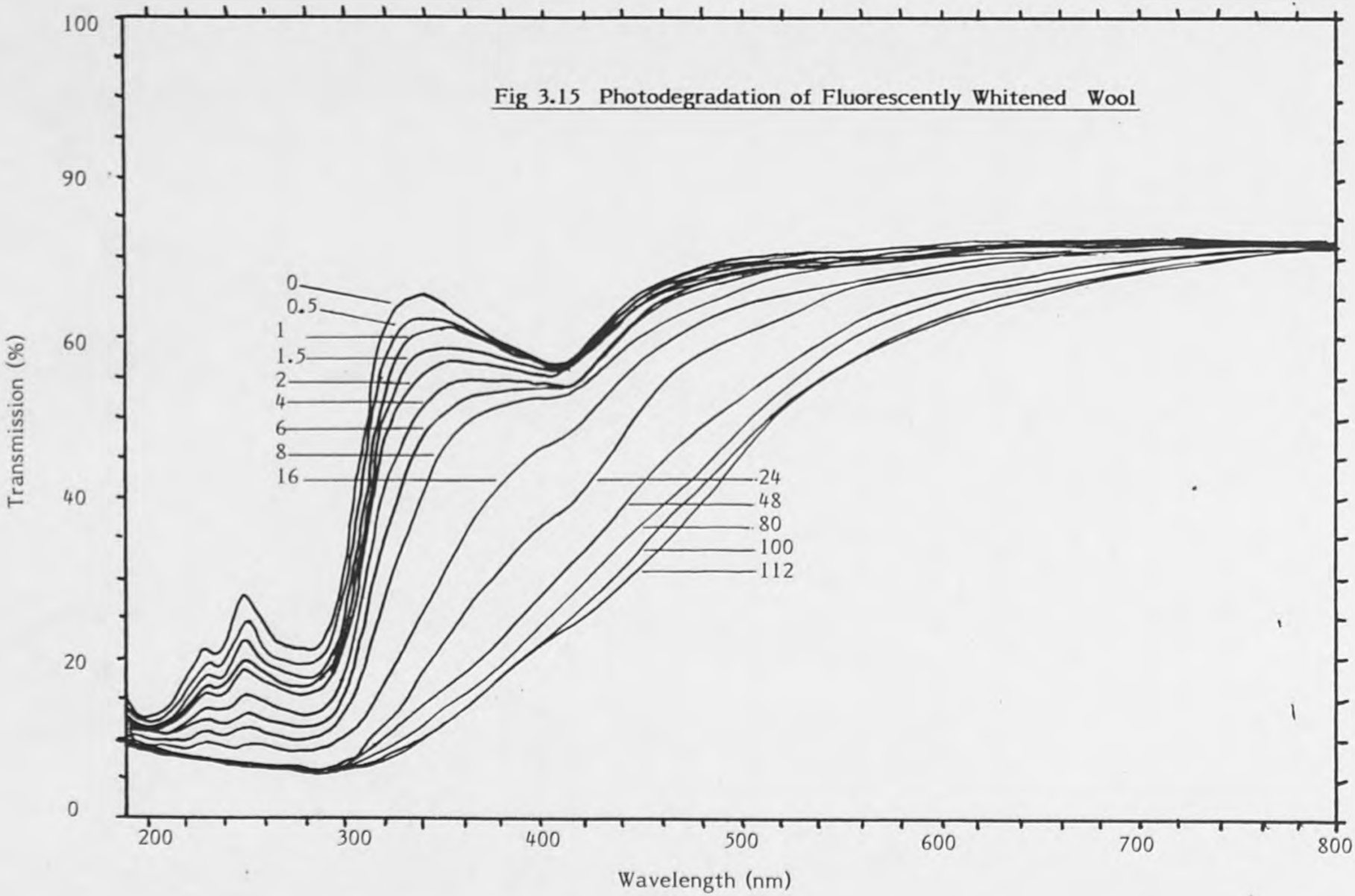


Fig 3.15 Photodegradation of Fluorescently Whitened Wool

Although yellowing is a primarily surface phenomenon, the light clearly penetrates through the bulk of the wool fabric to some extent, and in consequence photoyellowing takes place throughout the fibre. The extent of this is illustrated in an extreme case, shown in Fig 3.16, where Uvitex NFW treated serge was irradiated for 136 hours, causing it to become very yellow indeed. The spectra of the upper and lower surfaces were then run, and compared with that of the unde graded material.

3.6 The Effect of Treating Wool with Different FWA Concentrations

In normal usage, an FWA is used at a level of 1% owf for wool. This is because the level of brightness imparted does not increase proportionally above this amount but the acceleration in yellowing does! When levels of around 5% and upwards are used, a decrease in brightness is observed, due to an inner filter effect where the fluorescence emission is reabsorbed. Thus 1% owf is considered to be the optimum value for routine applications.

During this study it was considered to be instructive to determine the limits of sensitivity of the technique. To this end, salts serge wool fabric was treated with Uvitex NFW at the following levels owf: 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, 0.02%, 0.01% and 0.005%. The diffuse reflectance spectra of each of these was run on the same sheet of chart paper, and is shown in Fig 3.17. It is clear that 0.005% is at the detection limit of the system, and the spectra thus provide an index of the application level for Uvitex NFW on an unknown sample. This could be distinguished from a photoyellowed sample by the difference in spectral profile, particularly in the blue region of the spectrum.

3.7 Possible Reasons why Absorption Bands of FWA's Appear as Negative Peaks.

It has been generally observed throughout the work described in this chapter that the absorption bands of the fluorescent whitening agents appeared as decreases in absorption, ie. as negative peaks. Examples of this may be seen in figures 3.4-3.8.

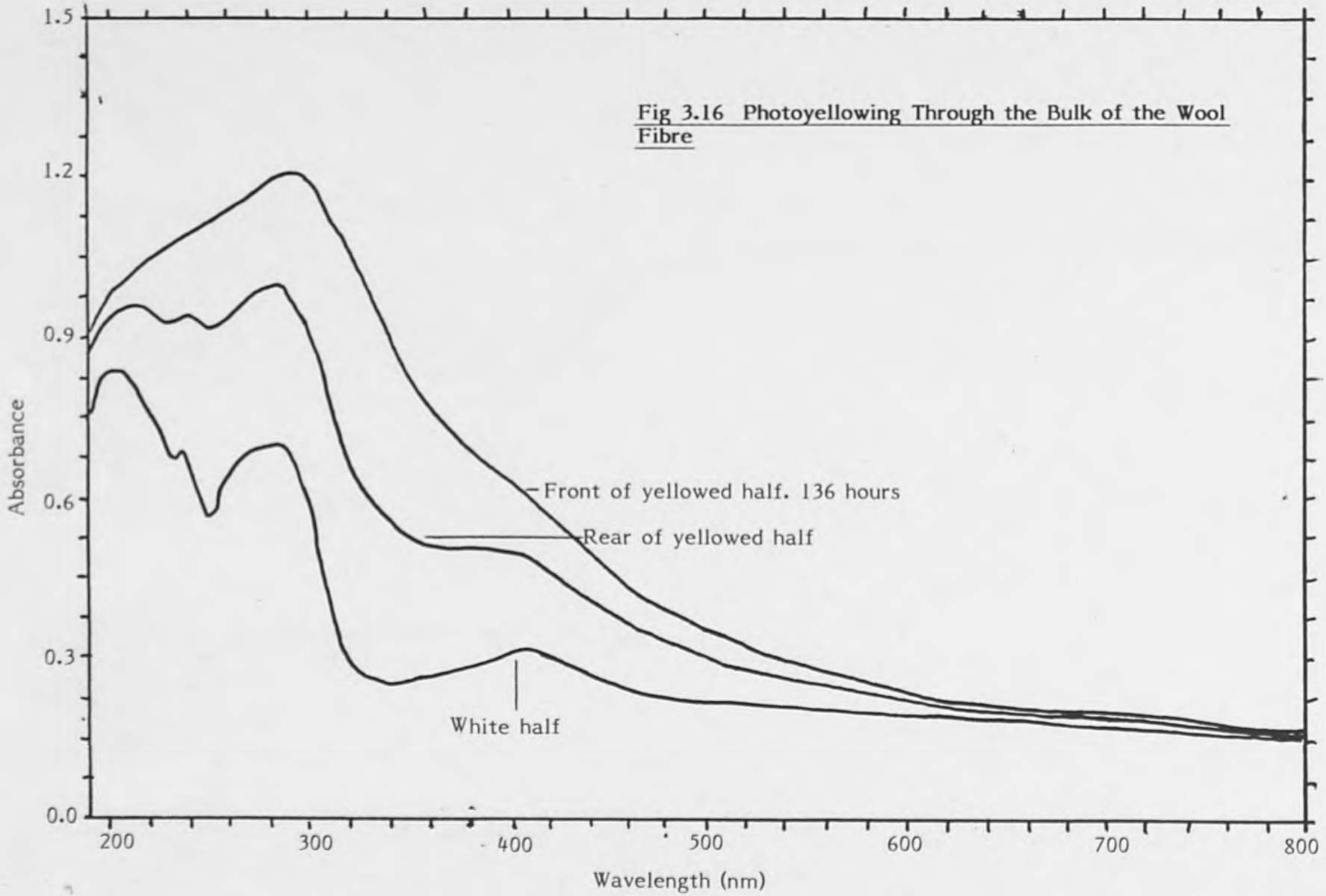


Fig 3.16 Photoyellowing Through the Bulk of the Wool Fibre

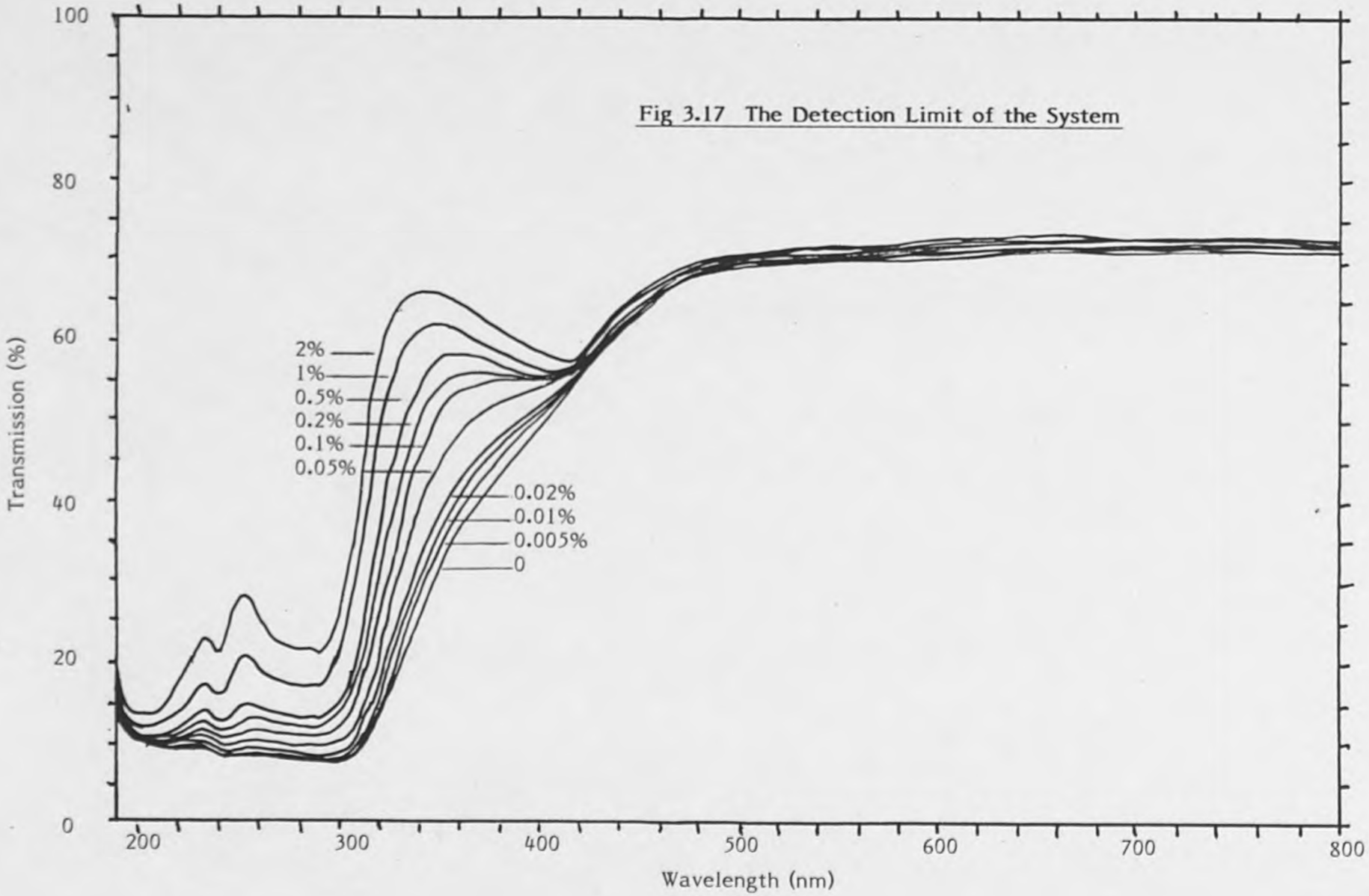
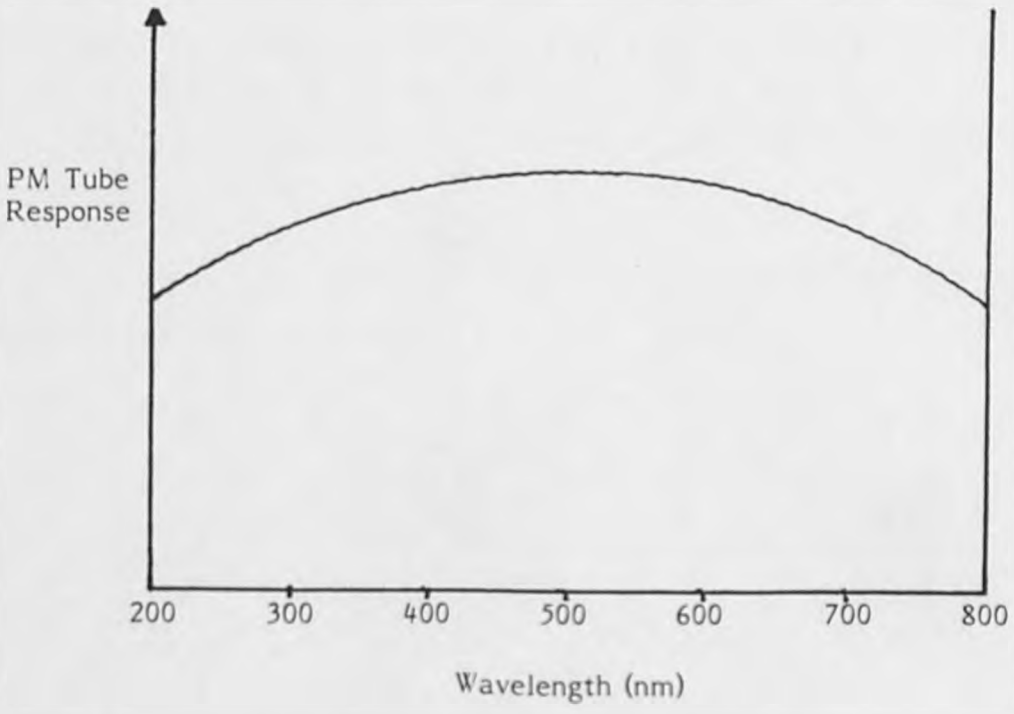


Fig 3.17 The Detection Limit of the System

This situation was originally believed to occur as a result of the particular optical system utilized in the Lambda 5 spectrophotometer, in which the sample is irradiated with a beam of monochromatic light, whilst the detecting photomultiplier tube is sensitive to all frequencies in the spectrum. In consequence when light is being absorbed at, for example, 350nm, and is also re-emitted as fluorescence, the photomultiplier tube detects both the reflected light and the fluorescence at the same time, thus diminishing the apparent absorption. Whilst this argument is undoubtedly true, it has the drawback that in the optimum case, where the fluorescence quantum yield is unity, this should still only result in the absorption and emission cancelling each other out, and the absorption peak disappearing. To explain the negative peaks requires some additional explanation.

The problem that exists is, therefore, that more light is apparently emitted than is absorbed. This clearly cannot be the case, and the reasons listed below are possible explanations. Probably the real answer is a combination of all three.

The first possibility is that the photomultiplier tube does not have the same level of response to photons of all energies;

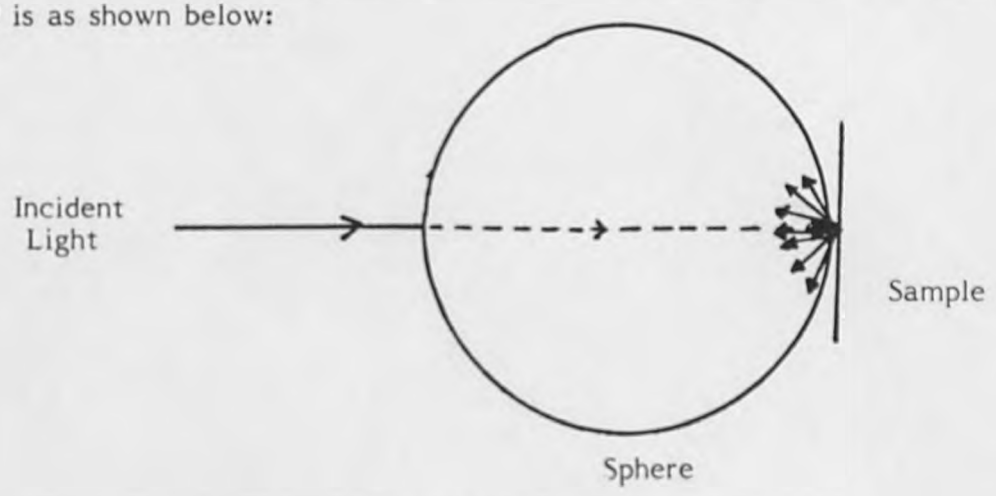


The tube used in the Lambda 5 is a 928 side-window model, which is known to have the response variation as drawn above. Perkin Elmer have stated that the "background correct" routine (run before the analytical spectra, to set up the instrument) should correct for any variation in PM tube response. However, any corrections made would be effective only at single wavelengths, and at 350nm, the correction would be for 350nm light, and not 420nm. Nevertheless this explanation is unlikely to be sufficient in itself because the wavelength difference is not much more than 70-80nm between the absorption and emission bands, and the PM tube variation over this narrow range is not expected to be such as to give the observed effect.

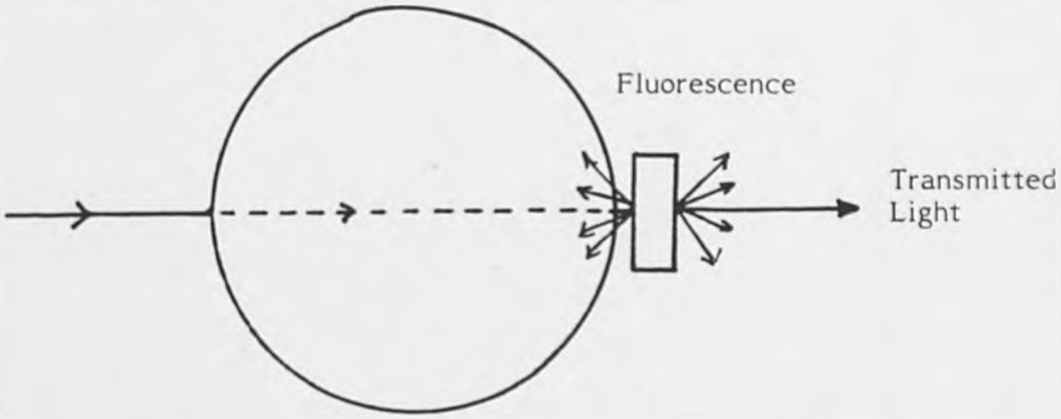
The second possible explanation concerns the light output of the spectrophotometer lamp. If the lamp output is low in the 400-420nm regions (the fringe of the visible spectrum), the spectrophotometer would compensate for this by increasing the sensitivity to these wavelengths so as to achieve a linear spectrum. A fluorescence emission in this region would then appear to be of an exaggerated intensity, apparently greater than that of the absorbed light.

A third but related explanation arises from the spectral range of the illumination sources. For UV wavelengths, a deuterium lamp is used, whilst for the visible region, a tungsten/halogen lamp is necessary. The intensity of light from the tungsten lamp is believed to be higher than that from the deuterium lamp, which the instrument duly corrects via the microprocessor and the "Background Correct" program. Thus, the response to blue light is amplified to compensate for the variation, and is recorded at an artifically high intensity level.

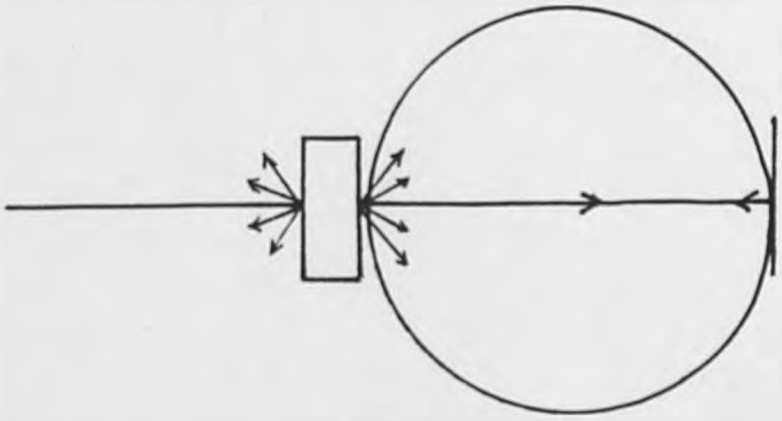
The normal optical system for the Lambda 5 with the integrating sphere fitted is as shown below:



It was decided to mount a quartz cuvette containing Uvitex NFW solution (aq) in the position of wool sample, with the fabric support plate removed. In this way, some of the fluorescence would enter the integrating sphere, but none of the transmitted light. The spectra were run using magnesium carbonate as the reference.



The result was a spectrum showing decreases in absorption at the wavelengths where the brightener absorbed. The situation was then changed, monitoring the quartz cuvette on the other side of the sphere, such that all transmitted light was detected, but only a small part of the fluorescence emission:



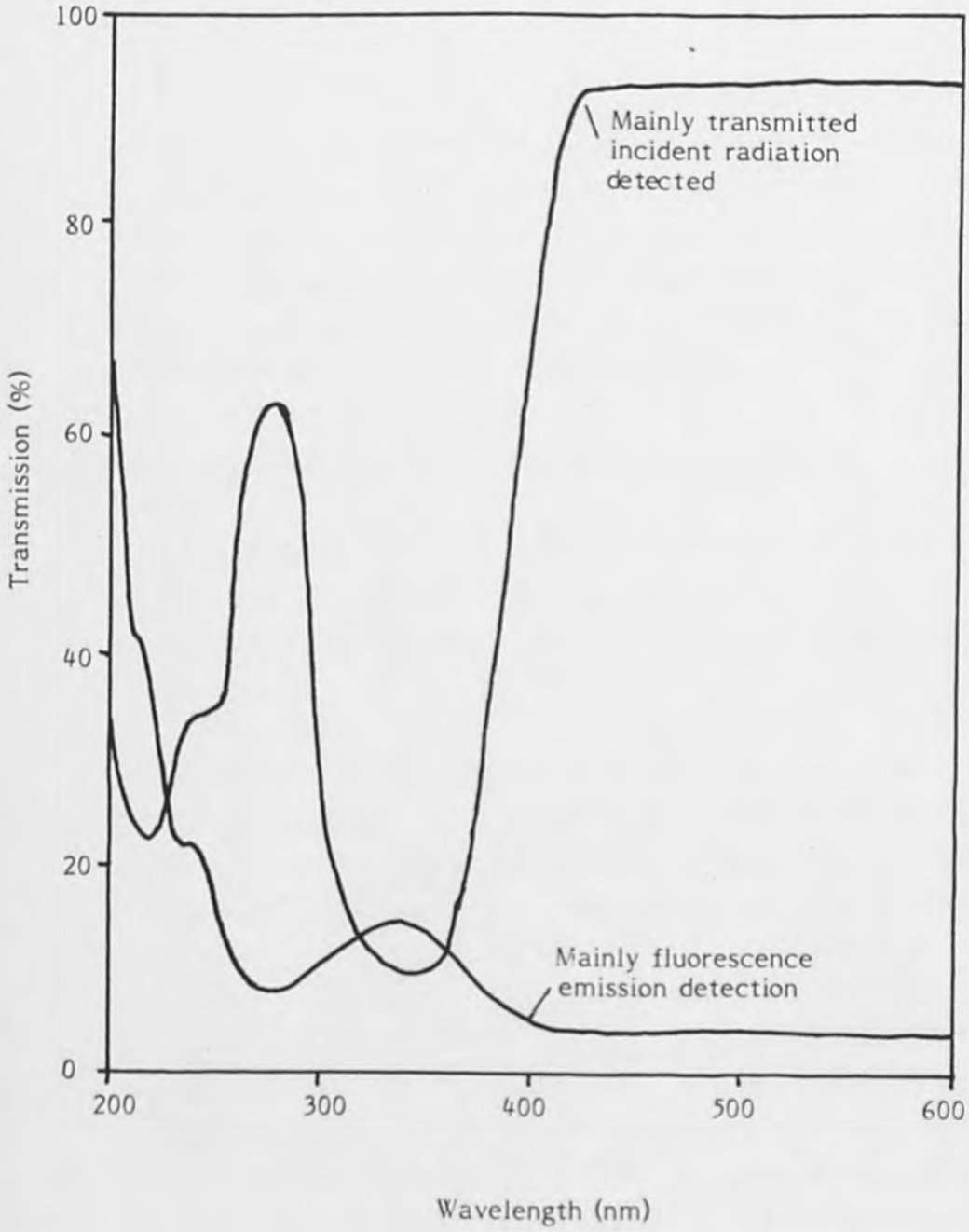


Fig. 3.18 Solution Spectra of Uvitex NFW

The result in this case was the inverse of the previous spectrum, the FWA absorption bands appearing as true absorption peaks. The spectra are shown in Fig 3.18.

The above results were obviously predictable, but served to prove that when the fluorescent emission detected exceeds in intensity that of the absorbed radiation, the result was the negative peaks. This seems to be the only source of the phenomenum, and the reasons for the effect are likely to be among those described earlier. This is not a drawback of the technique, but it is obviously necessary to allow for it when interpreting the resultant spectra of fluorescent compounds.

3.8 Application of Diffuse Reflectance to Dyed Fabrics

The diffuse relectance spectrum of a dyed wool sample is very easy to interpret, because the background wool profile is almost flat in the visible region. It is thus completely dominated by the absorption of the dye or dye mixture used.

The spectra were run of a wide range of dyed wool fabrics, the dyes having been obtained from a number of different manufacturers, and being available in most of the commonly used colours. The absorption bands (seen as true absorption) were clearly recognizable as coming from the originating dye colour. For example, Lanasol yellow showed a single absorption band, λmax 410nm. However, the spectra were distinctive for each individual dye, allowing several shades of blue, for example, to be positively identified. A total of ten different dyes were run on wool, and each could easily be detected by this method. These spectra are shown on the following pages. The technique therefore offers a rapid, non-destructive means of identifying both dyes and fluorescent whitening agents, with a high degree of sensitivity, and may well be of use in forensic applications, as well as textile research laboratories.

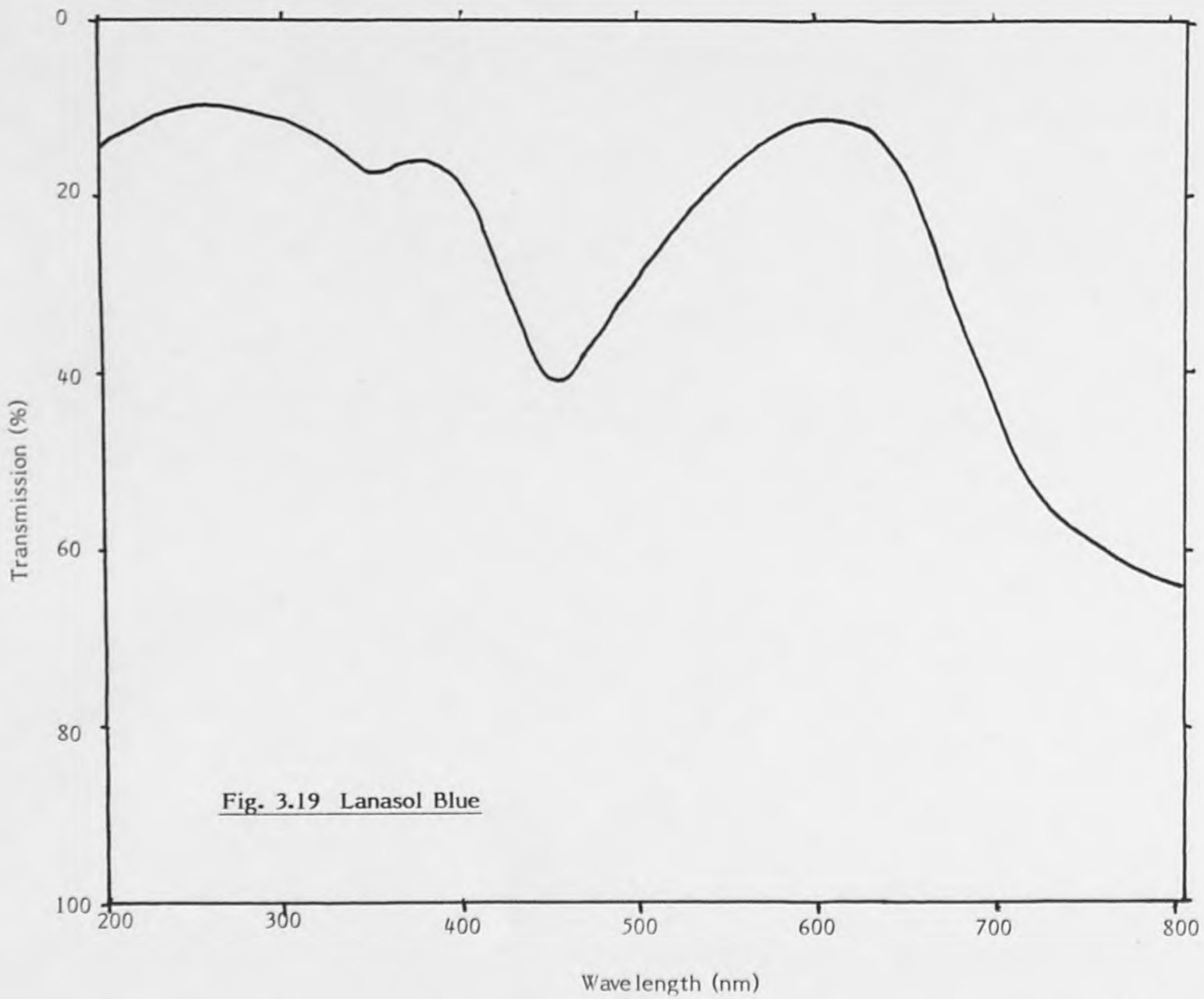


Fig. 3.19 Lanasol Blue

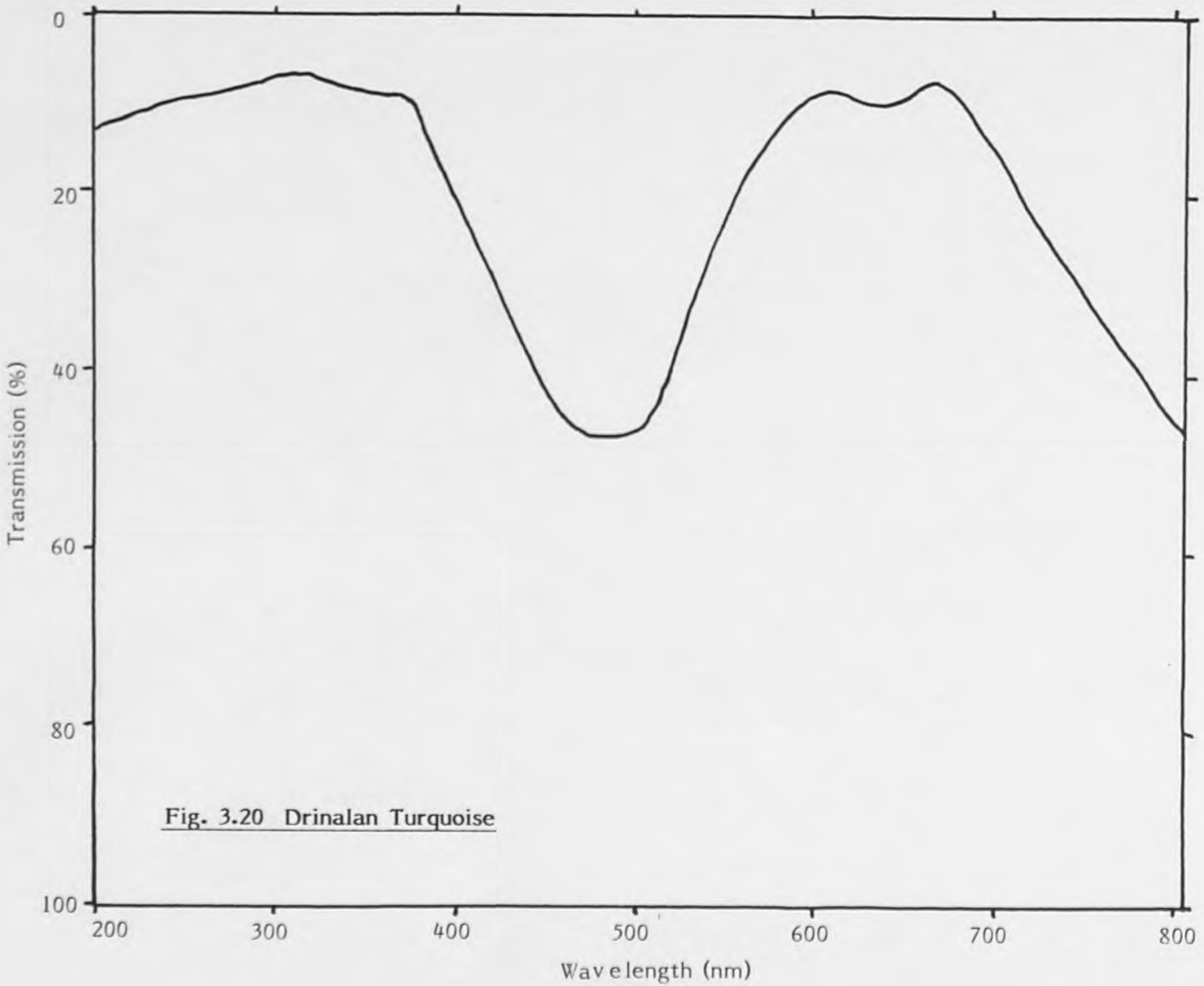


Fig. 3.20 Drinalan Turquoise

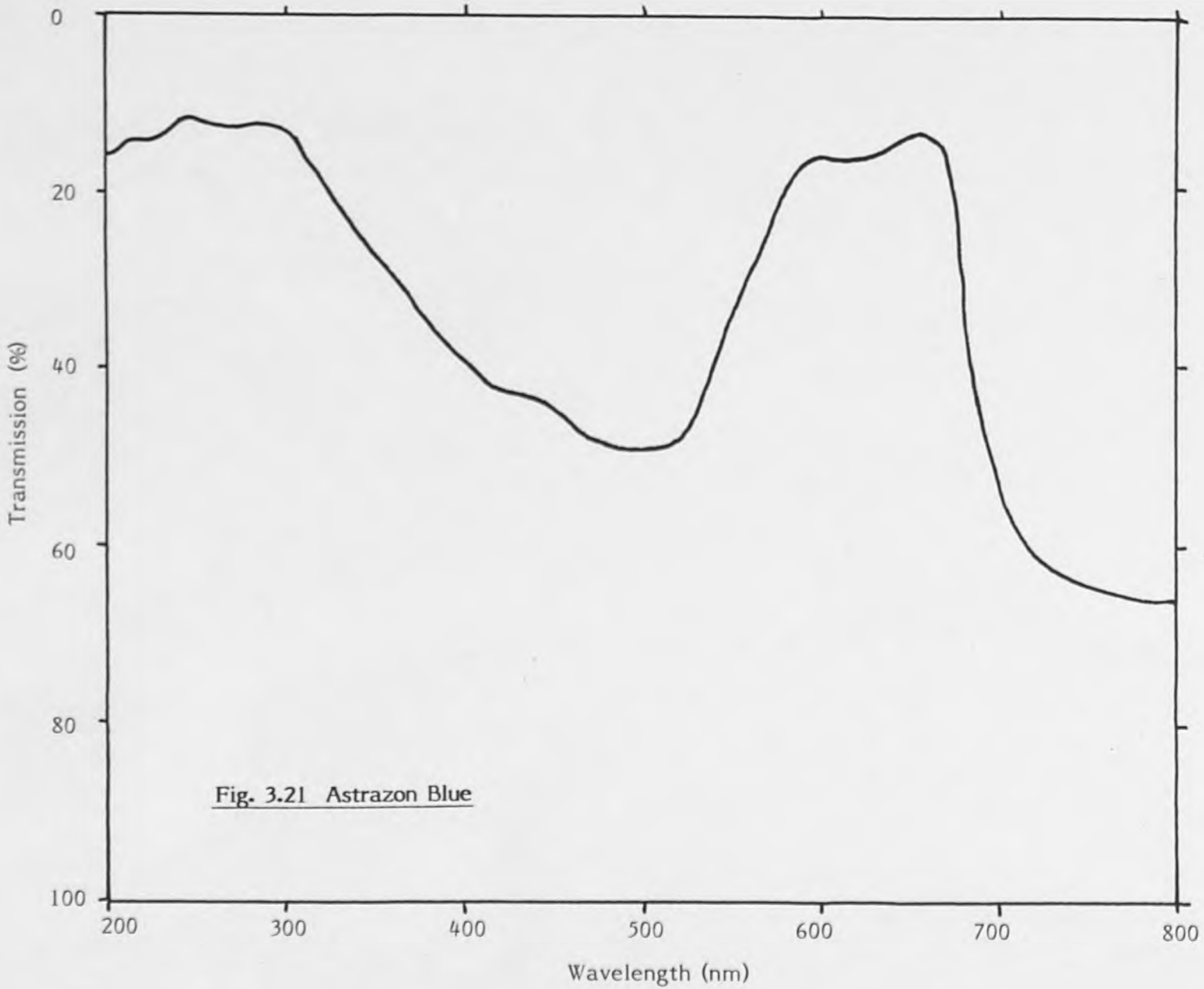


Fig. 3.21 Astrazon Blue

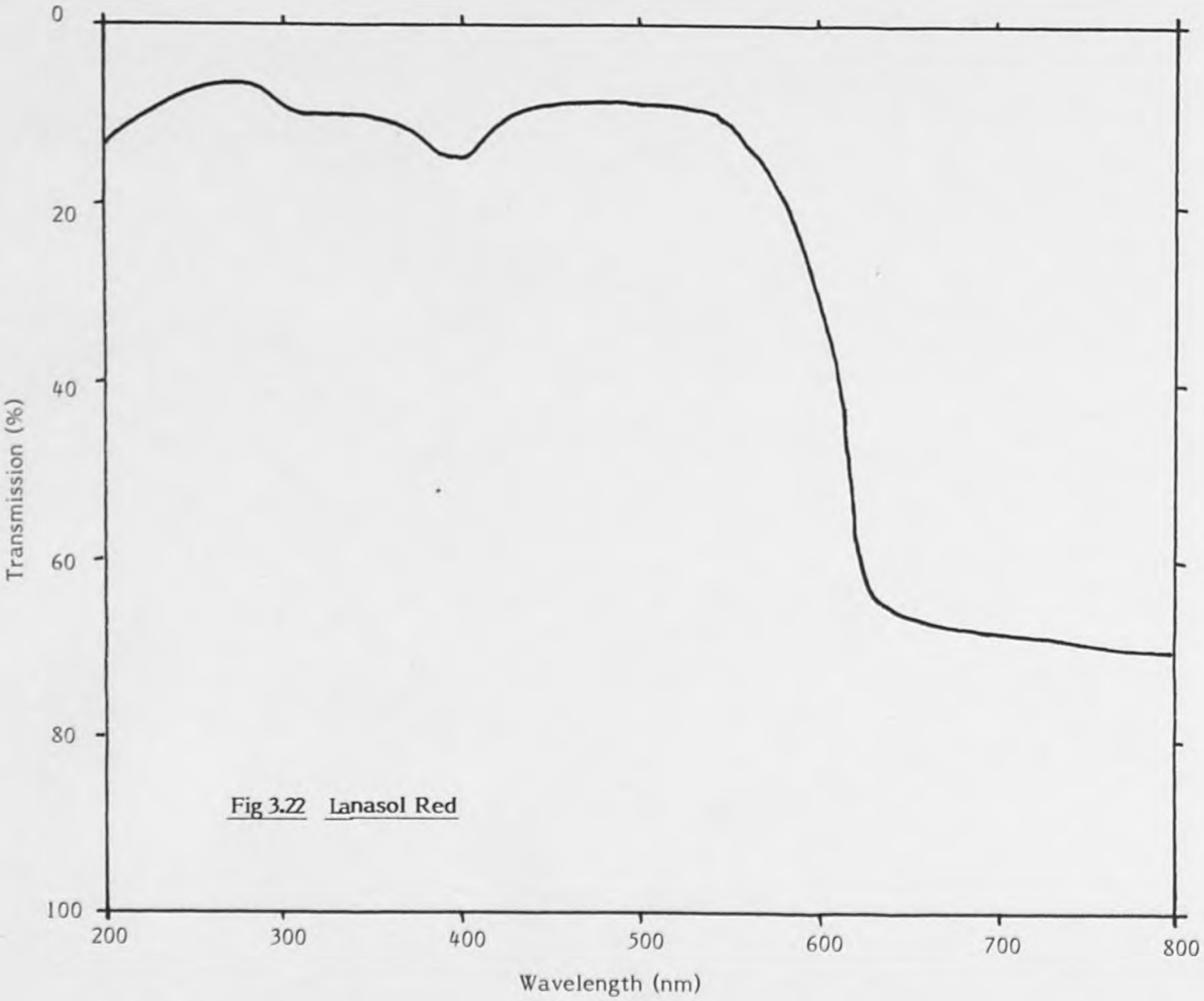


Fig 3.22 Lanasol Red

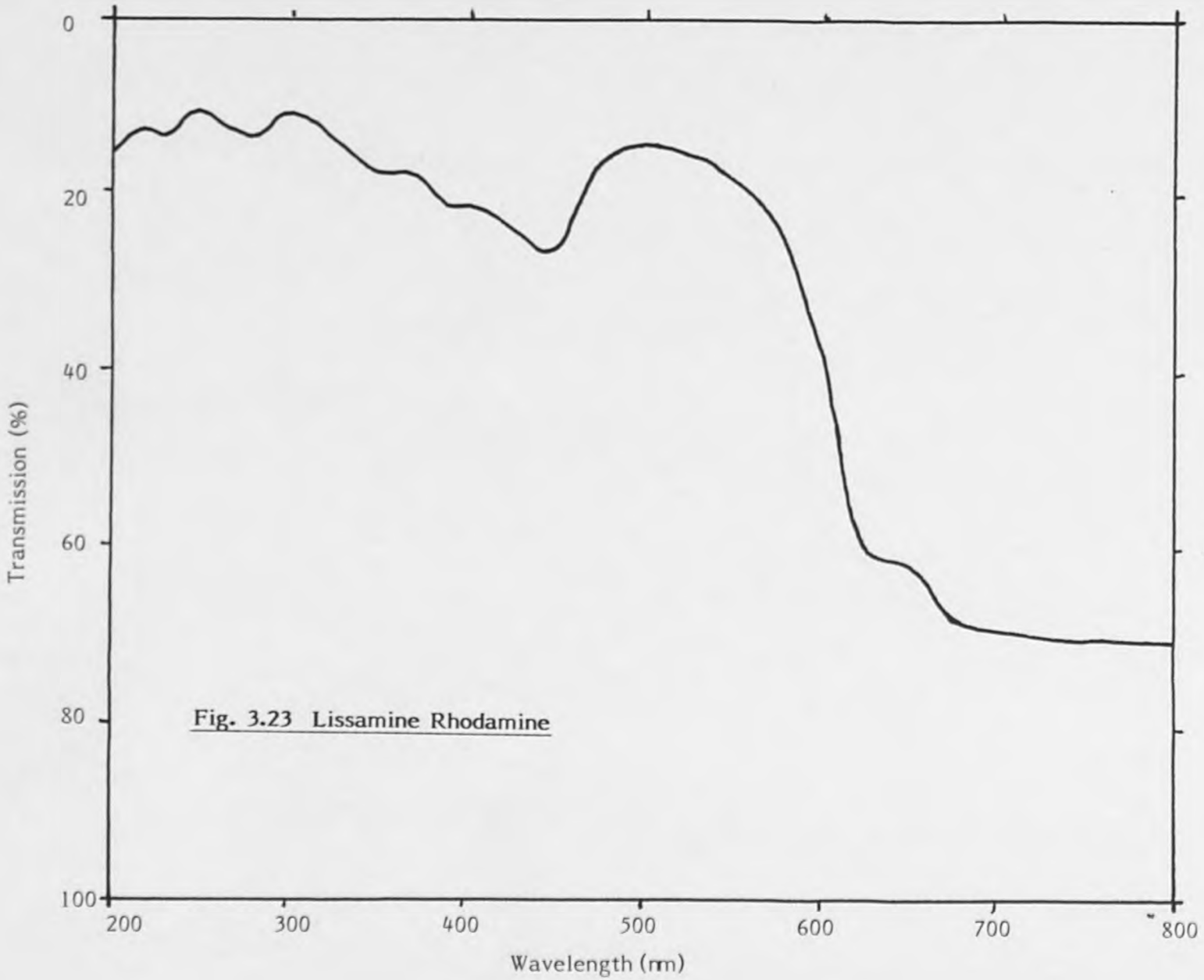


Fig. 3.23 Lissamine Rhodamine

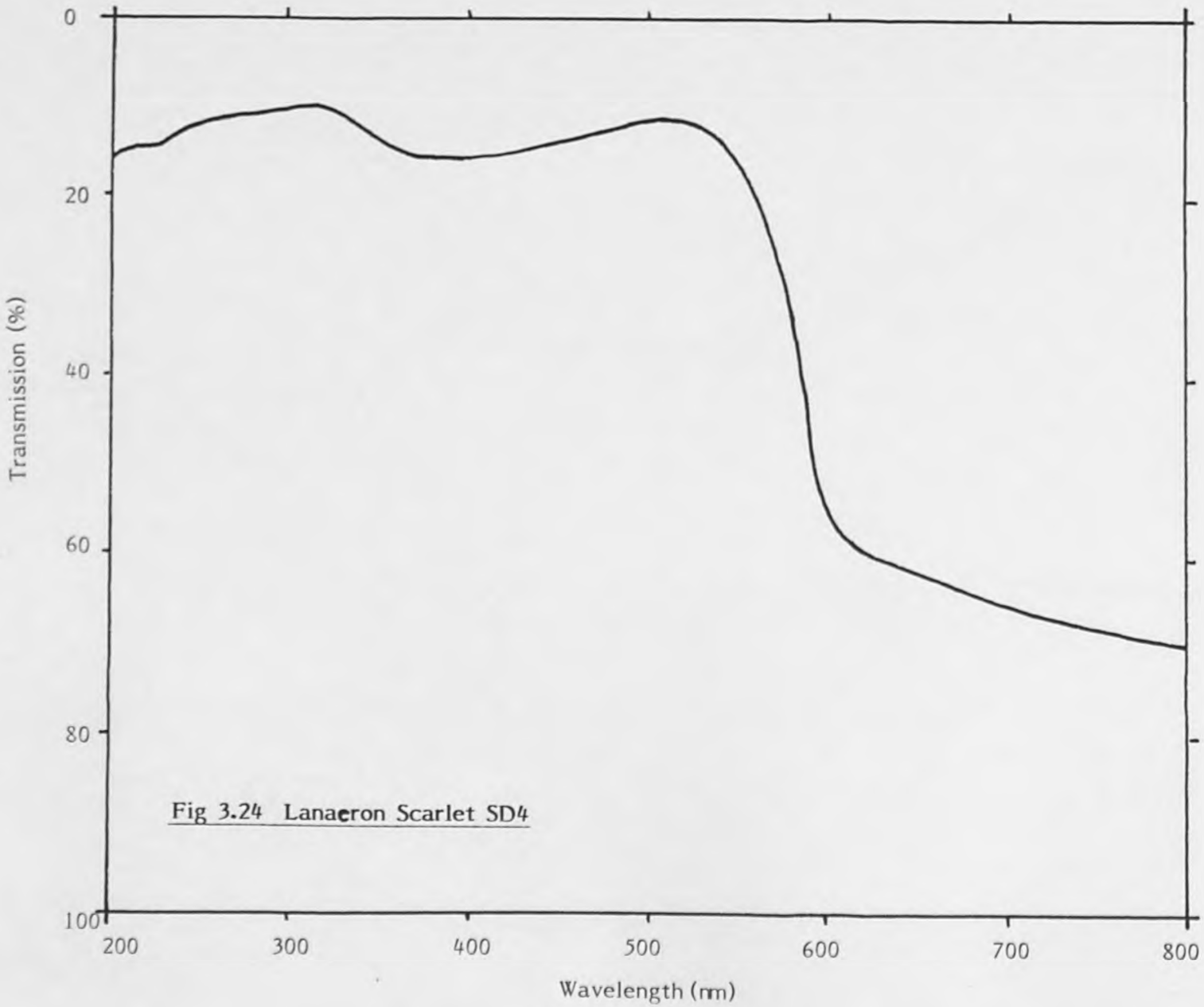


Fig 3.24 Lanaeron Scarlet SD4

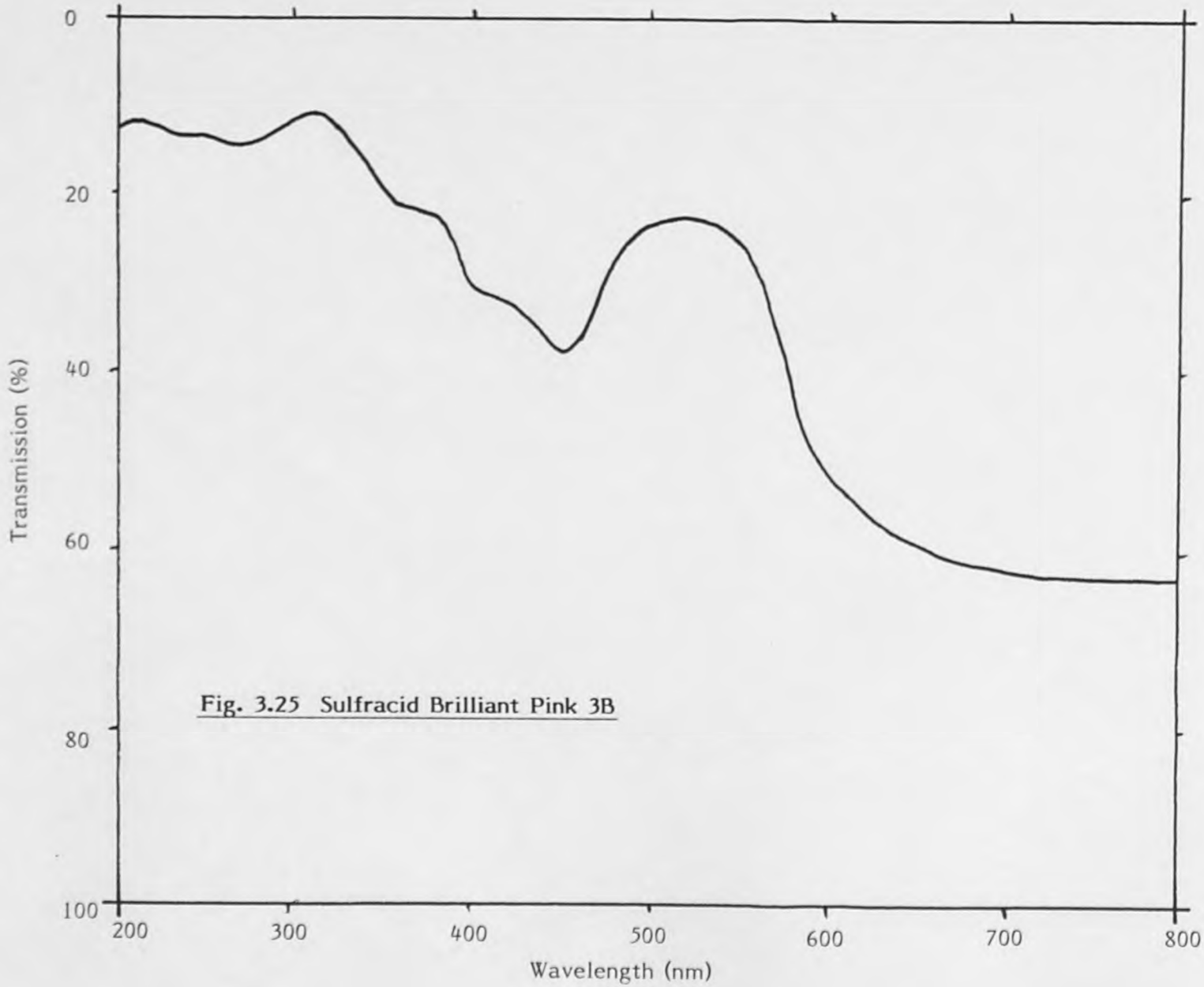


Fig. 3.25 Sulfracid Brilliant Pink 3B

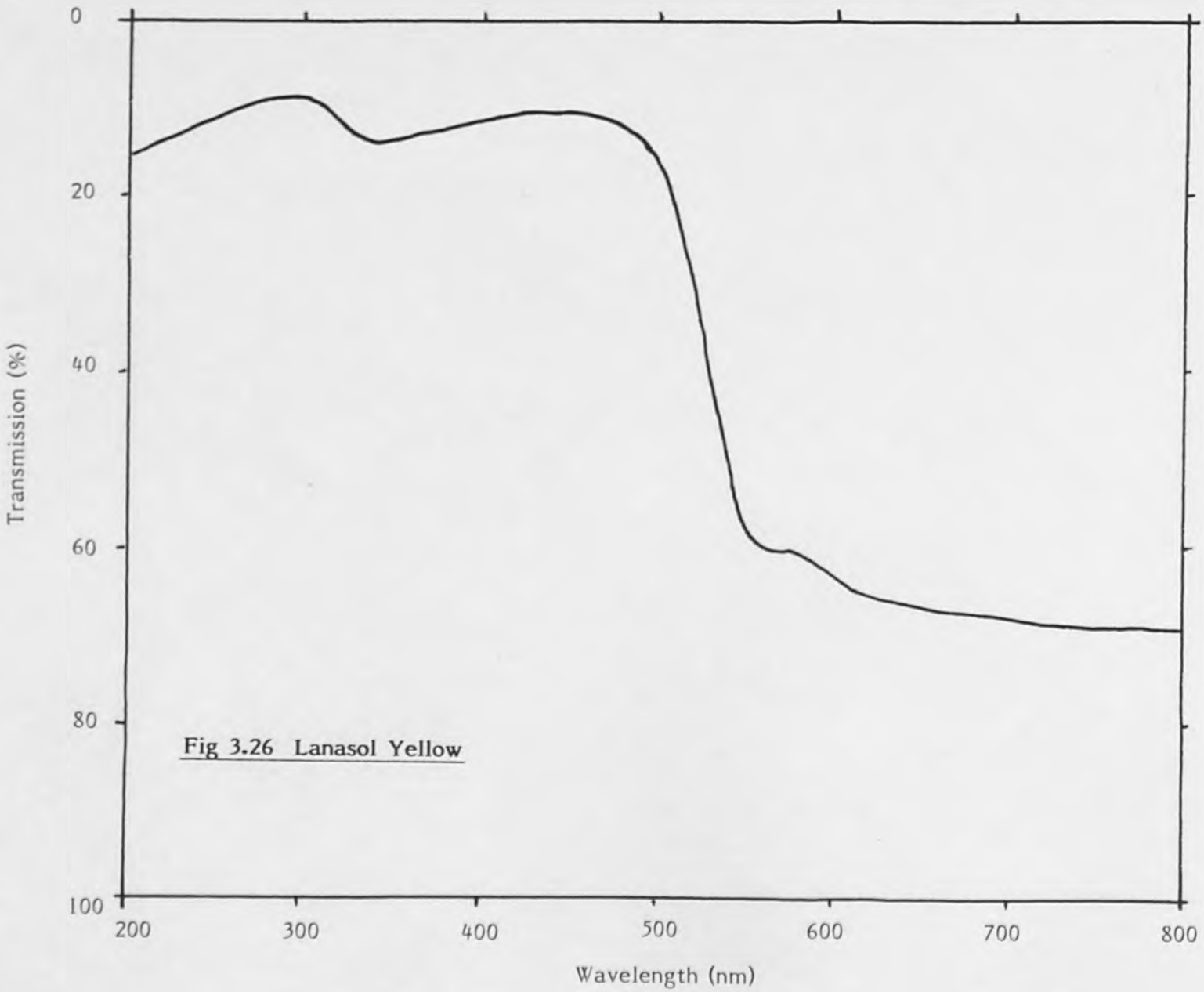


Fig 3.26 Lanasol Yellow

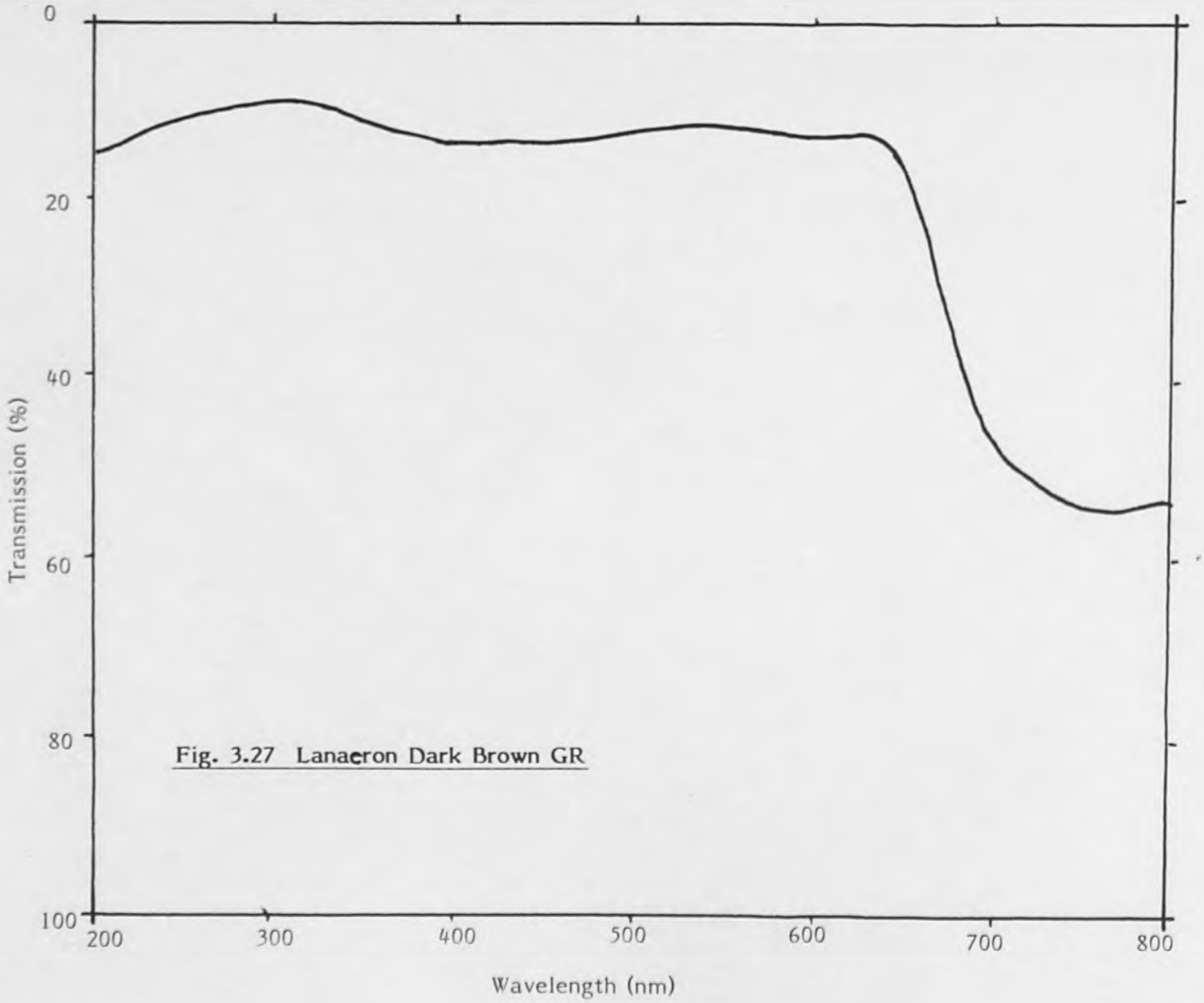


Fig. 3.27 Lanaeron Dark Brown GR

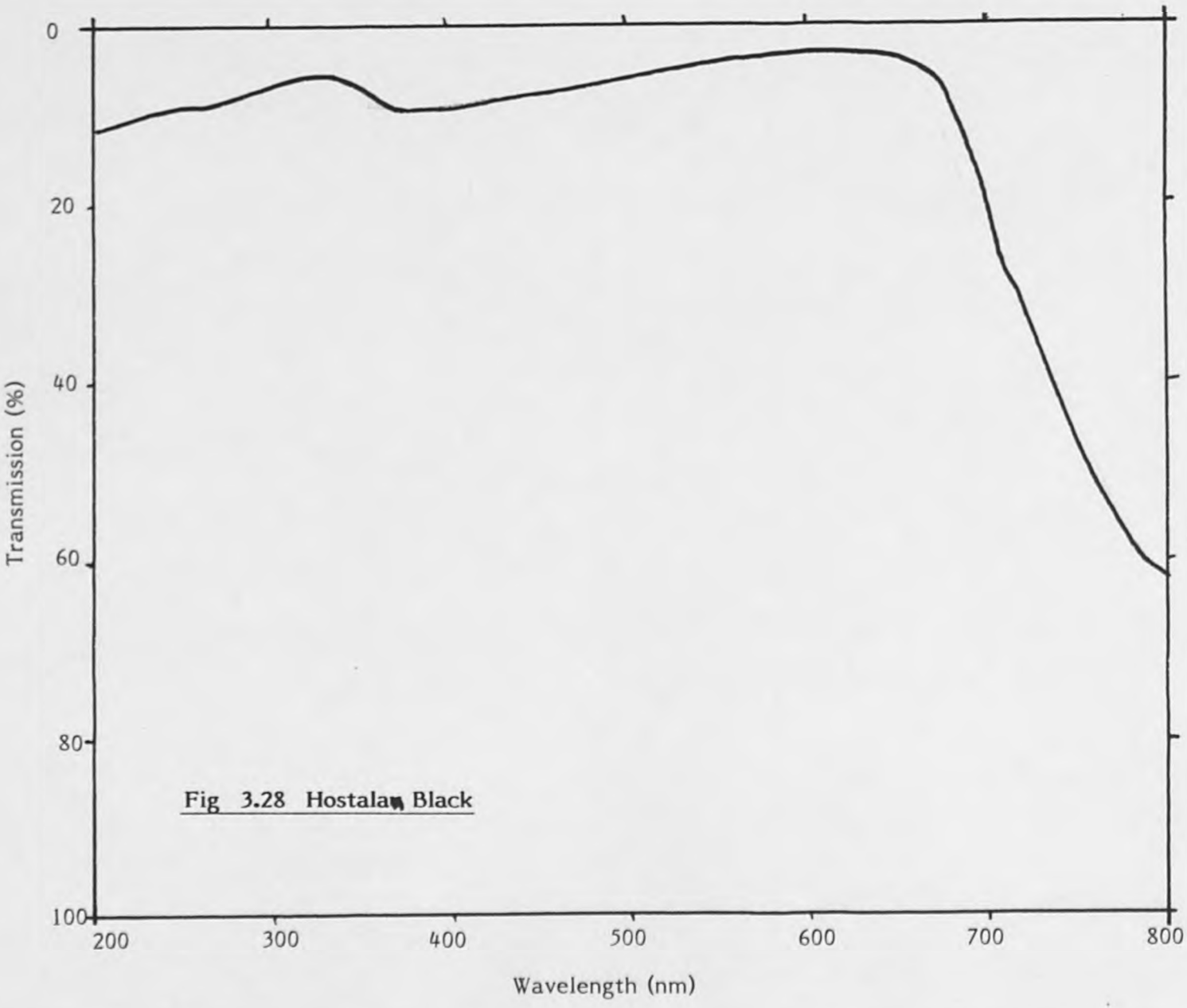


Fig 3.28 Hostalan Black

REFERENCES

1) B.Milligan and L.A.Holt, Aust.J.Chem., 1974, 27, 195

2) N.A.Evans, D.E.Rivett & P.J.Waters, Text.Res.J., 1976, 46, 214

3) I.H.Leaver, Aust.J.Chem., 1977, 30, 87

4) I.H.Leaver, Text.Res.J., 1968, 38, 729

5) I.H.Leaver & G.C.Ramsay, Photochem. Photobiol., 1969, 9, 359

6) M.Zahradnik, in "The Production & Application of Fluorescent Whitening Agents", Publ.John Wiley & Sons, Chichester, 1982

7) R.S.Davidson & D.King, J.de Phys., 1983, 44 (10), C6

8) N.A.Evans, D.E.Rivett & J.F.K.Wilshire, Aust.J.Chem., 1974, 27, 2267

9) J.Lin, D.E.Rivett & J.F.K.Wilshire, Aust.J.Chem., 1977, 30, 629

10) N.A.Evans, L.A.Holt & B.Milligan, Aust.J.Chem., 1977, 30, 2227

11) R.S.Davidson & J.E.Pratt, Photochem. Photobiol. 1984, In the Press

12) T.Oda, N.Kuramoto & T.Kitao, J.S.D.C., 1981, 97, 462

13) L.A.Holt & B.Milligan, Aust.J.Biol.Sci., 1974, 27, 23

14) McKeller & Allen, "Photochemistry of man-made Fibres", 1979, Applied Science Publishers, London

15) L.A.Holt, B.Milligan & L.J.Wolfram, Text.Res.J., 1974, 44, 846

CHAPTER 4

A Comparison of the Effects of Different Oxidizing Agents upon Wool

CHAPTER 4

A Comparison of the Effects of Different Oxidizing Agents Upon Wool

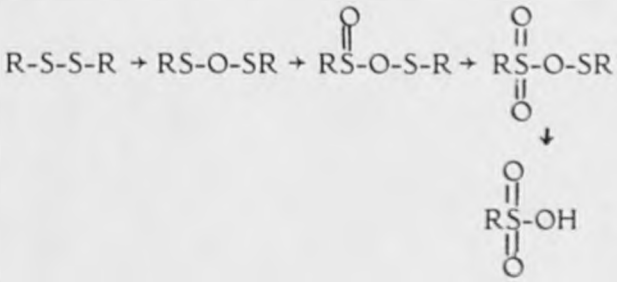
CHAPTER 4

A Comparison of the Effects of Different Oxidizing Agents upon Wool

4.1 Introduction

The complex and variable amino acid structure of wool keratin contains a number of sites which are susceptible to oxidation by a variety of reagents. Many oxidizing agents are used routinely on an industrial scale for bleaching or shrinkproofing of wool, and in laboratories for the preparation of soluble proteins from wool. The chemistry of these reactions has been described by a number of authors, and is summarized here by way of introduction. However, the reason for this study was to determine the nature of changes to the physical and optical properties of the wool fabric; very little information having been previously reported in this area.

Cystine, cysteine, methionine and tryptophan are the residues most susceptible to oxidation in wool and other proteins. Of these, cystine residues are the most abundant, and consequently, oxidants exert their major effect on wool by modifying cystine residues. Complete oxidation of cystine residues yields cysteic acid residues, but intermediate oxidation products often result:



Complete oxidation of cystine to cysteic acid residues provides a method for extracting "soluble" proteins (keratoses) from wool, and for the quantitative determination of cystine contents. Oxidative bleaching and shrinkproofing processes also involve the oxidation of cystine residues, but in these cases it is important to limit the extent of oxidation to avoid excessive fibre damage.

Although oxidizing agents react predominantly with the thiol and disulphide groups in wool, they may also modify other groups, such as thioether, (methionine),

imidazole (histidine), phenol (tyrosine) and indole (tryptophan). For example, the sulphide group in methioninyl residues is generally converted to the corresponding sulphoxide or sulphone [1]. Tryptophyl residues are also converted to N-formyl kynurenine or kynurenine, and many other products.

Apart from the above reactions, specific to particular amino acid residues, some oxidants cause cleavage of the peptide chains. This is especially true of those (eg. peroxide or persulphate) which induce the formation of free radicals on the protein backbone.

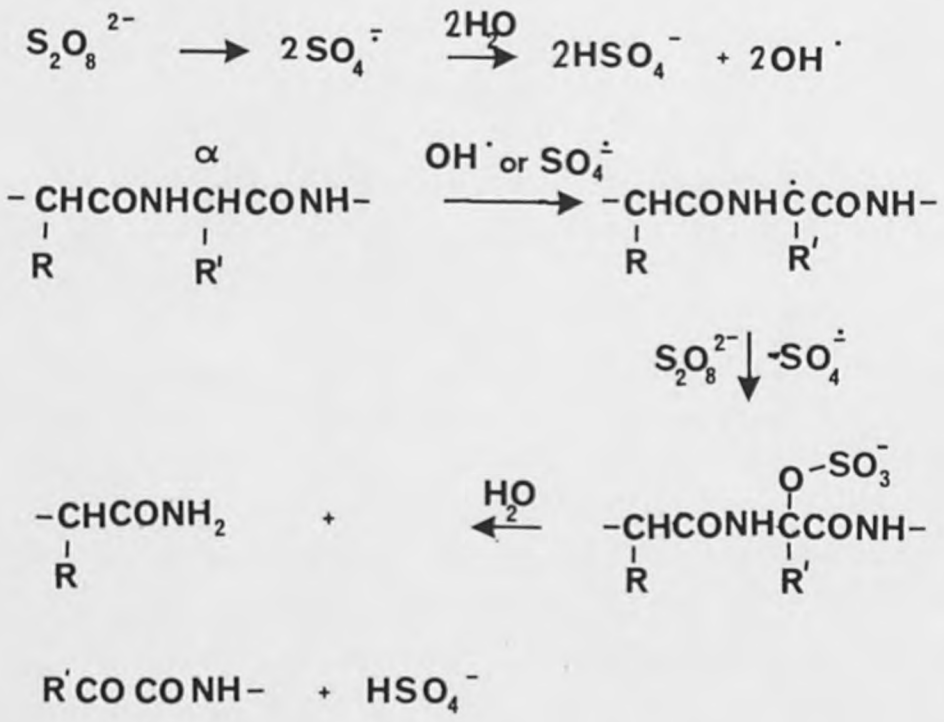


Fig 4.1 Reaction Scheme for Peptide Chain Cleavage During Persulphate Oxidation

The effects of the most commonly used oxidizing agents on the chemical composition of wool are discussed below. Further details of the reactions involved, and of other oxidizing agents, may be obtained from the original papers referenced in this chapter, or from the excellent review by N.H.Leon, [2], published in Textile Progress in 1975 and reprinted with minor changes in "The Chemistry of Natural Protein Fibres" in 1977 [3].

4.1.1 Hydrogen Peroxide

Hydrogen peroxide is a potent oxidant of many organic compounds. In proteins it attacks mainly 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 are affected by pH, oxidation of cysteine decreasing in rate as the pH is reduced, while the rate for methionine increases slightly under more acid conditions [4].

This reagent is widely used for the oxidative bleaching of wool, having largely superseded sulphur dioxide and the sulphites. It bleaches wool most effectively under alkaline conditions, [5], but unfortunately these are the very conditions which cause the most damage to wool. There is a progressive increase in the extent of oxidation of cystine residues with increasing pH, and protein is solubilized and extracted during treatment at pH10 and above [6]. The loss of cystine content correlates fairly well with the increase in alkali solubility of the wool, and this test is therefore used to assess the extent of wool damage during bleaching. [6,7,8,9]

The reaction of wool keratin with hydrogen peroxide is relatively slow, except under alkaline conditions. When wool is immersed in hydrogen peroxide solutions at pH9 or less, some of the reagent is initially absorbed on the amino or imino groups [10]. The absorbed peroxide seems to be remarkably stable and is removed by washing. Under alkaline conditions, hydrogen peroxide oxidizes cystine residues to cysteic acid residues, with few, if any, intermediate oxidation products present, probably because of their instability to alkali [11,11a]. These reactions are discussed by Zahn in his papers comparing the effects of a range of oxidizing agents [12,13].

Peptide bonds are also cleaved during peroxide treatments [14,15]. Elöd et.al. showed that both silk (which contains no cystine) and wool were completely dissolved by 3% hydrogen peroxide solution in three days at 60°C [15]. Only low molecular weight peptides were isolated.

Under the usual conditions of peroxide bleaching, amino acid analysis has shown that 30% of tryptophan residues are modified [16,17,18], together with 15-20% of tyrosine residues. However, there is no destruction of lysine or arginine residues [19]. The effects of peroxide oxidation on nineteen amino acid residues in wool have been described by Inglis et.al. [16].

The colour of hair, and of darkly coloured wool, is due mainly to the inclusion of discrete darkly coloured melanin granules in the keratinized cytoplasmic protein of the fibre-forming cells. During bleaching with hydrogen peroxide, an initial solubilization of the melanin occurs, followed by decolourization [20]. The morphological aspects of bleaching wool with peroxide have been investigated by Kaplin et.al., [21], who have shown that initially the surface cuticle alone is affected, but with time, the bulk of the fibre is oxidized. Clearly, the extent of reaction is therefore greatest at the wool surface.

Acting as a cation exchanger, melanin present in the fibre readily binds metal ions. Thus heavy metal ions generally catalyse the reaction between keratin and hydrogen peroxide. Whether the reaction is identical with that in the absence of these ions is uncertain. Bleaching of wool or hair involving pretreatment with aqueous ferrous sulphate solutions has been shown to yield the most satisfactory results (ie. maximum colour removal with minimum fibre damage). [22-24] This catalytic effect may be due to the promotion in the breakdown of hydrogen peroxide to free radicals; hydroxy radicals, for example have been observed in the presence of ferrous ions [25, 26].

A number of papers have been published describing methods of bleaching wool with hydrogen peroxide [27-31]. Many of these incorporate the use of a silicate or phosphate stabilizer, and in one case, the use of sodium lauryl sulphate is discussed [31].

4.1.2 Performic & Peracetic Acids

Organic peroxyacids are the most specific reagents for the oxidation of disulphide bonds in wool, performic and peracetic acids being the preferred reagents. They have the advantages of good solubility, ease of removal of excess reagent, and the irreversibility of their reaction:

$$\begin{matrix} | \\ CH\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}CH \\ | \end{matrix} + 6\ RCO_3H + H_2O \rightarrow 2 \begin{matrix} | \\ CH\text{-}CH_2SO_3H \\ | \end{matrix} + 6\ RCO_2H$$

Apart from the purposes of bleaching or shrinkproofing the wool, this reaction is also carried out to facilitate the electrophoretic separation of the peptides.

Performic and peracetic acid are both able to oxidize cystine residues in wool to cysteic acid residues, via intermediate oxidation products. When used in sufficient excess, performic acid leads to virtually complete oxidation of disulphide bonds, [32], whereas peracetic acid proceeds more slowly [32,33] and cleaves a small percentage of peptide bonds [32]. Peracetic acid has the advantage that it is stable in aqueous solution, and that dilute solutions dissolve very little wool, whereas performic acid is unstable in aqueous solution, and must be used in formic acid solution.

Under normal reaction conditions, only tryptophan, methionine and cystine are oxidized by organic peracids, although after prolonged treatment, peracetic acid destroys some of the arginine, lysine and histidine residues [34].

The destruction of tryptophan residues is almost complete by this treatment [1]. Tyrosine has been found to be modified during oxidation in solution, [35], but no evidence has yet been found to support these changes in wool. The influence of pH and irradiation on this process has been studied by Sweetman et.al. [36], and the use of the oxidation step to separate various protein fractions (keratoses) by others [37,38].

4.1.3 Perchloric and Periodic Acids

A vast number of references exist to the reactions of halogen compounds with wool, mainly due to the commercial use of chlorine in acid solution as a shrinkproofing reagent. This generates a number of oxidation products

from cystine residues [33,39], which are frequently used for further cross-linking reactions [40]. These reactions of chlorine with wool have been described by Schirte [41,42].

Perchloric and periodic acid cause cleavage of the disulphide bond and are often used as setting agents. The effectiveness of this action has been attributed to the formation of cysteic acid groups, which form strong electro-valent links of the type $-SO_3^{\ominus}$ - - - - $^{\oplus}NH_3$ - with the free amino groups in the fibre [42a].

The reactions of wool with periodic acid have been extensively studied by Kantouch and Bendak [43-45], who have found that the sulphur content of periodate-treated wool gradually decreases with oxidation. They suggested that periodate attacks mainly the carbon atom alpha to the peptide linkage, leading to bond fission and the formation of new carbamic acid derivatives, amide groups and aldehydes. Reactivity of the α-carbon is influenced by the adjacent side chain, a high degree of degradation being observed for the two hydroxyamino acids, serine and threonine, as well as for tyrosine and tryptophan.

Treatment of wool with perchloric acid has also been described [46,47].

4.1.4 Permonosulphuric Acid

Like the more reactive performic and peracetic acids, permonosulphuric acid oxidizes only cystine, tryptophan and methionine residues in wool under the normal conditions used to impart shrink-resistance, [48,49], but more vigourous oxidation can produce destruction of some tyrosine residues [50]. This is given by the authors an explanation for its superior shrink-resist properties over peracetic acid,.

Treatment of wool with permonosulphuric acid under normal conditions (0.1% solution, pH1.5, 22°C, 2hrs, liquour:wool ration 35:1) is claimed to produce a substantial increase in the dyeing rate of both acid and basic dyes [51], although others do not agree [52]. If true, this is probably because cleavage of disulphide bonds promotes fibre swelling and loss of cell membrane material, which outweighs the opposite effect of the introduced cysteic acid groups.

Shrinkproofing is the main use for permonosulphuric acid oxidation of wool [53], and in the industrial process, it is followed by a sulphite reduction [54]. In the presence of high concentrations of sodium sulphate, sufficient shrink-resistance is obtained from a single treatment with neutral permonosulphuric acid solution [53].

4.1.5 Persulphate

Treatment of wool with ammonium or potassium persulphate in aqueous solution causes quite extensive degradation and discolouration [55,56]. There is significant attack on cystine, methionine, arginine, histidine, proline, tyrosine and phenylalanine. Carbon dioxide and ammonia are liberated during the reaction. Two sources of oxidation were detected, viz (i) due to oxygen resulting from persulphate decomposition, and (ii) reduction of the persulphate by wool [56]. The reaction rates were found to be different.

Needles suggested [55,57] that the main damage is due to the attack of the peptide chain by hydroxyl or sulphate radicals, resulting from the decomposition of the persulphate. This is supported by Burke et.al., [26,58]. Abstraction of a proton from the α-carbon of an amino acid residue gives a radical that reacts with a persulphate ion to form an unstable sulphate peptide. Cleavage of the chain then takes place between the α-carbon and nitrogen, yielding an amide peptide and an α-oxoacyl peptide that may further decompose, liberating carbon dioxide and ammonia. See Fig.4.1. It has been shown by electron spin resonance spectroscopy that free radicals produced are on cystine, tyrosine and tryptophan residues [58]. It has been shown [56,59] that the content of sulphur-containing amino acids and lysine, histidine and arginine in wool, decreases on oxidation with persulphate. When studying human hair, Breuer and Jenkins [60] showed that the reaction is highly complex, and that the rate of reaction is proportional to the square root of the persulphate ion concentration. The reaction with cystine has also been reported [61].

4.1.6 Potassium Permanganate

Potassium permanganate has been used as an oxidizing agent to shrink-proof wool, and as an anti-felting agent [50]. The extent and course of oxidation depends upon whether acid or alkaline solution is used. Cystine residues

are destroyed under either conditions. At low pH, they are oxidized to cysteic acid residues and sulphate [33]. It was originally claimed that no cysteic acid residues were formed during treatment with alkaline permanganate [33,62]. However, this has now been disproved [63]. Both cysteic acid and lanthionine residues are formed under these conditions. An early claim that treatment of wool with excess permanganate at pH2 oxidized only 30% of the disulphide groups [62] has also been disproved [64]; oxidation is virtually quantitative.

Under alkaline conditions, tyrosine residues are significantly destroyed [62, 65], and tryptophan residues are also affected [65]. Many references exist to the application of neutral permanganate in the presence of sodium chloride, and various methods of treatment are given [66-73]. Inorganic salts added in relatively large amounts increase the degree of initial shrink resistance probably by preventing swelling and confining the reaction more to the cuticle layers, so that a greater surface modification of the fibre results [2].

Manganese dioxide is deposited in the fibre at the sites of reaction with permanganate [74]. This is usually removed with a "clearing" solution of an acidified bisulphite. The resultant sulfitolysis complicates subsequent analysis of the wool for residual cystine content.

4.1.7 Miscellaneous References

One of the prime uses for oxidation of wool is for bleaching, and this is further described in two anonymous articles in Wool Science Reviews [75,76]. The effect of oxidation by ozone is reported by Thorsen et.al., [77], and the use of oxidation to produce unsymmetrical disulphides, which may be of use when dyeing with reactive dyes, is also described [78].

Several methods for evaluating oxidized wools have been described. These include infrared spectroscopy [79,80], acid and alkali solubility measurement [81] and the determination of set and supercontraction properties [82].

4.2 Experimental Results & Discussions

The main aim of this particular work was to determine colour changes in the wool following oxidation with different oxidizing agents. In the context of this thesis, the effects of different oxidation methods upon the photo-

stability of the wool were also studied.

4.2.1 Hydrogen Peroxide

Wool is oxidized chemically on an industrial scale to achieve either shrink proofing or bleaching. Hydrogen peroxide is used for both of these functions, but primarily for the latter. Bleaching is a process used sometimes in its own right, but frequently as a precursorto the application of a fluorescent whitening agent, so that the whitener operates against a background of white wool. In this chapter, attention is confined to the bleaching procedure.

The wool fabric used for this work was 200gsm salts serge, pre-buffered to pH7. Hydrogen peroxide was obtained from BDH, 100 volume concentration, laboratory reagent grade. The stabilizer used was Tubotex PC (C.H.T. Tubingen, West Germany). This was a blend of sodium oxide, silica, alkalis and sequestering and wetting agents. No attempts were made to vary the nature of the stabilizer, and during this work it is referred to as "Stabilizer PC". Aqueous solutions of this stabilizer at the concentrations used have a natural pH of 11.5-12.0. However, to prevent fibre damage this was always reduced to pH8 in use, by the addition of formic or acetic acids.

A liquor to wool ratio of 30:1 was used as standard. The hydrogen peroxide concentration was set at 2.8 volumes, which involved diluting 100 volume stock by 1:36. The actual amount used was determined by the weight of the wool sample to be bleached.

The method of application used is summarized below, with figures included as an example:

1) Weighed out wool sample eg.10g.
2) Liquor:wool ratio 30:1, so required 300ml.
3) Hydrogen peroxide concentration 2.8 volume, so required 2.8ml/100ml solution. ie. 8.4ml.
   Diluted 8.4ml 100vol H_2O_2 to approximately 200ml.
4) Dissolved required weight of stabilizer PC to achieve an overall concentration of 5g/litre in a small amount of warm water, and added to the hydrogen peroxide solution. This was then diluted to required volume (300ml).

5) The pH was adjusted to 8 by the addition of concentrated formic or acetic acid.
6) The wool sample was pre-wetted with Lissapol N and rinsed thoroughly.
7) The solution was heated to 40°C in a Jeffreys dyemaster and the wool sample introduced. This temperature was maintained for five hours.
8) On completion of the treatment, the wool sample was rinsed with copious amounts of cold water, washed briefly with dilute acetic acid at pH5, and rinsed again before drying in air at room temperature.

Having bleached wool using this method, the Yellowness Index and Whiteness Index values were measured, as described in Chapter 2. Measurements were made on a Zeiss RFC-3 spectrophotometer, and the yellowness calculated according to the following equation.

Yellowness Index

$$Y.I. = 100 \frac{(1.28X - 1.06Z)}{Y}$$

Jacquemart Whiteness Index

$$W.I. = [(100-Y)^2 + 5.5 (X-Z)^2]^{\frac{1}{2}}$$

Decreasing values of Y.I. and W.I. indicate a decrease in the yellowness of the wool ie. an increase in whiteness. W.I. values were included because the yellowness index scale does not differentiate adequately between different levels of greyness in white wool.

These results are listed in Table 4.1. For comparison, wool was also reductively bleached. This did not give such a good whitening effect, but also did not result in the marked decrease in photostability observed following the peroxide bleach.

The reducing agent used was Blankit D (zinc formaldehyde sulphoxylate, described in detail in Chapter 2). A 3g/litre solution of Blankit D was adjusted to pH3 with acetic acid, and a 10g sample of wool fabric introduced. This was heated to 80°C for 2.5 minutes, following which the wool was rinsed thoroughly in cold water and dried in air at room temperature. Yellowness Index and Whiteness Index results for this material are also given in Table 4.1.

In an industrial environment, it is not uncommon to carry out an oxidative bleach with hydrogen peroxide, followed by a reductive bleach as described above, to improve the final whiteness and photostability of the wool fabric. This was performed on a further 10g wool sample, and the yellowness measured.

Each of the bleached samples prepared above was photoyellowed for 24 hours in the wet state, as described in Chapter 2. The yellowness of these samples was then redetermined, and the results listed in Table 4.1.

Table 4.1 The Effects of a Peroxide Bleach on Wool

	Y.I.	Δ Y.I.	W.I.	Δ W.I.
1) Untreated Wool	24.5	0	38.9	0
2) Peroxide Bleached	11.9	-12.6 for bleach	22.0	-16.9
3) (i) Peroxide Bleached, (ii) Photoyellowed 24hrs.	24.8	12.9 for hν	39.2	17.2
4) Blankit D Bleach	19.9	-4.6	33.8	-5.1
5) (i) Blankit D Bleach, (ii) Photoyellowed 24 hrs.	25.5	5.6 for hν	40.8	7.0
6) (i) Peroxide Bleached, (ii) Blankit D Bleached	10.2	-14.3	20.0	-18.9
7) (i) Peroxide Bleached, (ii) Blankit D Bleached, (iii) Photoyellowed 24hrs.	22.1	11.9	34.1	14.1

NOTE: Negative Δ values indicate a whitening of the wool fibre

The results in Table 4.1 show clearly that the peroxide bleach gives a superior whiteness to that obtained using the reductive bleach method. However, the photstability of the wool is quite seriously affected, and marked yellowing occurs during subsequent irradiation with 350nm light. It was also found that by following the peroxide bleach with a reductive bleach, a small further increase in whiteness was achieved, and at the same time slightly improving the photostability.

It was shown in Chapter 2 that the amino acid primarily responsible for photoyellowing of wool is tryptophan. Oxidation with peroxide is known to attack primarily the cystine residues, but since cysteic acid is colourless, it is likely that peroxide enhances the rate of yellowing by modifying the tryptophan residues into a more unstable form. A proposed photooxidation pathway for tryptophan is given in Chapter 1 (Fig. 1.9), and this includes several hydroperoxide intermediates, which may also be formed when wool is bleached with hydrogen peroxide. If so, this would be an explanation of the enhanced photoyellowing derived from this treatment.

By varying the peroxide bleach method described earlier, it was found that serious damage to the fibre occurred when any one or more of the following was true:

(1) The operating pH of the liquor was set at a higher value than 8;
(2) The temperature used was higher than 40°C;
(3) The peroxide concentration was increased;
(4) The treatment duration exceeded approximately seven hours.

Under these conditions, protein was extracted and the wool became hard and boardy. The tensile strength was also greatly diminished. This was believed to be due to an increase in the level of peptide bond fission. It was therefore critical that the described method was carefully followed. Reducing the temperature, pH, treatment duration or the peroxide concentration resulted in a poor whiteness being achieved. Even when the method was carefully followed a marked decrease in the tensile properties of the wool was observed. This was attributed to the loss in disulphide cross-links on the cystine residues following oxidation.

In the introduction, the alkali solubility test was referenced as a means for the determination of the extent of fibre damage resulting from an oxidative treatment. This test was not used routinely in this study, but was used to compare the effects of a peroxide bleach with the reductive bleach described earlier. The method used for this determination is described below. It should be noted that an average value for the alkali solubility of untreated serge is 10-11%.

Alkali Solubility test Method

All analyses were carried out in duplicate.

1) A 35ml porosity 1 sintered glass crucible was heated in an oven at 105°C for 1 hour. It was then removed, cooled in a desiccator and weighed to three decimal places (C1).

2) Approximately 1.1g of wool was weighed accurately (to 1mg) into the preweighed crucible (W1), and placed in an oven at 105°C for 3 hours. This was then removed, cooled in a desiccator and weighed (to 1mg) (W2). It was replaced in the desiccator until required.

3) 100ml of 0.1M sodium hydroxide was transferred by pipette into a 100ml stoppered conical flask. This was placed in a Grant waterbath at 65°C $\pm$ 0.5°C, and allowed to reach the temperature of the bath.

4) Once at temperature, the dried wool sample was carefully added. The flask was shaken to ensure complete wetting of the sample, and replaced in the waterbath for 60 minutes.

5) After this time, the flask was removed from the bath, and the contents filtered through the preweighed crucible under suction, with washings of demineralized water to transfer all fibrous material from the flask. The residue was washed 6 times with demineralized water. The vacuum was then disconnected, and the sample washed with dilute acetic acid twice by allowing it to stand for 1 minute and then sucking the acid through. Finally, the sample was washed six times again with demineralized water. The crucible and contents were dried at 105°C for 3 hours, removed and cooled in a desiccator, and reweighed (W3).

6) Calculations:

(i) The moisture content of the original sample: $= \frac{(W1-W2)}{(W1-C1)} \times 100\%$

(ii) The Alkali solubility of the wool: $= \frac{(W2-W3)}{(W2-C1)} \times 100\%$

Test samples were cut in half. One half was used to determine the moisture content, so that the other half (to be tested) need not be heated to dryness, possibly causing fibre damage.

The alkali solubility results obtained are listed below:-

WOOL SAMPLE	ALKALI SOLUBILITY
Untreated Wool	12.2% ± 0.1%
Peroxide Bleached Wool	20.7% ± 0.4%
Reductively Bleached Wool	7.7% ± 0.2%

These results show that fibre damage does occur, as expected, when wool is oxidized with hydrogen peroxide. However, they also show that not only does reductive bleaching not increase the alkali solubility, but a small decrease is seen.

Finally, fluorescence spectroscopy was used to observe the effect of peroxide oxidation on the long wavelength luminescence of wool. This is discussed in some detail in Chapter 6, but may be summarized here. The luminescence originates from an as yet unknown species present in wool which absorbs at 360nm and fluoresces in the blue region of the spectrum at 435nm. The intensity of this fluorescence or phosphorescence emission is decreased by approximately 30% after photoyellowing, but is increased by 20% following oxidation with peroxide. However, when the peroxide treated wool is photoyellowed, a 60% decrease in the fluorescence emission is observed, giving an emission intensity 35% less than that obtained for photoyellowed untreated wool. It may therefore be deduced that the long wavelength species is photodegraded by ultraviolet light during yellowing, but is also generated, although in an unstable form, by the peroxide oxidation.

4.2.2. Potassium Permanganate

Much work has already been done to study the effects of potassium permanganate on wool. It is generally applied as a shrink-resist reagent,

although it has largely been superseded by polymer treatments such as Lankrolan SHR-3 (Diamond Shamrock), which offers a superior level of protection to the wool.

The wool used in these experiments was again 200g/m^2 salts serge, and the potassium permanganate and sodium chloride were obtained from BDH.

The wool samples used weighed 20g, and solutions were made up to contain 5% owf potassium permanganate. A liquor to wool ratio of 40:1 was used. 1g of permanganate was dissolved in 800ml distilled water and adjusted to pH4 with sulphuric acid. The wool was pre-wetted with Lissapol N, rinsed thoroughly, and introduced to the dyemaster tube containing the solution. This was agitated at 60rpm for 10 minutes at room temperature, by which time the solution was virtually colourless. During the reaction, the wool became brown due to the deposition of manganese dioxide at the sites of reaction. After exhaustion of the potassium permanganate, the manganese dioxide was removed by treating with sodium hydrosulphite ($Na_2S_2O_5$), 0.05M, for 10 minutes.

The brown colour obtained by the initial treatment indicated that the application was uneven, an effect observed by previous workers **[65,69]**. They suggested that the unlevel results may have resulted from the high affinity of potassium permanganate for wool producing a precipitation of manganese dioxide on the outer layers of the wool fibre, with a gradual decrease in the permanganate available for reaction with the inner layers. The phenomenon may also, of course, have been caused by inadequate circulation of the permanganate solution during application.

Following clearing with sodium hydrosulphite or acid bisulphite, the wool was found to be whiter than the untreated fabric. All previous workers who have remarked on this aspect have made the same observation. However, this is not surprising, since hydrosulphite is a reductive bleach. The wool was found to be somewhat less white than a sample of untreated wool, also exposed to the same concentration of sodium hydrosulphite for the same time period. These results suggested that instead of whitening the wool, oxidation with permanganate caused a slight degree of yellowing. To confirm this, a sample of brown wool was cleared of manganese dioxide by treatment with hydroxylamine hydrochloride (HO NH_3 Cl), 0.05M. As expected, this

yielded a wool sample that was yellower than that cleared with hydrosulphite, and yellower too than untreated wool. See Table 4.2.

The permanganate oxidation of wool, described in the literature [62-73] has been carried out under acid, alkaline and neutral conditions, in the presence and absence of sodium chloride. This process was therefore repeated under each of these conditions to determine whether any variation in residual colour change occurred. A small difference was observed for treatments at different pH values, greatest yellowing occurring under alkaline conditions and a small decrease in the yellowing effect was observed in the presence of high concentrations of sodium chloride. This was considered surprising, since the object of adding salt to the shrinkproofing process was to minimize fibre swelling and to confine the reaction to the cuticle layers, thereby achieving maximum surface modification. However, it was also observed that a more even deposition of manganese dioxide was formed under these conditions, so the effect may simply be due to the prevention of excessive oxidation occurring in certain parts of the fabric. Possibly a more likely explanation was found by Andrews et.al., [68] to be that less degradation of tryptophan and tyrosine residues occurred under these conditions.

The photostability of wool is slightly reduced by the hydrosulphite bleach process, and was found to be further reduced by the permanganate oxidation. When the oxidation process was followed by cleaning in hydroxylamine hydrochloride solution, the increase in photoyellowing was less than that observed following the hydrosulphite after treatment, but more than that of untreated wool. Thus it was shown that this method of oxidation does reduce the photostability of wool, but to a considerably smaller extent than does peroxide. Results are listed in Table 4.2.

Reference to previous authors reveals that the alkali solubility of wool treated with permanganate is approximately 21%, under these conditions [65], which is very similar to the value obtained with hydrogen peroxide. It was also noted from the same reference that up to 50% of tryptophan and 45% of tyrosine may be destroyed during oxidation with potassium permanganate at pH4.

Table 4.2 The Oxidation of Wool with Potassium Permanganate

WOOL SAMPLE	Y.I.	Y.I.After Photoyellowing	Δ Y.I.
1) Untreated Wool	24.1	29.5	4.4
2) Hydrosulphite Bleached	19.0	24.8	5.8
3) (i) $KMnO_4$, pH4 (ii) $Na_2S_2O_5$	22.7	30.9	8.2
4) (i) $KMnO_4$, pH4 (ii) HO NH_3Cl	26.5	33.6	7.1
5) (i) $KMnO_4$, pH7 (ii) $Na_2S_2O_5$	22.6	30.7	8.1
6) (i) $KMnO_4$, pH7 (ii) HO NH_3Cl	26.5	33.5	7.0
7) (i) $KMnO_4$, pH10 (ii) $Na_2S_2O_5$	23.8	32.4	8.6
8) (i) $KMnO_4$, pH10 (ii) HO NH_3Cl	29.4	36.0	6.6
9) (i) $KMnO_4$, pH7, Na Cl (ii) $Na_2S_2O_5$	20.3	27.0	6.7
10) (i) $KMnO_4$, pH7, Na Cl (ii) HO NH_3Cl	24.9	30.9	6.0

4.2.3 Ammonium Persulphate

Like potassium permanganate, persulphates are used as shrinkproofing and antifelting agents for wool. The reaction with wool is probably the least specific for any particular functional group or amino acid residue of all the commercially used oxidizing agents. Much previous work on this reaction has been reported in the literature, and yet the pathways and mechanisms are far from clear.

The wool used for this reaction was again the 200g/m^2 salts serge, and the Ammonium persulphate used was obtained from BDH, Analar grade.

Wool was treated with a 1% w/v solution of ammonium persulphate at 50°C for 2 hours in a Jeffreys dyemaster. To ensure an even reaction, the wool samples were prewetted with Lissapol N, and thoroughly rinsed before use. 10g wool samples were used, with a liquor to wool ratio of 50:1. The reaction was set at pH4.5 in one tube and pH1.5 in another for comparison.

It was found that the wool was severely degraded and discoloured by reaction with persulphate under these conditions. The final colour of the fabric was orange - brown, being a darker colour in the case of reaction at pH 1.5.

By way of comparison, further wool samples were oxidized by persulphate, having been pretreated by various standard processes, viz., peroxide bleaching, reductive bleaching and fluorescent whitener application. Yellowness Index values were determined for these samples although in the case of such severe degradation, visual inspection revealed greater colour differences than were suggested by the analytical figures. It was found that untreated wool became the yellowest, and Blankit D bleached fabric yellowed the least, although the change in yellowness index was similar in each case. See Table 4.3.

A sample of persulphate oxidized wool was taken and reductively bleached, using Blankit D, under the conditions described in section 4.2.1. It was found that virtually all of the discolouration was removed, but reappeared upon further oxidation with persulphate. This suggested that the chemical modifications resulting in the overall discolouration were reversible under reducing conditions. It was likely that changes in colour of that severity would be due

to modification of tryptophan and tyrosine residues, these amino acids being responsible for much of the yellowing occurring under photooxidation conditions. Graham & Stathan [17] have reported a loss of up to 55% of tryptophan residues during persulphate oxidation, and this has been confirmed by Burke & Nicholls [58], who described tryptophan as the amino acid most readily degraded by persulphate. Substantial losses in tyrosine have also been observed [56], again up to approximately 55% destruction [55]. The mechanism of reaction, and the nature of the coloured degradation products remain obscure, although it has been suggested on several occasions that a radical mechanism is involved [26, 58]. Nicholls postulated the indole nucleus as the location of the free radicals generated [58], but no further suggestions have as yet been made.

Table 4.3 Oxidation of Wool with Ammonium Persulphate

WOOL SAMPLE	Y.I.	Y.I. After Persulphate Oxidation
1. Untreated Wool	24.5	54.9
2. Peroxide Bleached	13.3	53.6
3. Reductive Bleached	19.2	49.6
4. Fluorescently Whitened	2.5	50.2
5. Blankit D Irradiated	13.7	53.8
6. (i) Persulphate Oxidized (ii) Reductive Bleached	28.4	55.1
7. Persulphate Oxidized and Photoyellowed	57.9	-

It was likely that if tyrosine residues were degraded by the persulphate treatment, dityrosine residue would be oxidized as well. Particular interest was shown in these residues, since they have been shown to generate strongly yellow products during photooxidation (see Chapter 2).

As a qualitative method of confirmation that these aromatic amino acid residues generate coloured products when oxidized by persulphate, the reactions in aqueous solutions were examined. Dilution solutions (0.1%,

w/v) of N-acetyl tryptophan, tyrosine and biphenol (as a model for dityrosine) were added to 1% solutions of ammonium persulphate. All solutions were clear and colourless initially, but became dark brown within 5 minutes. Although providing little information about the reaction pathway, this clearly demonstrated that all three amino acid residues were involved in the yellowing of the wool under these conditions.

A sample of persulphate-yellowed wool was photoyellowed with 350nm ultraviolet light to assess the photostability of the wool subsequent to oxidation. It was very interesting to observe that the wool became very significantly more brown during the 24 hour irradiation period. This information was particularly noteworthy because wool which has been severely photoyellowed shows very little colour change after a further 24 hours irradiation. The implication was, therefore, that persulphate oxidation follows a different pathway to photooxidation, and that residual undegraded amino acid residues were still available for photochemical degradation.

This conclusion was supported by a further test. Wool which had been photostabilized by irradiation in Blankit D solution (as described in Chapter 2) was oxidized with persulphate using the method previously given. It was observed to yellow just as severely as untreated wool, even though its stability to photooxidation was markedly increased.

Wool oxidized by ammonium persulphate was also examined using fluorescence spectroscopy to determine the effect of the treatment upon the long wavelength emission, referred to earlier. Again, this is discussed in more detail in Chapter 6.

It was found that when samples of the treated wool were scanned using excitation wavelengths of 340nm, 360nm and 380nm, the emission at 430nm decreased in intensity by 80-85% over untreated wool. It was thus probable that the one or more species responsible for this long wavelength emission from wool are severely degraded by persulphate oxidation. Other suggestions may be that an inner filter effect could be operating, reabsorbing the emitted fluorescence before it was detected, or that little penetration by 350nm light occurs. In reality, both may well be true. If the assumption is correct that the species responsible for the fluorescence is destroyed

by persulphate, it is consistent with the possibility that dityrosine may be involved, although this has yet to be proved unequivocally.

4.2.4 Permonosulphuric Acid

Permonosulphuric acid, and its corresponding sodium and potassium salts, are used commercially as part of a shrinkproofing treatment, which, if carried out under acid conditions, is followed by a treatment with sodium sulphite. This confers a high degree of protection, with relatively little colour change.

Permonosulphuric acid is formed either by the action of hydrogen peroxide upon sulphuric acid, or by acidifying potassium permonosulphate with concentrated sulphuric acid. A sample of "Dylanize Salt" was obtained from Precision Process (Textiles) Limited, which was primarily potassium hydrogen permonosulphate ($KHSO_5$), also containing potassium bisulphate and potassium sulphate. 2g of this salt was dissolved in 990ml of water, and 10ml of concentrated sulphuric acid added giving an approximately 0.2% w/v solution of permonosulphuric acid. The acidity of this solution was pH1.5, and the reaction with wool carried out at room temperature. A wool sample (10g) was treated with 500ml of this solution in a Jeffreys dyemaster for 2 hours, (liquor:wool ratio 50:1). The wool was then thoroughly rinsed with cold water and dried in air.

It was found that the wool was yellowed by this process, although not as strongly as observed with the persulphate treatment. The colour of the wool was a pinkish-yellow, a colour often associated with tryptophan degradation products. This was not altogether unexpected, since previous workers have reported cystine, tryptophan and methionine as the main centres of attack, with some degradation to tyrosine residues **[48-50]**. Cystine and methionine oxidation products are nearly all colourless, and hence it is reasonable to assume that most of the coloured products observed arise from tryptophan residues.

Permonosulphuric acid is considerably less reactive towards wool than is persulphate, but as yet, mechanisms have only been proposed for the reactions with the disulphide cross-link in cystine residues. This is not

of major concern in the field of wool yellowing, because apart from some thiols, no coloured products of significance are formed from cystine degradation [83]. Wool which has been reduced and the resulting thiol groups carboxymethylated has not been found to be more phtostable than untreated wool [83]. The permonosulphate oxidized wool was found to "photoyellow" when irradiated subsequent to the chemical treatment. The results are listed below in Table 4.4, and show that this sample had yellowed in excess of what would normally be expected, considering its initial yellowness. Like the persulphate treated sample, this suggests that the permonosulphate oxidation occurs at a different site from the photooxidation process.

In summary, therefore, it has been shown that oxidation of wool with the reagents tested causes a variety of different reactions to occur, no two oxidizing agents causing the same effect. Only peroxide was found to whiten the wool, whilst permanganate caused slight yellowing, and persulphate and permonosulphate extensive discolouration. All treatments were found to decrease the photostability of the fibre, possibly due to the formation of intermediates in the tryptophan photodegradation pathway.

Table 4.4 The Action of Permonosulphate on Wool

WOOL SAMPLE	YELLOWNESS INDEX, Y.I.
Untreated Wool	25.1
Permonosulphate Oxidized Wool	40.2
Photoyellowed Persulphate Treated Wool	46.4

REFERENCES - Chapter 4

1) C.H.W.Hirs, Methods Enzymol., 1967, 11, 197

2) N.H.Leon, Textile Progress, 1975, 7, No.1, 1-81

3) "The Chemistry of Natural Protein Fibres", Chapter 5, Edited by R.S. Asquith. Pub. John Wiley & Sons Limited, London, 1977

4) N.P.Neumann, Methods Enzymol, 1972, 25, 393

5) J.Cegarra, J.Gacen and J.Ribe, J.S.D.C. 1968, 84, 457

6) A.L.Smith & M.Harris, Am.Dyest.Rep., 1936, 25, 180p

7) Milton Harris & Arthur Smith, Am.Dyest.Rep., 1936, 25, P542

8) R.Merkle, Textil Rund Schau, 1964, 19, 655

9) R.Merkle, Textil Rund Schau, 1965, 20, 51

10) P.Alexander, D.Carter & C.Earland, Biochem.J., 1950, 47, 251

11) J.Nachtigal & C.Robbins, Text.Res.J., 1970, 40, 454

11A) C.Robbins, J.Soc.Cosmet.Chem., 1971, 22, 795

12) H.Zahn, J.Soc.Cosmet.Chem., 1966, 17, 687

13) H.Zahn, Textilveredlung, 1982, 17, No.10, 421

14) E.Elöd, H.Nowotny and H.Zahn, Melliand Textilber, 1940, 21, 617

15) E.Elöd, H.Nowotny and H.Zahn, Melliand Textilber, 1942, 23, 313

16) A.S.Inglis & J.H.Leaver, Text.Res.J., 1967, 37, 995

17) D.R.Graham & K.W.Statham, Text.Res.J., 1962, 32, 908

18) P.Miro, Investigacion e Informasion Text., 1964, 7, 223

19) H.A.Rutherford & M.Harris, Am.Dyest.Rep., 1938, 27, P173

20) L.J.Wolfram, K.Hall & I.Hui, J.Soc.Cosmet.Chem., 1970, 21, 875

21) I.J.Kaplin & J.M.Marzinkowski, J.Text.Inst., 1983, 74, (3), 155

22) G.Laxer and C.S.Whewell, J.S.D.C., 1953, 69, 83

23) G.Laxer and C.S.Whewell, Chem. and Industr., 1954, 127

24) G.Laxer, J.Sikovski, C.S.Whewell & H.J.Woods, Biochim Biophys Acta. 1954, 15, 174

25) C.J.Delgado and E.Slobodian, Biochem. Biophys. Acta, 1972, 268. 121

26) M.Burke, P.Kenny & C.H.Nicholls, Nature, 1962, 196, 667

27) J.Cegarra, J.Gacen & M.Caro, J.S.D.C., 1978, 94, 85

28) M.J.Palin, D.C.Teasdale & L.Benisek, J.S.D.C., 1983, 99, 261

29) J.Cegarra & J.Gacen, Wool Science Review, 1983, No.59

30) L.Benisek & M.J.Palin, J.S.D.C., 1983, 99, 154

31) J.Cegarra, J.Gacen & M.Caro, J.Text.Inst., 1983, 74, 351

32) E.O.P. Thompson & I.J.O'Donnell, Aust.J.Biol.Sci., 1959, 12, 282

33) P.Alexander, M.Fox, & R.F.Hudson, Biochem.J., 1951, 49, 129

34) C.Earland & C.S.Knight, Biochim.Biophys.Acta, 1956, 22, 405

35) E.O.P. Thompson, Biochim.Biophys.Acta., 1954, 15, 440

36) B.J.Sweetman, J.Eager, J.A.Maclaren & W.E.Savige, IIIe Int.Wool Text. Res.Conf.Paris, 1965, 2, 85

37) I.J.O'Donnell & E.O.P. Thompson, Aust.J.Biol.Sci., 1959, 12, 294

38) V.G.Kulkarni, Text.Res.J., 1974, 44, 918

39) G.Valk, Proc.Int.Wool.Text.Res.Conf., Paris, 1965, II, 371

40) E.H.Hinton, Text.Res.J., 1974, 44, 233

41) C.Schirle, Bull.Inst.Text.France, 1953, 41, 21

42) C.Schirle, Bull.Inst.Text.France, 1953, 42, 43

42A) R.S.Candlin, Ph.D. Thesis, University of Leeds, 1965

43) A.Kantouch & A.Bendak, Text.Res.J., 1967, 37, 772

44) A.Kantouch & A.Bendak, Text.Res.J., 1969, **39, 851**

45) A.Kantouch & A.Bendak, Text.Res.J., 1969, 39, 858

46) R.C.Shah & R.S.Gandhi, Text.Res.J., 1968, 38, 1066

47) K.K.Juneja, A.D.Sule & H.R.Chipalkatti, Text.Res.J., 1965, 35, 950

48) B.J.Sweetman & J.A.Maclaren, Text.Res.J., 1965, 35, 315

49) J.A.Maclaren, W.E.Savige & B.T.Sweetman, Aust.J.Chem., 1965, 18, 1655

50) P.Alexander, R.F.Hudson & C.Earland, "Wool - Its Chemistry & Physics", Chapman & Hall, 2nd Ed., 1965, Page 270

51) C.S.Whewell, C.B.Stevens & S.C.Amin, Appl.Polym.Symp., 1971, 18, 387

52) Anon., Wool Sci.Review, 1967, 32, 1

53) V.A.Williams, Text.Res.J., 1964, 34, 79

54) F.M.Stevenson & M.C.Stevenson, J.Text.Inst., 1962, 53, P649

55) H.L.Needles, Text.Res.J., 1965, 35, 298

56) A.A.Kantouch, I.Rusznak & A.N.A.Bendak, Proc.Int.Wool.Text.Res. Conf., Paris, 1965, 2, 459

57) H.L.Needles, Text.Res.J., 1965, 35, 953

58) M.Burke & C.H.Nicholls, Text.Res.J., 1968, 38, 891

59) A.Kantouch & A.Bendak, Text.Res.J., 1967, 37, 483

60) M.M.Breuer & A.D.Jenkins, Proc.Int.Wool.Text.Res.Conf., Paris, 1965, 2, 447

61) F.R.Andrews, Ph.D. Thesis, University of Sussex, 1969

62) P.Alexander, R.F.Hudson & M.Fox, Biochem.J., 1950, 46, 27

63) J.E.Kearns, D.J.Kilpatrick and J.A.Maclaren, unpublished results.

64) C.Earland & D.J.Raven, Biochem,Biophys.Acta., 1958, 27, 41

65) A.Kantouch, A.Bendak & M.Sadek, Text.Res.J., 1978, 48, 619

66) J.R.McPhee, Text.Res.J., 1960, 30, 358

67) V.A.Williams, Text.Res.J., 1962, 32, 977

68) M.A.Andrews, A.S.Inglis, F.E.Rotheray & V.A.Williams, Text.Res.J., 1963, 33, 705

69) J.R.McPhee, Text.Res.J., 1964, 34, 1111

70) C.Earland, Text.Res.J., 1964, 34, 177

71) M.W.Andrews, Text.Res.J., 1965, 35, 676

72) K.R.Makinson, Text.Res.J., 1969, 39, 1072

73) D.H.Tester & K.R.Makinson, Text.Res.J., 1982, 52, 227

74) P.Alexander, D.Carter & R.F.Hudson, J.S.D.C., 1949, 65, 152

75) Anon, Wool Sci.Rev., 1966, 30, 16

76) Anon, Wool Sci.Rev., 1967, 31, 1

77) W.J.Thorsen & R.Y.Kodami, Text.Res.J., 1967, 37, 975

78) J.A.Maclaren & A.Kirkpatrick, J.S.D.C., 1968, 84, 564

79) H.H.Steiss & J.Guarnascio, Text.Res.J., 1959, 29, 492

80) R.C.Shah & R.S.Gandhi, Text.Res.J., 1968, 38, 874

81) D.Klee, P.Lerschmacher & H.Zahn, Textil Praxis Int., 1984, 23

82) R.S.Gandhi & R.C.Shah, Text.Res.J., 1969, 39, 1088

83) A.Kirkpatrick & J.A.Maclaren, Text.Res.J., 1964, 34, 1082

CHAPTER 5

Attempts to Develop a Photostable White Wool

CHAPTER 5

5.1 Attempts to Develop a Photostable FWA for Wool

5.1.1 Introduction

It is well known that when wool is whitened by the application of a fluorescent whitening agent, the stability to photooxidation is dramatically reduced [1]. Maximum yellowing occurs during irradiation with those wavelengths which excite the whitener to maximum fluorescence (generally 350-400nm), rather than with the shorter wavelengths which promote maximum yellowing of unwhitened wool. However, the decomposition of the whitener is believed to play only a small part in the photoyellowing of fluorescently whitened wool [2]. This is substantiated by the observations that whitened wool yellows at much the same rate, regardless of the stability of the applied whitening agent [3]. It has also been found that the sensitization of wool yellowing by an FWA is very pronounced in the presence of water. Extensive yellowing may occur, even though most of the whitener remains unchanged [4].

Much work has been done to elucidate details of the mechanism for the sensitization of wool yellowing by FWA's, [5] but although many proposals have been made, no real solution to the problem has been found.

It would seem reasonable to categorize the potential approaches to the problem as follows:-

1/ To develop a new, stable FWA which does not sensitize wool yellowing;
2/ To chemically modify the wool to make it less susceptible to sensitization by the brightener, or to inhibit the natural yellowing pathways;
3/ To isolate the FWA from the wool, eg. by application in a polymer film; and
4/ To screen the wool from certain harmful UV wavelengths.

As may be imagined, there are problems with each of these approaches. In the first case, it is quite possible that any FWA which absorbs in the near UV region (necessary to achieve the required blue fluorescence) will sensitize the wool to yellowing, regardless of its own photostability. However, this is by no means certain, and there are many classes of fluorescent compounds which may yet be of use as fluorescent whitening agents.

In the second instance, the obvious problem is that the yellowing process almost certainly includes a multitude of different reactions of many amino acids to varying degrees, and the likelihood of finding a process which successfully affects a sufficient number of these reactions to improve the photostability of the wool is not great. Many reagents have been tested in this context already, but only a few gave even a slight improvement. For example, Holt et.al. [2] tested a number of reducing agents with reasonable success, but observed no lasting protection to the wool. Others discovered the thiourea/formaldehyde process [6,7], which offered some protection though this was not durable to washing. Fruen and Inglis tried the inclusion of aluminium sulphate and sodium formaldehyde sulphinate with the FWA application and again slightly improved the whiteness of the wool [8,9].

A blanket testing of 270 varied reagents was carried out by Kirkpatrick & Maclaren [10]. After photoyellowing, only 16 showed even marginal inhibition of yellowing. A wide range of sulphur-containing compounds were applied to fluorescently whitened wool by Tucker [11] in search of a protective effect. The most effective were thiols and sulphides which contain a free carboxyl group, the best being thioglycollic acid, 2-mercapto-propionic acid, S-ethylthioglycollic acid and N-acetyl cysteine. Compounds containing sulphur in higher states of oxidation were much less effective. A range of triazine thiols were synthesized and evaluated as potential photoprotective agents by Karen Röper [12], but unfortunately, none of those tested showed any effect as a photoprotective agent. When applied in higher concentrations, the wool was actually yellowed by the process.

The third approach involved the separation of the FWA from the wool by incorporation into a polymer film. The potential problem with this process is that it may well be that the fluorescence of the FWA could be adversely affected by the polymer, and that the required whiteness could not then be achieved. It does have the advantage, however, that the FWA is confined to the surface of the fibre, where it has maximum effect. A number of polymers have been found to be suitable for this treatment, and many FWA/Polymer combinations have been evaluated [13,16] and found to lead to good whitening, though few provide a satisfactory stability to light, and few are as white as could be obtained by conventional methods of application. A further problem encountered was the harsh

handle conferred on the wool by the polymer, and thus, combined with the limited level of success in improving the photostability of the fabric, has damped the commercial interest in the treatment.

The last category was the protection of wool by the applications of U.V. screens and related materials. These were discussed in Chapter 1 and are the least likely to produce an answer to the photoinstability of fluorescently whitened wool, since the U.V. light is necessary for the FWA to function on the wool.

The work described in this section falls into the first group of approaches to a solution of the problem, the development and application of a photostable FWA to wool. Commercially available FWA's were used, which were known to have a very high light stability but were normally used only for application to other types of fibre, such as polyester.

5.1.2 The Evaluation of Dispersed FWA's for Wool

It may be observed from certain manufacturers technical data sheets that the photostability of dispersed FWA's used for the whitening of polyester, polypropylene and polyvinyl chloride fibres, was very much greater than that of the FWA's commonly used for wool. Dispersed FWA's are so-called because of their insolubilities in water, which necessitates the production of fine dispersions prior to application. Using the standard lightfastness scale, where 1 represents very poor photostability and 8, excellent stability, Uvitex NFW (Ciba Geigy), has a value of 2 when applied to wool. However, a typical dispersed FWA such as Uvitex EBF (Ciba Geigy), has a value of 7-8 when applied to Polyester. Uvitex ERN-P has a lightfastness of 7 on Polyester, while Uvitex EN has a value of 6. Thus it is clear that when these FWA's are used as designed, with man made fibres, a very stable white fabric results.

Not a great deal is known about the structure of these FWA's, but Uvitex EBF and ERN-P are benzoxazole derivatives, while Uvitex EN is a styryl-benzoxazole. Other dispersed whitening agents are available from other manufacturers, such as Blankophor DCR (Bayer), which is a pyrazoline derivative, and Hostalux ETR (Hoechst). These are not claimed to have quite such a high lightfastness, and so were not evaluated in this study.

These FWA's are applied to man-made fibres by exhaustion methods from aqueous dispersions or by incorporation via the thermasol process at temperatures up to 200°C. The brightener may thus be encapsulated by the fibre, giving an excellent washfastness. Clearly the high temperature applications could not be used with wool, because extensive thermal degradation would occur. Attempts were therefore made to apply them via exhaustion processes from an Aqueous dispersion.

5.1.3 Application of FWA's by Exhaustion

The one method of application of dispersed FWA's which was described in the manufacturers technical data sheets as being applicable to all fabrics was that of exhaustion. This involved the preparation of a solution containing a predetermined quantity of FWA, which was subsequently discharged onto the wool surface at an elevated temperature.

The three dispersed FWA's used were claimed by the manufacturer, Ciba Geigy, to be best applied to Polyester from weakly acid, neutral or alkaline baths, giving optimum whiteness under high temperature conditions. For wool, the alkaline conditions and high temperature were obviously disadvantageous, so the exhaustion was carried out in the presence of citric acid at 80°C.

A 1% v/v dispersion of the concentrated FWA was prepared in warm water (45°C), stirring thoroughly. Wool samples (10g) were cut from a roll of salts serge, and solutions were made up as follows:-

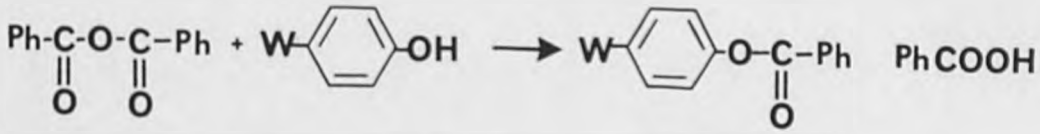
Citric Acid, 2% solution 5ml
FWA, 1% dispersion, 10ml
Solution made up to 200ml and a few drops of Lissapol N added a wetting agent for the wool

The FWA's used were Uvitex ERN-P, Uvitex EN and Uvitex EBF, described earlier. Each solution was heated to 80°C in a Jeffreys Dyemaster, and the wool agitated at 60 rpm for 60 minutes. Each sample was then rinsed in running cold water and dried in air.

It was found that none of the samples was any whiter as a result of the treatment, but had yellowed by 1-2 units on the Yellowness Index scale. The conclusion was thus drawn that under these conditions, the FWA had little or no affinity for wool and that an alternative application method was required.

5.1.4 Benzoylation of Wool with Benzoic Anhydride

To increase the hydrophobicity of wool, with the intention of increasing the affinity for dispersed FWA's, benzoic anhydride was applied [17,18] Benzoic anhydride acylates primary and secondary amino groups in wool, such as those in lysyl or arginyl residues, and hydroxyl groups, such as those in tyrosyl or seryl residues.



The reaction can only ever be 50% efficient, because half of the molecule is always converted to benzoic acid, which is removed by subsequent rinsing in alkaline solution. Dimethyl formamide and dimethyl sulphate have been found to be excellent solvents for the acylation of wool with anhydrides, because they permit a reasonable rate of acylation at temperatures

as low as 60-60°C with very little discolouration [19,20]. At low levels of acylation, where N-acylation is predominant, the result is a decrease in the internal pH of the fibre by the conversion of cationic $-NH_3^+$ groups into neutral benzoylamino groups. However, treatment with benzoic anhydride usually results in O-acylation as well as N-acylation, and a marked increase in the hydrophobicity of the wool fibre [21].

Wool samples were treated with benzoic anhydride using the following method, based on that given by Lewis et.al. [17].

1/ Wool samples of approximately 10g were accurately weighed.
2/ Wool samples were pre-dried at 100°C for 30 minutes, to prevent reaction of water with benzoic anhydride.
3/ A 10% w/w solution of benzoic anhydride (Aldrich, 98% pure) was prepared in DMF.
4/ The liquor : Wool ratio was 30:1, so for a 10g wool sample, 300ml of benzoic anhydride solution was used.
5/ 10g samples of salts serge from Salts of Saltaire, Bradford, were treated in a Jeffreys dyemaster for 4 hours at 70°C.
6/ Treated samples were washed thoroughly in 10% w/v sodium carbonate to neutralize the residual benzoic acid.
7/ The wool was rinsed well under running tap water and dried in air at room temperature.
8/ When dry, the wool was reweighed, and the weight gain used to assess the extent of reaction.

This process was repeated on several occasions, and weight gains of 16-20% were experienced. These compare favourably with those of ca.14% observed by Lewis & Pailthorpe, and may be due to the higher liquor to wool ratio used.

The benzoylation process was found to yellow wool slightly, and it was thus necessary to bleach the wool following the treatment. Even this failed to recover the whiteness of untreated wool, as is shown in Table 5.1.

Table 5.1 The Effect of Benzoylation upon Wool

Wool Samples	Yellowness Index
Untreated Wool	29.5
Benzoylated Wool	37.6
Benzoylated Wool Bleached with Peroxide	33.5
Benzoylated Wool Bleached with Blankit D/hν	30.3

This yellowing was clearly a severe disadvantage, but it was considered worthwhile to continue and test the treated fabric. Lewis & Pailthorpe had tested benzoylated wool for its affinity for dispersed dyes, and found that the colour yield was directly proportional to the degree of benzoylation (as measured by weight gain) [17]. They observed that a weight gain of at least 13% was required to give adequate colour yields, and calculated that this corresponded to 1238μ mol of benzoyl groups per gram of wool.

5.1.5 Application of Dispersed FWA to Benzoylated Wool

To the 10g samples of benzoylated wool, Uvitex ERN-P was applied, using the same method as described for untreated wool. 1% owf of FWA was applied by the exhaustion process, and the samples rinsed and dried at room temperature. Along with benzoylated wool, two samples were included which had been bleached subsequent to the application of the benzoic anhydride. Each piece of wool was then photoyellowed for 24 hours.

Yellowness Index measurements for each sample were recorded, and these are listed in Table 5.2.

The results given in Table 5.2 are somewhat curious. It is clear that the application of benzoic anhydride had increased the affinity of the wool for the dispersed FWA, as anticipated. Yet for some reason,

the wool samples which had been bleached as an intermediate process had been markedly yellowed by the application of the FWA. The reason for this is not clear, although the results were confirmed by a repeat experiment. However, the most important conclusion from this work is that even with a high concentration of benzoic anhydride applied to the fabric, the whiteness obtained with the dispersed FWA is very poor compared to the brilliant whites achievable with the reactive brighteners such as Uvitex NFW or Uvitex CF. Further, it was noted that even with a low percentage of dispersed whitener applied, the lightfastness was vastly inferior to the levels obtained when the FWA was exhausted onto synthetic fibres. The conclusion is thus drawn that dispersed FWA's do not offer anything to the process of whitening wool.

Table 5.2 The Effect of a Dispersed FWA on Benzoylated Wool

Wool Sample	Y.I.	Y.I. After Application of Uvitex ERN-P	Y.I. After Photoyellowing
Untreated Wool	29.5	31.4	34.8
Benzoylated Wool	37.6	24.6	32.2
Benzoylated Wool Bleached with Peroxide	33.5	37.2	38.5
Benzoylated Wool Bleached with Blankit D/ hν	30.3	40.4	41.0

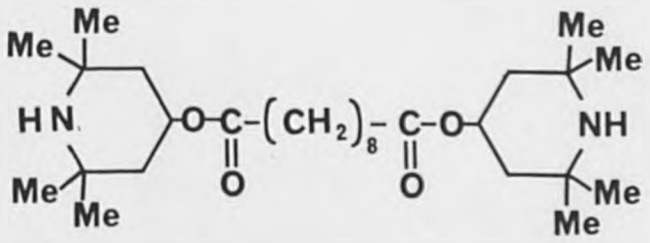
5.2 The Application of a U.V. Stabilizer

5.2.1 Introduction

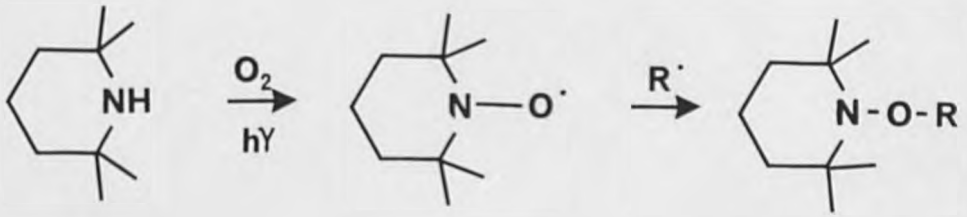
Hindered amine compounds derived from piperidine are a relatively new addition to the range of commercial ultraviolet stabilizers for bulk and fibrous polymers, such as polythene or polypropylene. In comparison with other types of stabilizer, the hindered piperidines have proved to be by far the most effective systems [22]. Of these, the commercially

used derivative shown below has attracted widespread interest. It is known as "Tinuvin 770".

"Tinuvin 770"



It has been suggested [22] that the hindered piperdines do not operate by mechanisms of optical screening or excited state quenching. Essentially, their stabilizing effectiveness depends upon their ability to form a stable nitroxyl radical, which then scavenges any alkyl radicals [R•] produced during photooxidation.



In polyolefins they also inhibit the photo-reactions of carbonyl chromophores, and react with hydroperoxides.

The theories of photoyellowing have been described in Chapter 1, and it seems likely that a singlet oxygen mechanism is involved. However, it is not possible to totally discount the participation of radicals, and for this reason, the effect of Tinuvin 770 upon wool was studied.

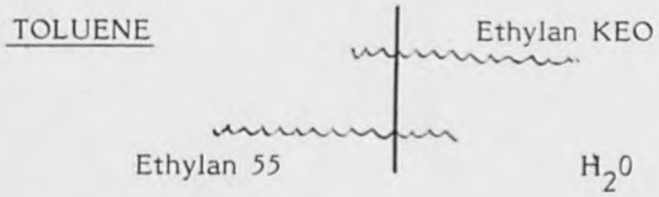
5.2.2 Application of Tinuvin 770 to Wool

Tinuvin 770 was virtually insoluble in water, and for this reason could not be applied from a dyebath. The problem was overcome by exhausting a solution in toluene onto the wool from an emulsion.

The emulsifier system chosen was as follows:-

20% w/v Tinuvin
3% w/v Ethylan KEO (Diamond Shamrock)
3% w/v Ethylan 55 (Diamond Shamrock)
2% w/v Arylan CA (Diamond Shamrock)
Made up to 100% in toluene.

Ethylan KEO and Ethylan 55 were short chain ethylene oxide moieties. Ethylan KEO was slightly more soluble in water than toluene, and Ethylan 55 slightly more soluble in toluene than water. Arylan CA was believed to be calcium dodecylbenzene sulphonate.



Once the emulsifiable concentrate had been prepared, as above, the mass of Tinuvin required to give the desired concentration on the wool was calculated, and from this, the volume of concentrate required. This was then made up to the desired volume in water. Tinuvin concentrations of 5 and 10% owf were applied, using a liquor to wool ration of 30:1.

On addition of the emulsifiable concentrate to water, it was possible to obtain two phases, or a stable emulsion, or anything in between. The stability of the emulsion was critical, and could be controlled by adjusting the Arylan CA concentrations. Arylan CA is an anionic surfactant, which is very much more soluble in toluene than in water. Droplets of toluene thus acquire negative charges and repel each other, thereby favouring an emulsion rather than two phases. Increasing the concentration of Arylan

CA increases the emulsion stability. However, if the emulsion was too stable it would not exhaust onto wool, and for this work a 2% Arylan CA concentration was found to be satisfactory.

Initially, Tinuvin 770 was applied at a level of 5% owf, from the emulsion at the boil for 30 minutes. Three tubes were used in a Jeffreys dyemaster, applying the stabilizer to untreated, bleached and fluorescently whitened wool. The results are shown in Table 5.3, from which it may be seen that the wool was slightly yellowed by the process. Small improvements in the photostability of the wool were observed compared to unstabilized samples, but these differences may only be the result of the poorer initial whiteness of the fabric.

Table 5.3 Wool Samples Treated with Tinuvin 770

Wool Sample	Tinuvin Concentration (%)	Initial Y.I. (1)	Y.I.After Treatment (2)	Y.I.After Photoyellowing (3)	ΔY.I. (3)-(2)
Untreated Wool	5	27.5	33.2	36.1	2.9
Blank	N/A	27.6	N/A	33.1	5.5
Bleached Wool	5	16.2	21.4	31.5	10.1
Bleached Blank	N/A	16.2	N/A	28.4	12.2
Fluorescently Whitened	5	8.2	18.1	45.6	27.5
FWA Blank	N/A	8.2	N/A	40.9	32.7
Untreated Wool	10	27.6	29.2	32.4	3.2
Blank	N/A	27.6	N/A	33.2	5.6
Bleached Wool	10	16.3	17.8	28.0	10.2
Bleached Blank	N/A	16.3	N/A	28.4	12.1
Fluorescently Whitened	10	8.2	10.2	39.4	29.2
FWA Blank	N/A	8.1	N/A	41.7	33.6

To improve the protective effect, the concentration of Tinuvin was doubled to 10% owf, and at the same time, the emulsion was applied at 80% for 60 minutes, rather than at the boil. Following the treatment, the wool samples were again dried in air at room temperature. They were then photoyellowed for 24 hours alongside untreated blank samples for comparison. The results are given in the lower half of Table 5.3, from which it is clear that the milder treatment conditions certainly reduced the yellowing conferred by the treatment, but did not eliminate it completely. The photostability of the resultant samples was again slightly better as a result of the stabilizer application, but as before, the difference was not of great consequence because of the initial yellowness of the treated fabric. After photoyellowing the yellowness indices show that both treated and untreated wool were approximately the same colour! Thus it was concluded that although some protection was probably conferred upon wool by the application of Tinuvin 770, the improvement was at best modest, and of little practical value because of the concurrent yellowing of the wool by the initial stabilizer application.

5.3 Application of a Singlet Oxygen Quencher

5.3.1 Introduction

If wool degradation occurs via a singlet oxygen mechanism, it would seem logical to stabilize photoyellowing by the application of a singlet oxygen quencher. It has been reported [23] that such an approach has already been tried in an attempt to improve the photostability of jute. Two singlet oxygen quenchers, 1,4-diazabicyclo (2,2,2) octane (DABCO) and β-carotene, were applied by padding to bleached jute fabric from solutions in water and toluene respectively. After drying at room temperature. Abdullah et.al. [23] exposed treated and untreated samples to U.V. light for various lengths of time and measured the extent of photoyellowing. They found that the colour change on exposure of the fabric treated with DABCO was essentially identical to that of untreated jute, and no stabilizing effect could be detected. The colour changes observed for the β-carotene treated jute were more complex, since the fabric was given a yellow colour which was then found to be unstable in UV light. However, the end result was that again, no evidence of singlet oxygen quenching was observed.

For the purposes of stabilizing the photodiscolourations of white wool, the use of an orange pigment clearly undesireable! So in this study, only DABCO was considered. No reference could be found to this material having been applied to white wool, so the potential of a singlet oxygen quencher as a potential stabilizer was evaluated.

5.3.2 Application of DABCO to Wool

A 3% w/v solution of DABCO was prepared by dissolving 3g in 100ml Ethanol. This was applied to samples (10g) of untreated salts serge and fluorescently whitened wool by the pad/dry method, achieving 10 % pick up;ie. 10g wool absorbed 10g of solution, thus obtaining a 3% owf application of DABCO. The samples were then dried in air at room temperature, and photoyellowed alongside untreated blank samples. The results are given below in Table 5.4.

Table 5.4 The Effect of DABCO Upon Wool

Wool Sample	Initial Y.I. (1)	Y.I.After Treatment (2)	Y.I.After Photoyellowing (3)	Δ Y.I. (3)-(2)
Salts Serge	28.4	N/A	31.3	2.9
Salts Serge + DABCO	28.4	27.9	31.2	3.3
FWA Treated Wool	4.3	N/A	37.6	33.3
FWA Wool + DABCO	4.3	7.1	38.5	34.2

It may be observed from those results that no stabilization of wool against photoyellowing was achieved with DABCO. The reason for this is not clear, though it is possible that the majority of tryptophan residues in wool are so located as to be inaccessible to the quencher molecules applied in this way.

5.4 Some Investigations of the Processes Occurring During Yellowing

5.4.1 Introduction

It has been observed that no amino acid has a significant U.V. absorption above 300nm, and that very little light below 300nm reaches the earth; and yet amino acids are photodegraded by sunlight. The experiments described in this section were undertaken to gain a further insight into the processes which take place during the irradiation of wool by sunlight.

5.4.2 Coating of Wool with Free Amino Acids

It is well known that in solution it is tryptophan which undergoes the most extensive discolouration and degradation during irradiation with 300nm light. To establish the effect of light upon the different amino acids in wool, free amino acid solutions were applied to the surface of wool by the pad/dry method. The particular amino acids chosen were those known to degrade during photoyellowing from amino acid analysis results.

Solutions of the individual compounds (2% w/v) were prepared in 0.880 ammonia, for solubility reasons. These were padded onto wool, with 100% pick up. The amino acids used were phenylalanine, methionine, leucine, tryptophan, arginine, histidine, cystine, cysteine, proline, tyrosine, isoleucine and threonine. Following the pad/dry process, the treated wool samples were irradiated in individual trays under water with 350nm light for 24 hours. None of the amino acids absorbed 350nm light, but the light source was not monochromatic, and some shorter wavelengths were also present. Unlike amino acid analysis, which monitors the degradation of the individual amino acids, this experiment made it possible to determine which species caused the most extensive yellowing of wool. The results are shown in table 5.5.

Table 5.5 The Treatment of Wool with Free Amino Acids

Amino Acid Used	Y.I.	Y.I. After Photoyellowing	Δ Y.I.
Phe	20.8	27.4	6.6
Met	19.7	24.3	4.6
Leu	19.9	29.5	9.6
Trp	20.9	45.0	24.1
Arg	20.8	28.0	7.2
His	20.5	25.4	4.9
CySS	19.4	27.4	8.0
CySH	20.1	25.7	5.6
Pro	20.4	28.8	8.4
Tyr	19.6	27.3	7.7
Iso	20.7	29.4	8.7
Thr	20.8	24.1	3.3
Blank	23.7	28.9	5.2

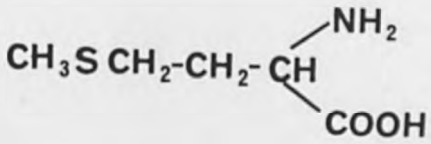
The most marked result is that for tryptophan. This is not unexpected because tryptophan has the highest UV absorption wavelength of all the amino acids in wool (280nm), and is known to produce far more coloured products. It is interesting to note, however, that tyrosine, which has a UV absorption maximum at 274nm, showed no marked sensitization of the wool to yellowing. In Chapter 1, the possibility of tyrosine absorbing energy and transferring it to tryptophan was discussed, yet it showed no evidence of this here. It is probable that tryptophan and tyrosine need to be close, if not adjacent, in the peptide chain for this to occur.

Another interesting observation was that methionine and threonine appear to have actually stabilized the wool to photoyellowing. That they themselves should not degrade is not suprising, since their UV absorption maxima are both below 200nm, and it may be that they protected the wool by physically screening it against the irradiation. However, the stabilizing influence was considered to be worth investigating.

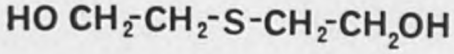
5.4.3 Irradiation of Wool in Thiodiglycol Solution

The poor solubility of methionine made it desireable to find a similar compound with a higher water solubility, such that wool could be irradiated in a solution of that material, and its effect upon yellowing monitored.

Thiodiglycol was chosen as a chemically similar structure, with good water solubility:



Methionine



Thiodiglycol

By irradiating wool under a solution of thiodiglycol, it would be possible to determine whether there was indeed a chemical stabilization occurring, or whether the result obtained with methionine was purely due to physical screening.

A 3% aqueous thiodiglycol solution was prepared, and under this were irradiated samples of untreated wool for 24 hours. After this period, the wool was removed, rinsed and dried in air. It was found that the wool had yellowed just as much as a control sample irradiated in distilled water.

The conclusion was thus reached that thiodiglycol offered no stabilization at all to wool, and if methionine acted in a similar way, it was probable that its protection in the solid phase was purely due to physical screening.

5.4.4 The Application of Two Free Amino Acids to Wool

The possibility existed that when two amino acids occurred together in the peptide chain of wool, the may exert a synergistic effect on the promotion of wool photoyellowing. If true, then it was likely that it would be tryptophan and one other amino acid, so these possibilities were investigated first.

Solutions of amino acids were again made up in 0.880 Ammonia, this time containing 1.5% tryptophan and 1.5% of the other amino acid. These

solutions were applied to wool by the pad/dry method, and dried in air at room temperature. Each wool sample was then irradiated with 350nm UV light for 24 hours. The yellowness index results for each sample are listed in Table 5.6.

Table 5.6 Treatment of Wool with Amino Acid Mixtures

Amino Acids Used	Y.I.	Y.I. After Photoyellowing	Δ Y.I.
Trp + Thr	21.4	46.9	25.5
Trp + Phe	21.5	52.0	30.5
Trp + CySS	19.1	44.2	25.1
Trp + CySH	19.8	45.0	25.2
Trp + Tyr	20.2	46.8	26.6
Trp + Met	20.5	51.2	30.7
Trp + Leu	21.5	49.0	27.5
Trp + His	22.1	53.7	31.6
Trp + Iso	22.3	46.6	24.3

Again it was found that the expected synergistic effect of tryptophan and tyrosine was not observed. Surprisingly, it was methionine, phenylalanine and histidine which produced the greatest discolouration. These three samples were visibly browner than all the others. Histidine and phenylalanine have been observed to be degraded during photoyellowing (by amino acid analysis) and yet they showed little discolouration when applied to wool alone. It may be that they, or their degradation products sensitize the photodecomposition of tryptophan residues. However, the involvement of methionine is unclear.

Much speculation has been made concerning the participation of cystine in the photoyellowing process. For this reason a series of amino acids were applied to wool with cystine using the same method as described for the tryptophan mixtures. The high level of yellowing obtained with tryptophan was not expected, but the application of cystine alone had caused one of the higher changes in yellowness after irradiation. Each of the treated wool samples were irradiated as before, and the results listed in Table 5.7.

These results again showed some variations, and it was the unexpected amino acids which caused the most and the least yellowing. Apart from tyrptophan, it was found to be arginine which gave the most extensive yellowing when applied with cystine. Both arginine and cystine accelerated the rate of yellowing when applied individually to wool, but neither are known to produce coloured degradation products when irradiated at 350nm. It was therefore concluded that these two amino acids together stimulated the yellowing of the wool, rather than generated the discolouration by their own decomposition.

The smallest amount of yellowing was found to arise from the combination of phenylalamine and cystine. This was most surprising because both amino acids caused a higher level of yellowing than the control sample, when applied individually to wool. The explanation for this observation is not known.

Table 5.7 Application of Cystine to Wool in Conjunction with other Free Amino Acids

Amino Acids Used	Y.I.	Y.I. After Photoyellowing	Δ Y.I.
CySS + Leu	20.3	26.7	6.4
CySS + Tyr	22.2	32.6	10.4
CySS + Iso	20.2	29.8	9.6
CySS + Phe	20.8	25.2	4.4
CySS + Arg	21.1	35.6	14.5
CySS + Thr	20.2	30.2	10.0
CySS + Met	19.6	26.5	6.9
CySS + His	20.4	26.0	5.6
CySS + Pro	19.3	26.1	6.8
CySS + Trp (from previous expt.)	19.1	44.2	25.1
CySS alone (from previous expt.)	19.4	27.4	8.0

5.4.5 The Photoyellowing of Wool Using Light with no Component Below 335nm

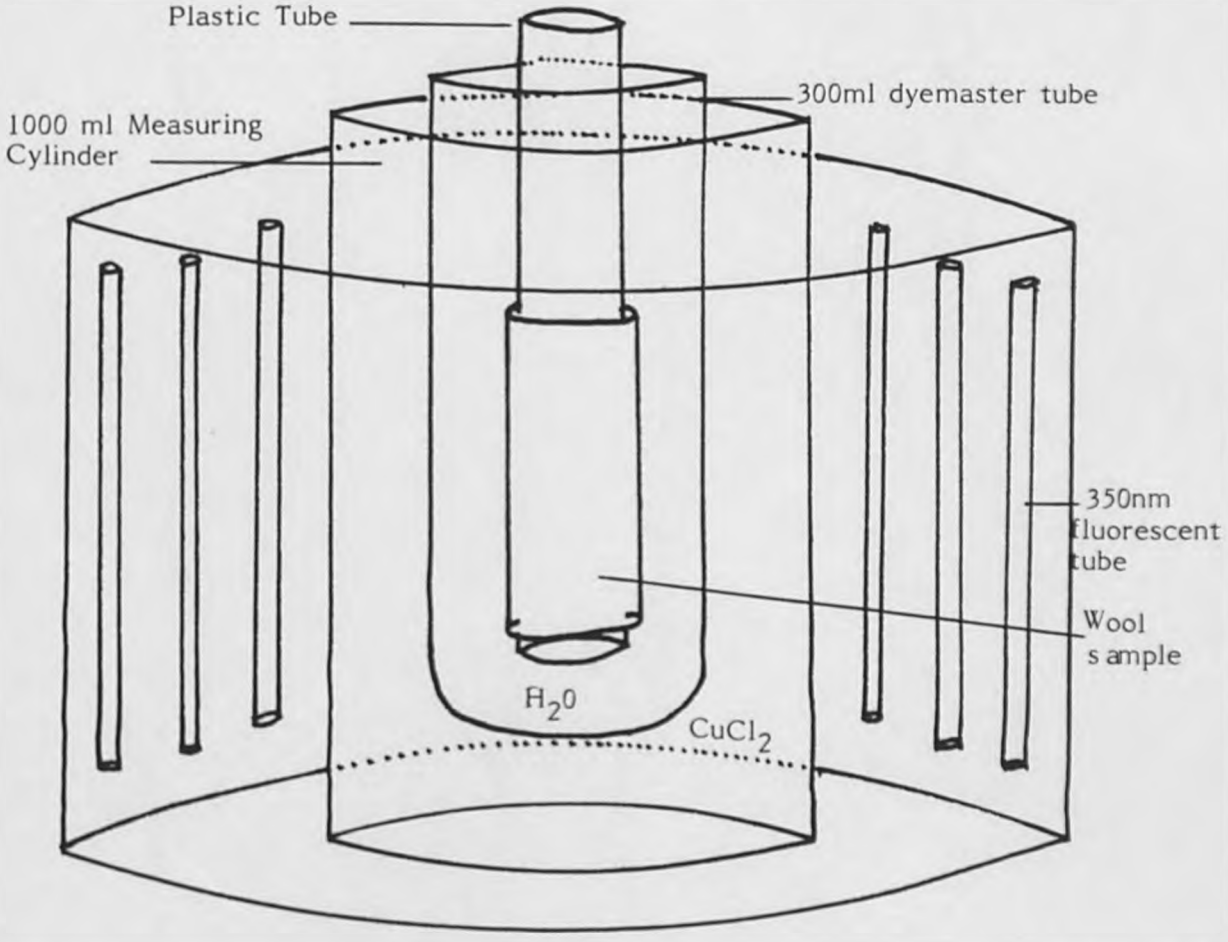
The irradiation sources used for wool photoyellowing did not have a sharp UV cut-off at 350nm, but emitted light at much lower wavelengths, albeit with a very low intensity. To prove that wool was yellowed by 350nm light, the component below 335nm was removed by filtering the radiation

through 0.1M copper chloride solution ($CuCl_2$). This solution had a UV cut-off at 335nm. It was not practical to immerse wool in the solution, because the blue colour would influence all subsequent yellowness measurements. To overcome this, a new design of photochemical reactor was employed.

In Chapter 3, a reactor design was described which consisted of a vertical aluminium cylinder, around the inside of which were positioned a circular array of 16x8W fluorescent tubes. These served as a light source with a λmax of 350nm, and were used for this experiment.

Inside this cylinder was a 1000ml measuring cylinder, centrally positioned and filled with the copper chloride solution. Within this cylinder was suspended a 300ml tube from a Jeffreys dyemaster, containing distilled water, and inside this was a plastic tube, around the outside of which the wool sample was mounted, as shown below in Figure 5.1.

Fig 5.1 The Irradiation of Wool with Light Filtered Through Copper Chloride Solution



Thus the wool sample was maintained in the wet state and irradiated with light containing no wavelength components under 335nm or over 670nm.

A sample of salts serge was irradiated under these conditions for 46 hours, and the change in yellowness index measured. Fluorescently whitened wool was not tested because the FWA would absorb strongly the filtered light. As a control, a sample of untreated wool was irradiated in this apparatus for the same time period without the presence of the copper chloride, and the change in yellowness measured. The longer time periods were necessary because some of the shorter wavelengths would have been absorbed by the glass tubes, which would also have resulted in light scattering, decreasing the intensity of the light incident upon the wool.

The results for this experiment are presented in Table 5.8.

Table 5.8 The Effect of Irradiating Wool with Light Filtered Through Copper Chloride Solution

Wool Sample	Initial Y.I.	Y.I. After Irradiation	Δ Y.I.
Untreated Serge irradiated with filtered light	32.0	36.8	4.8
Untreated wool, irradiated through glass only	32.0	38.1	6.1

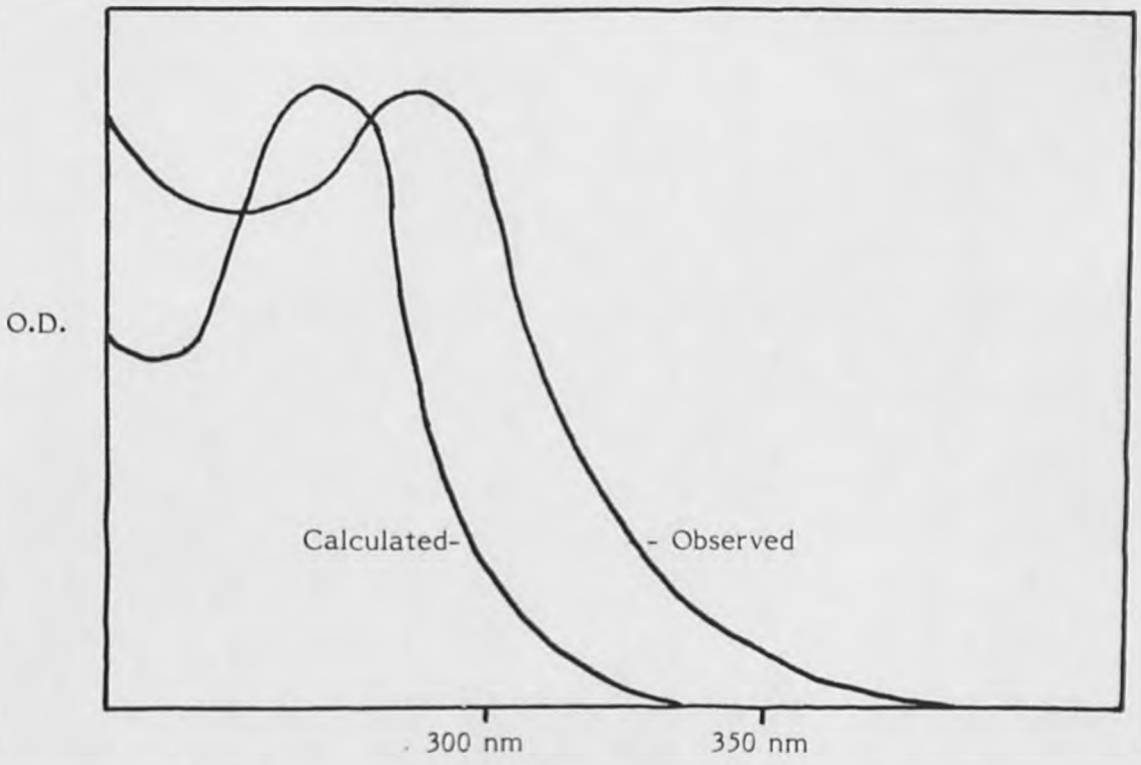
The magnitude of these yellowing results is of little significance when compared to previous values because the method of irradiation was completely different and the wool was treated under glass. However, the principle was established that the component in wool which absorbs at longer wavelengths in the U.V. spectrum is definitely involved in photoyellowing, such that when irradiated with wavelengths absorbed only by this species, yellowing still occurs. It may be that this component of wool is degraded itself to form yellow products, or it may only absorb energy and transfer it to other species in the wool, such as tryptophan.

5.5 Investigation of the Long Wavelength Fluorescence Emission of Wool

5.5.1 Introduction

It has been established [24-28] for a number of years that wool contains one or more unknown species which absorb ultraviolet light at around 350nm and fluoresce in the blue region of the visible spectrum. Nicholls & Pailthorpe [25] measured the UV spectrum of wool and compared it with the theoretical spectrum calculated from the amino acid composition, as reproduced below in Fig 5.2.

Fig 5.2 The Calculated & Observed UV Spectra of Wool



The experiment reported in section 5.4.5. showed clearly that wool is yellowed by light having no component below 335nm, and it therefore

is likely that this species is involved in the photoyellowing process.

Various explanations for this absorbing moity have been proposed. Dunlop et.al. [29] and Bendit [30] have both suggested that it is a degradation product of the wool keratin, resulting from weathering or sunlight damage prior to the wool reaching the laboratory. Whilst possible, this would seem unlikely since the fibre tip is all that is really exposed to sunlight during growth on the sheep, and the fibre tips are discarded when the wool is processed. Another possible explanation was the existence of pigment precursors in the wool [31]. Crossweiner studied the luminescence of protein from the eye [27] and observed a similar long wavelength fluorescence, which he attributed to the presence of N-formyl kynurenine.

The reason for the interest in the luminescent species in this work arose from the reports that dityrosine is present in the cuticle of wool, and absorbs in this region of the spectrum. The UV spectrum of dityrosine shows a marked pH and solvent dependence, and its contribution to the spectrum of wool is thus unclear. However, some contribution to the observed luminescence was virtually inevitable, and hence it was necessary to study the effect of photoyellowing upon this abosrbance.

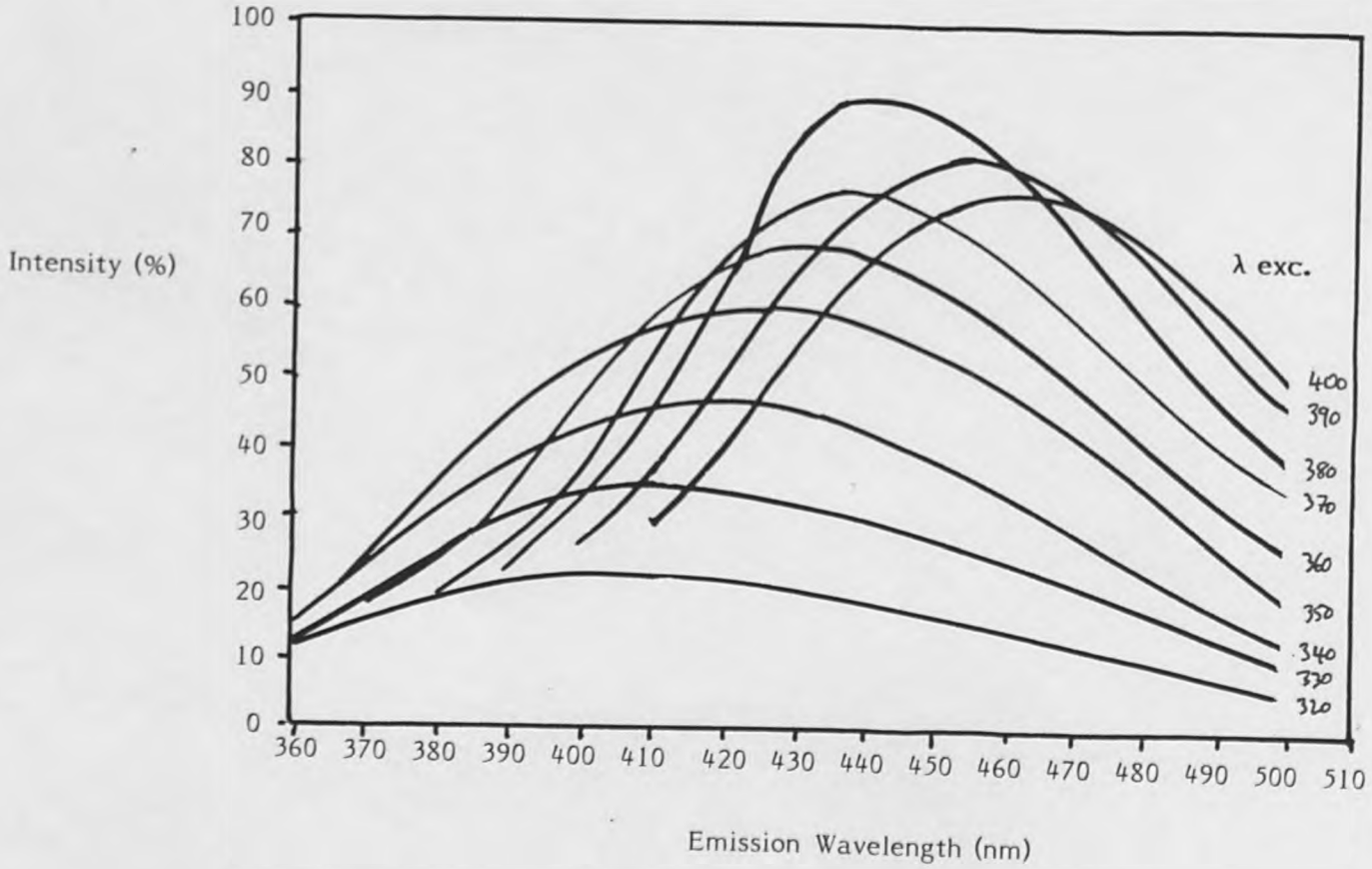
5.5.2 The Wavelength Dependence of the Luminescence of Untreated Wool

The wool used was salts serge ($200gm^{-2}$). A sample was mounted in a Perkin Elmer MPF-4 spectrofluorimeter using an attachment for solids, designed and made at the City University. This was similar to that described by McKellar and Allen [32]. To avoid reflection of the excitation wavelength, the wool samples were positioned at 10° to the plane normal tothe incident rays.

The fluorescence of the wool sample was measured by scanning the fluorescence emission for a series of excitation wavelengths. The results are shown below in Table 5.9 and Fig 5.3. The emission intensity i_S measured at λmax from the peak height, setting full scale deflection equal to 100%.

It is clear from Table 5.9 that increasing the excitation wavelength resulted in a corresponding red shift in the emission λmax. This suggested that more than one species was responsible for this emission. The most intense

Fig 5.3 Fluorescence Spectra of Wool over a Range of Excitation Wavelengths



emission was obtained when the excitation wavelength was 380nm.

Table 5.9 The Wavelength Dependence of the Fluorescence of Wool

Excitation Wavelength (nm)	Emission λmax (nm)	Emission Intensity (%)
320	402	22
330	408	35
340	420	47
350	425	60
360	432	69
370	438	77
380	440	90
390	455	82
400	461	77

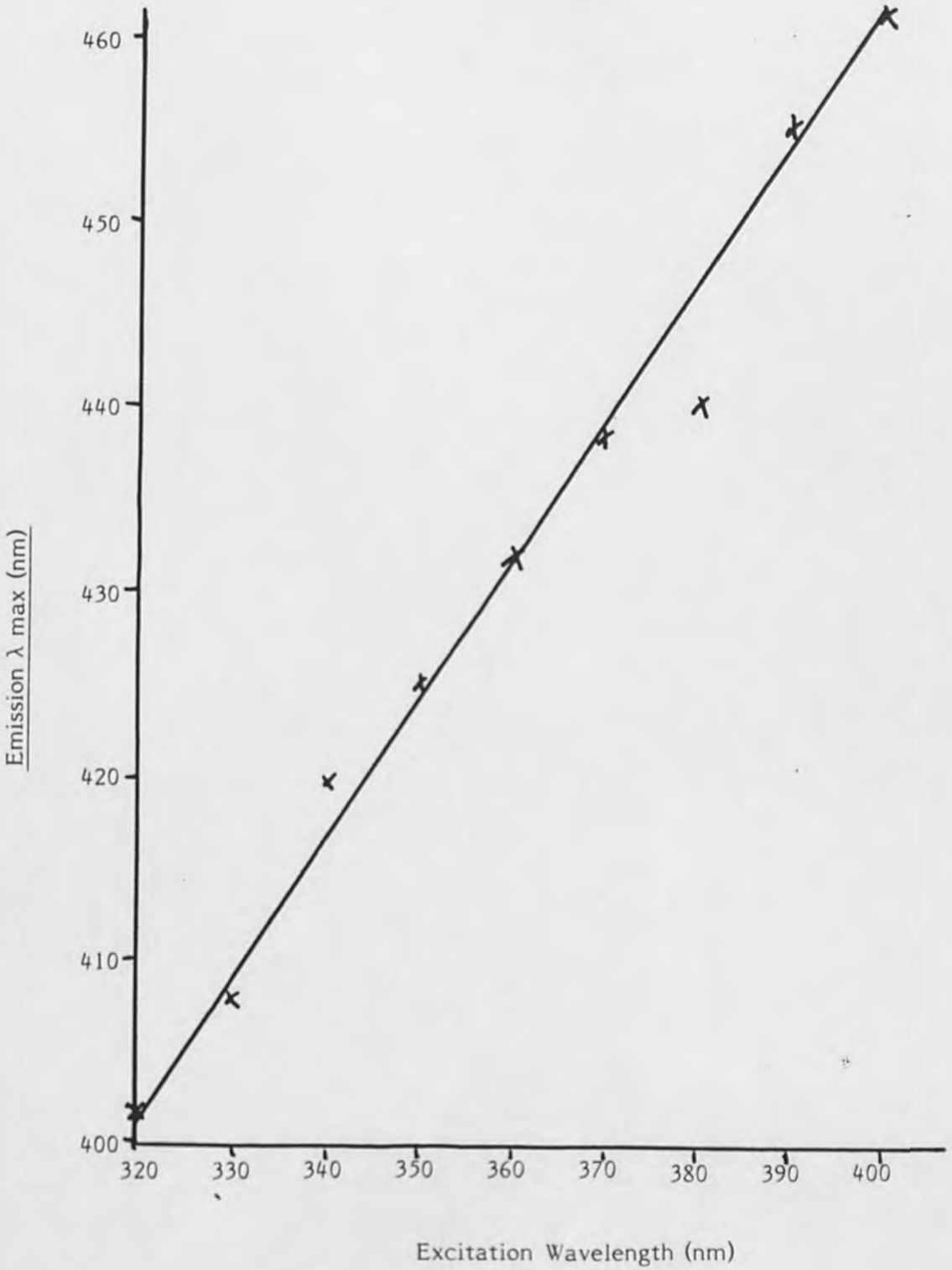
It has been reported [28] that there is some variation in the luminescence maxima of different types of wool. Fourteen different wools were tested by excitation at 360nm, and the emission maxima found to vary between 425-440nm.

Analysis of Table 5.9 and Fig.5.3 shows that the red shift in λmax which results from increasing the excitation wavelength is almost linear over the range of wavelengths studies. This is represented graphically in Fig. 5.4. Since all spectra were run on the same wool sample, without changing its orientation between spectra, it was certainly a photochemical effect in the wool, and not an artefact of the system used to monitor the fluorescence.

5.5.3. The Effect of Photoyellowing & Chemical Modification on the Luminescence of Wool

Samples of untreated wool, Blankit D treated, peroxide bleached and persulphate oxidized wool were taken, and half of each sample photoyellowed under 350nm light for 24 hours. The fluorescence of each of these samples was then measured for both the yellowed and unyellowed halves. As before,

Fig 5.4 The Variation in Fluorescence λ max with Increasing Excitation Wavelength



the peak intensities are recorded as percentages of full scale deflection under constant spectrofluorimeter conditions for all samples. In some cases the λmax changed following photoyellowing, and hence the λmax at which the intensity was measured is quoted in each case. The change in fluorescence intensity following photoyellowing is measured at the λmax of the wool before the yellowing took place. The results are given in Table 5.10.

Table 5.10 The Effect of Photoyellowing & Chemical Modification upon the Luminescence of Wool

		Before Yellowing		After Yellowing		Δ I (at initial λ max)
Wool Sample	λexc(nm)	Emission λmax	Emission Intensity	Emission λmax	Emission Intensity	
UNTREATED WOOL	340	430	36%	430	23%	-36%
	360	430	76%	437	53%	-33%
	380	435	89%	443	71%	-24%
BLANKIT D TREATED WOOL	340	425	17%	435	17%	-6%
	360	430	40%	435	43%	+5%
	380	433	48%	440	59%	+19%
PEROXIDE BLEACHED WOOL	340	410	48%	435	15%	-25%
	360	428	84%	437	34%	-62%
	380	433	97%	440	47%	-64%
PERSULPHATE OXIDIZED WOOD	380	445	5%	445	5%	0%

Discussion

One conclusion which may be drawn from the results in Table 5.10 is that whatever the species is that absorbs in the longer UV wavelength region, it is degraded by photooxidation. The sample of untreated wool showed that approximately one third of the fluorescence was lost after 24hrs photoyellowing. Peroxide bleached wool showed a marked increase over this, when nearly two thirds of the fluorescence intensity was lost. This

was perhaps to be expected, since oxidative bleaching promotes wool yellowing, and would at least be consistent with the proposal that this component of wool is involved in photoyellowing. It may be that it is a precursor to one of the yellow products formed by wool under UV irradiation. If so, it is significant to observe that it is apparently photostabilized by treatment with Blankit D, as described in Chapter 2.

It is curious that treatment with Blankit D should give such a marked decrease in the fluorescence intensity of the wool, as compared to the untreated sample. In Chapter 2 it was shown that Blankit D reacted with tryptophan and dityrosine by the addition of a methyl group, which did not affect the fluorescence at all. This would therefore suggest that dityrosine is not the sole species responsible for the observed luminescence. It may be, for example, that the fluorescence originates from two components, or groups of components; one of which is destroyed by the Blankit D treatment, and the other is stabilized against further irradiation. One of the mechanisms by which the wool fibre is stabilized by Blankit D may be to destroy 50% of the UV absorption of the wool at the longer wavelengths.

Another interesting observation is that oxidative bleaching enhances the initial absorption and fluorescence in this region. This may possibly be by the conversion of tyrosine residues to dityrosine, though no evidence is available to support this suggestion. Since dityrosine has been shown to yield such highly coloured photodegradation products, if the suggestion is true it may be a reason why peroxide bleaching enhances photoyellowing.

Oxidation of wool with persulphate causes extensive yellowing to occur, and appears to almost totally eliminate the long wavelength fluorescence emission. It has been shown to degrade tryptophan and biphenol (Chapter 4) to give yellow products, and it is likely that it should also give the same effect with dityrosine.

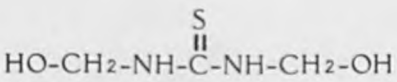
However, the existence of an inner filter effect cannot be discounted, since the blue fluorescence would be very rapidly reabsorbed by the yellow wool.

5.6 The Synthesis & Application to Wool of Thiouronium Compounds

5.6.1 Introduction

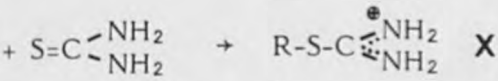
Many reagents have been applied to wool, in the search for inhibitors of sunlight yellowing [10]. Of these, very few have been found to give any protective effect at all, with the exception of certain thiols, [11], and other sulphur containing species. Perhaps the most effective has been thiourea in conjunction with formaldehyde, [33-36] followed by thermal curing. This offers a significant increase in lightfastness, though more than half of the effect is lost when the wool is washed. Thiourea or formaldehyde offer little protection when used alone.

Variations on the thiourea process have so far had very little to offer. It has been suggested [33] that the thiourea and formaldehyde bind to wool as methylol thiourea:



This has been found to improve the stability of wool, but polymerizes, limiting its usefulness in this respect.

In this work, thiourea was reacted with a series of halogenated molecules to form thiouronium salts:



These cationic surfactants were then applied to wool under alkaline conditions, and the photostability determined. The pH was then increased and the free thiol obtained by alkaline hydrolysis:

5.6.2 The Synthesis of Alkyl Thiouronium Bromides

Initially, thiourea was reacted with bromoalkanes. Functioning as a surfactant, the resultant thiouronium salt would thus have an affinity for wool and could be applied from aqueous solution. Once on the wool, the material could be hydrolysed to leave a hydrophobic thiol trapped within the fibre. If this gave a protective effect, one would expect it to be reasonably wash fast.

Two bromoalkanes were used, namely bromodecane and bromododecane.

a) $CH_3(CH_2)_9\,Br + S{=}C\begin{matrix}NH_2\\NH_2\end{matrix} \rightarrow CH_3(CH_2)_q\text{-}S\text{-}C^{+}\begin{matrix}NH_2\\NH_2\end{matrix}\;Br^{\ominus}$

M.Wt. 221.19	76.11	297.30
Used 22.12g (0.1M)	7.61g(0.1M)	29.73g (theoretical)

Bromodecane (22.12g, 0.1M) was dissolved in 200ml isopropyl alcohol. Thiourea (7.61g, 0.1M) was dissolved separately in a further 200ml isopropanol, and with vigourous stirring the two solutions were mixed, adding the bromodecane solution dropwise to the thiourea. A white precipitate was formed immediately. Once all the bromodecane had been added, the mixture was left stirring for 15 minutes before filtering out the product. This was then washed with isopropanol at 60°C (3x50ml). The thiouronium salt was dried in a vacuum desicator, and then applied to wool without further purification. The yeild was 28.82g (97% of the theoretical yield).

b) $CH_3(CH_2)_{11}\,Br + S{=}C\begin{matrix}NH_2\\NH_2\end{matrix} \rightarrow CH_3(CH_2)_{11}\text{-}S\text{-}C^{+}\begin{matrix}NH_2\\NH_2\end{matrix}\;Br^{\ominus}$

M.Wt. 249.42	76.11	325.53
Used 24.94g (0.1M)	7.61g (0.1M)	32.55g (theoretical)

The experimental conditions were repeated exactly. In this case, the yield was again almost quantitative; 31.48g were obtained (97% of the theoretical yield).

5.6.3 The Application of Alkyl Thiouronium Bromides to Wool

The thiouronium salts were applied to wool from an aqueous isopropanol

solution, adjusted to pH7. As a cationic surfactant, a high pH was obviously desirable to generate anionic sites on the wool protein. However, a pH in excess of 8 was found to hydrolyse the thiouranium salt, giving a white precipitate of the appropriate thiol.

The salts were applied in the Jeffreys dyemaster by the long liquor treatment. A 10% owf loading was found to be needed to achieve any noticeable effect. Three 300ml dyemaster tubes were filled with 50ml isopropanol and 250ml distilled water. 10g wool samples were used, one each of untreated peroxide bleached and fluorescently whitened wool. To achieve the desired concentration on wool, 1.0g of the decylthiouronium bromide was added to each tube and the pH adjusted to 7 with 1% ammonium hydroxide. The wool samples were introduced to each tube and the dyemaster heated to 50°C. This temperature was maintained for 45 minutes.

At the end of this time, half of each wool sample was removed and dried in air, while the other half was placed in ammonium hydroxide solution adjusted to pH9. Slight traces of white precipitate were observed as the thiouronium bromide was converted to the insouble thiol. Each sample was dried and the photostability determined by yellowing under 350nm light for 24 hours. The results are given on Table 5.11, where the thiouronium bromide is abbreviated to C10TUB, and the thiol to C10SH. From this table it is clear that the thiouronium salt gave little or no protection against yellowing, whilst a low level of protection was achieved with the thiol compounds. In the case of the bleached samples, this appeared to be a real improvement but for the fluorescently whitened wool, the improvement may have arisen because the wool was partially yellowed by the application of the thiol. It appeared that these materials offered little prospect in stabilizing the wool fibre to yellowing.

The dodecyl thiouronium bromide was applied to wool using the same method. Results are given in Table 5.12, and shown a very similar trend to those in the previous section. This thus confirms the conclusion drawn above that alkyl thiols and thiouranium salts do not confer sufficient protection to warrant further study.

Table 5.11 The Stabilizing Effects of Decylthiouronium Bromide & Decanethiol

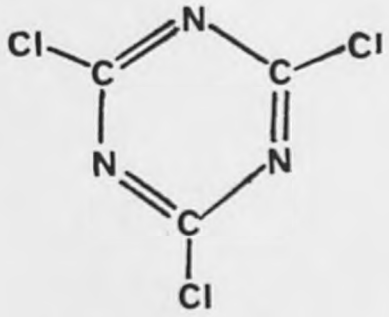
WOOL SAMPLE	Initial Y.I.	Y.I.After Yellowing	Δ Y.I.
Untreated Wool	20.9	24.7	3.8
Untreated Wool + C10 TUB	21.0	24.8	3.8
Untreated Wool + C10 SH	21.9	25.6	3.7
Peroxide Bleached Wool	14.8	24.4	9.6
Bleached Wool + C10 TUB	15.0	24.8	9.8
Bleached Wool + C10 SH	16.3	23.1	6.8
FWA Wool	-1.8	23.1	24.9
FWA Wool + C10 TUB	1.3	25.1	23.9
FWA Wool + C10 SH	6.3	26.3	20.0

Table 5.12 The Stabilizing Effects of Dodecyl Thiouronium Bromide & Dodecane-thiol

WOOL SAMPLE	Initial Y.I.	Y.I.After Yellowing	Δ Y.I.
Untreated Wool	20.9	24.8	3.9
Untreated Wool + C12 TUB	21.2	25.0	3.8
Untreated Wool + C12 SH	22.0	25.7	3.7
Peroxide Bleached Wool	14.8	24.4	9.6
Peroxide Bleached Wool + C12 TUB	14.9	24.8	9.9
Bleached Wool + C12 SH	16.1	24.1	8.0
Fluorescently Whitened Wool	-1.6	23.5	25.1
FWA Wool + C12 TUB	1.9	25.6	23.7
FWA Wool + C12 SH	6.1	26.2	20.1

5.6.4 The Synthesis of Triazine Thiouronium Salts

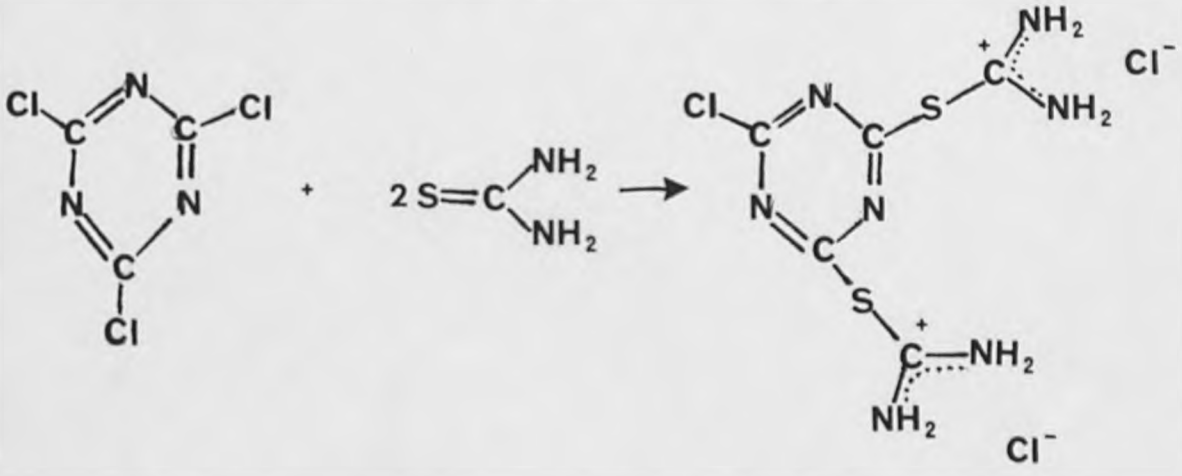
A possible way of increasing the concentration of modified thiourea on the surface of wool was to incorporate it in a smaller, more reactive molecule. To achieve this, the triazine structure was selected:-



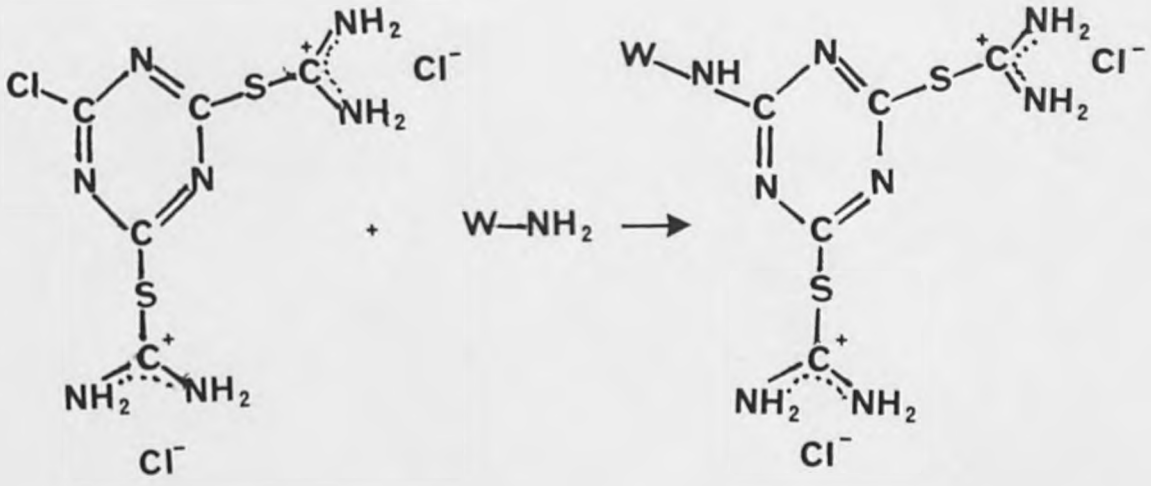
Cyanuric Chloride

This is very reactive towards wool, [12, 37-41] and is used as the reactive functional groups in many reactive dyes, such as Procion M (ICI), Cibacron (Ciba Geigy), Verofix (Bayer), and Drimalan F (Sandoz) [42]. It will react with one, two or three equivalents of thiourea, [43] and may be applied to wool either as the thiouronium salt, prior to hydrolysis to the thiol, or to react with amino groups in the wool at higher temperature. If two carbon atoms in the triazine ring remain unsubstituted, it may be introduced in the form of cross-links in the fibre, thereby increasing the stability of any protective effect obtained.

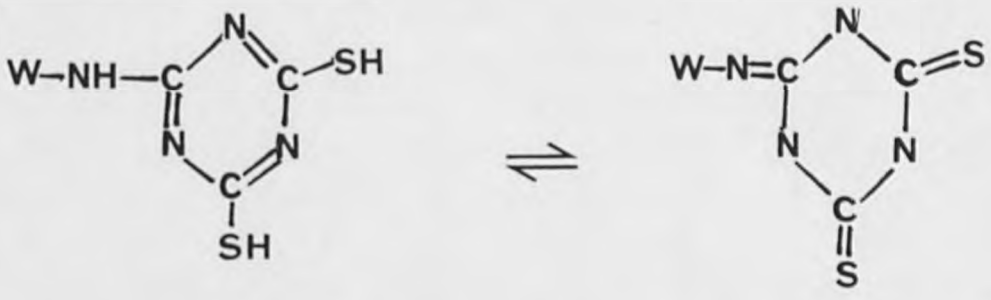
Cyanuric chloride could be reacted with thiourea as follows:-



This could then react with wool:



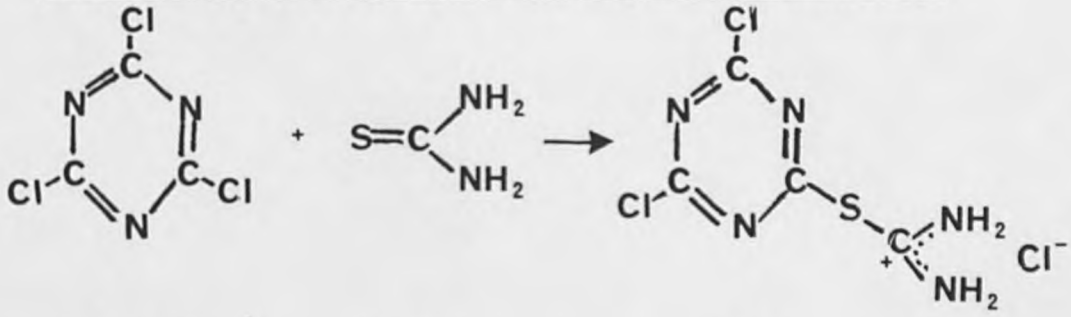
Increasing the pH yield the thiol, allowing the following equilibrium:



Thus if the C=S bond in thiourea is responsible for the protective effect with thiourea/formaldehyde, this could be reproduced in a species which was covalently bound to wool, thereby improving washfastness.

Triazine thiols have been found to stabilize polymers with good effect, [44-46] and the following study assessed their effectiveness with wool. Cyanuric chloride was reacted with one, two and three equivalents of thiourea, and the products applied to wool.

Reaction of Cyanuric Chloride with one Equivalent of Thiourea:



MW. 184.41	76.12	260.53
Used 18.4g (0.1M)	7.6g (0.1M)	26.1g (theoretical)

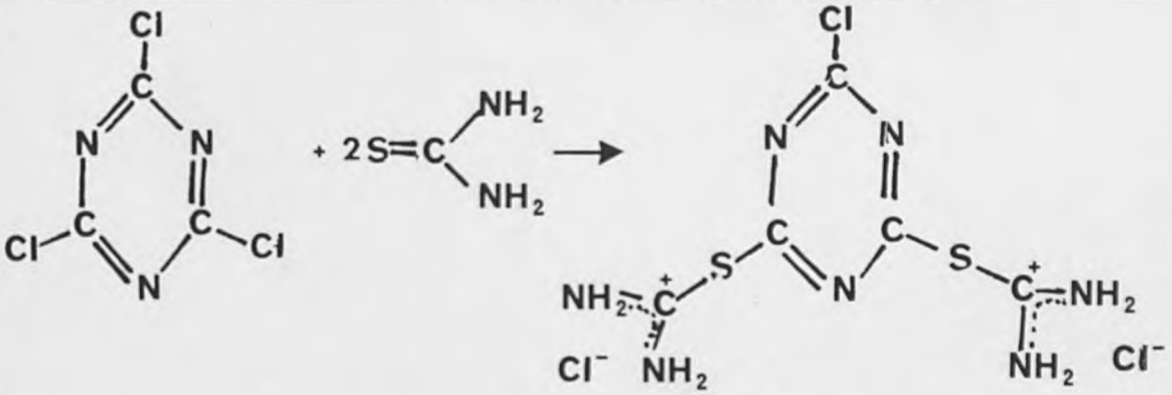
Cyanuric chloride (18.4g, 0.1M) was dissolved in acetone (400ml) and stirred in an ice bath until the temperature reached 0°C. Thiourea (7.6g, 0.1M) was dissolved in a further 400ml acetone and the temperature reduced to 0°C in an ice bath. The thiourea solution was then added dropwise from a separating funnel to the cyanuric chloride solution, whilst stirring rapidly and maintaining the temperature at 0°C. A white precipitate was observed to form following an immediate reaction. Once the addition of thiourea was complete, the reaction was stirred for 1 hour at the same temperature.

The reduced temperature had been found to be necessary by Lewis et.al. [47] to restrict the reaction to one site on the cyanuric chloride molecule. Two equivalents could be reacted at room temperature, and all three chlorine atoms substituted at 50°C.

After stirring for one hour, the precipitate was filtered, and washed with hot acetone (2x50ml at 50°C) followed by isopropanol (1x50ml at room

temperature) and cold acetone (1x50ml, r.t.). The product was dried in a vacuum desiccator. The yield was 22.4g (86% of theoretical yield). This product was labelled 1M. Melting point 140-141°C.

Reaction of Cyanuric Chloride with Two Equivalents of Thiourea

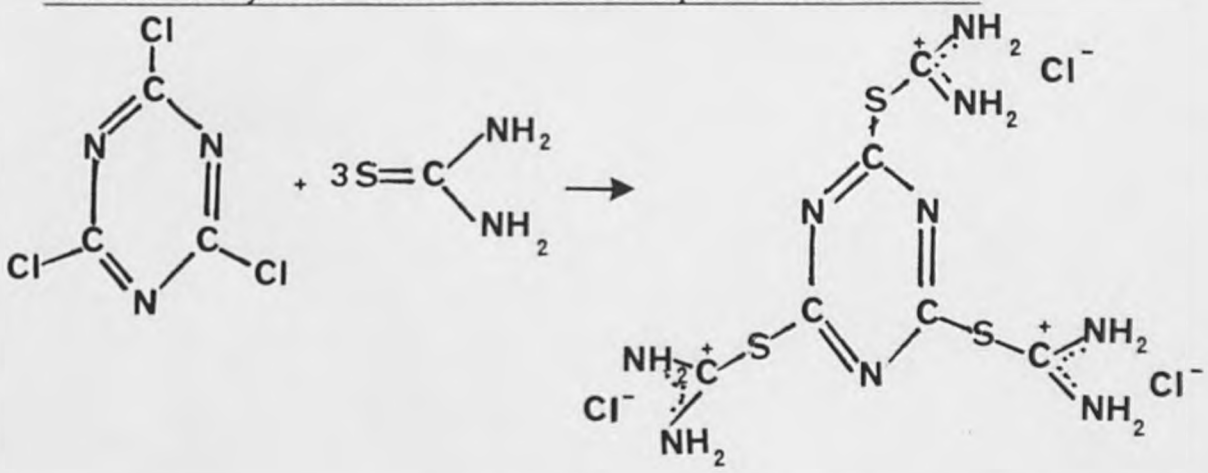


M.Wt. 184.41	76.12	336.65
Used 18.4g (0.1M)	15.2g (0.2M)	33.6g (theoretical)

Cyanuric chloride (18.4g, 0.1M) was dissolved in acetone and chilled to 5°C. Thiourea (15.2g, 0.2M) was dissolved in acetone (400ml) and also chilled. The two solutions were then removed from the ice batch, and the thiourea added dropwise to the cyanuric chloride with constant mechanical stirring. During this time, the temperature was allowed to gradually reach ambient, and the reaction mixture stirred for one hour after the addition of the two reagents was completed.

The product was filtered and washed with acetone, isopropanol and more acetone as before, followed by drying in a vacuum desicator. The yield of this product was 27.2g (81% of theoretical yield), and it was labelled 2M. Melting point 133-135°C.

Reaction of Cyanuric Chloride with Three Equivalents of Thiourea



M.Wt. 184.41	76.12	412.77
Used 18.4g (0.1M)	22.8g (0.3M)	41.2g (theoretical)

Cyanuric chloride (18.4g, 0.1M) was dissolved in 300ml acetone and heated to 50°C. Thiourea (22.8g, 0.3M) was dissolved in 800ml acetone and added from a separating funnel to the cyanuric chloride, stirring vigourously and maintaining the temperature at 50°C. Once the two solutions were completely mixed, the reaction was stirred at 50°C for 1 hour. Finally, the product was filtered and washed with cold isopropanol (1x100ml) followed by acetone (5x100ml), and then dried under vacuum. The yield was 35.2g (85% of theoretical yield) and the material labelled 3M. Melting point 130-131°C.

5.6.5 Characterisation of Thiouronium Triazines 1M, 2M and 3M

Elemental Analysis

1M		C	H	N
	Theoretical:	18.44%	1.55%	26.88%
	Found:	18.39%	2.37%	28.09%
	Difference:	0.05%	0.82%	1.21%

2M		C	H	N
	Theoretical:	17.84%	2.40%	29.12%
	Found:	17.65%	3.03%	29.28%
	Difference:	0.19%	0.63%	0.16%

3M		C	H	N
	Theoretical:	17.46%	2.93%	30.54%
	Found:	17.01%	3.12%	29.98%
	Difference:	0.45%	0.19%	0.56%

These values are clearly not ideal. However, Roper et.al. [12] and Lewis et.al. [37] experienced similar problems when synthesizing triazine derivatives, and in spite of numerous attempts at purification, these figures could not be improved. It is possible that water was present, and also that traces of mono-, di- and trisustituted triazines were present as contaminants. This was not considered to be a problem, since in this work aqueous solutions were used, and trace quantities of the other two materials were unlikely to make any difference to the photostability of treated wool samples.

Mass Spectrometry

These molecules were extremely unstable in the mass spectrometer, giving no molecular ion and an array of very low intensity ions at almost every mass number. Virtually no information could be obtained from these spectra regarding the structure of the products.

Infra Red Spectroscopy

These three compounds were found to give very poor KBr discs, and

hence were run as nujol mulls.

Symmetrical triazines [48] have at least one strong band between 1520-1580cm^{-1}, at least one band at 1350-1450cm^{-1} and at least one weak band at 775-860cm^{-1}. This last band is due to an out-of-plane deformation of the ring, the others being due to in-plane stretching vibrations. The C-Cl stretch is normally observed at 450-500cm^{-1}, and hence is not detected on these spectra. The C-S stretch occurs between 620-720cm^{-1}, and appears as a very weak band on these spectra. C=N stretching absorbs at 1600-1625^{-1} and is seen on all four spectra. The primary amino groups show a broad N-H stretch at 3100-3250, which is seen on all the product spectra but not, of course, on that for cyanuric chloride. The spectra are thus consistent with the proposed structures of products 1M, 2M and 3M.

5.6.6 Application of the Thiouronium Triazines to Wool

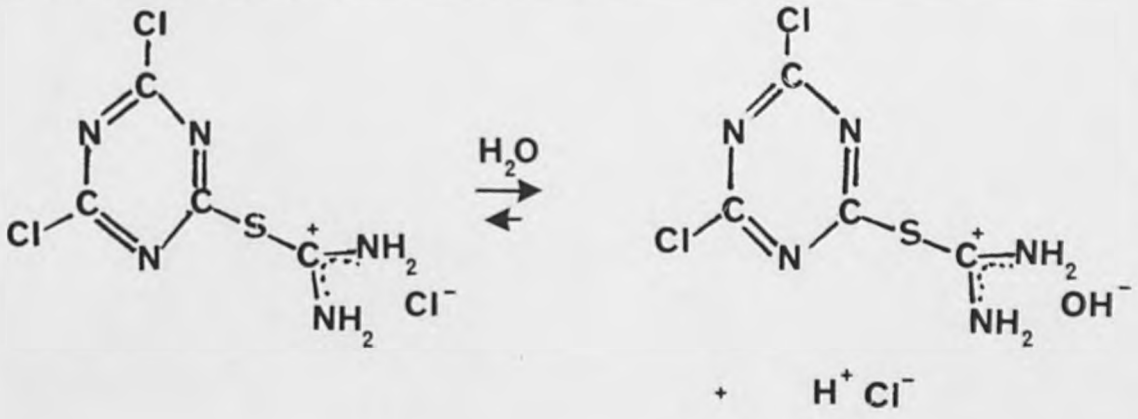
Exhaustion from Aqueus Solution

The first method of application was exhaustion from aqueous solution. Bearing in mind the results obtained with the alkyl thiouronium salts, a 10% owf concentration was used. 1.0g of the triazine was used with 10g wool samples. The liquor:wool ratio was 30:1, so 9 x300ml dyemaster tubes were filled with distilled water, and 1g of the triazine added. The wool samples tested were untreated, peroxide bleached and fluorescently whitened wool, a piece of each being treated with each of the three compounds, 9 tubes in total. The natural pH of the triazine thiouronium products was 2.5. Ideally the pH should have been buffered to 7 to increase the affinity of these species to wool, but the solutions began to turn cloudy above pH 3.0. It was thus necessary to carry out the exhaustion at pH3. The treatment was run for 45 mintues at 50°C with constant agitation by the dyemaster. At the end of this period, the pH of the solutions had increased to 6.5-7.0, and all solutions remained clear, although a yellow tint was observed in the tubes containing the untreated and bleached wool samples.

To convert the thiouronium products to the corresponding thiols, 1% ammonium hydroxide was added until pH 10.0 was obtained. No precipitation

was caused in any tube, which was interpreted to mean that the triazine was totally exhausted onto the wool. After standing for 15 minutes, each sample was washed in warm tap water and dried in air overnight.

The acidity of the aqueous solutions of the triazine thiouronium chlorides was attributed to the following equilibrium, which infers that the positive charge is preferentially solvated by hydroxide ions. It is supported by the fact that $H^+ Cl^{\ominus}$ is very stable in aqueous solution, and hence the equilibrium is probably displaced towards the right hand side:



Addition of ammonia will decrease the concentration of hydroxide ions, reducing the number available for solvation of the triazine. This may account for the low solubility at netural pH.

Since the purpose of the treatment was to ascertain whether these species conferred any photoprotective effect upon wool, the treated samples were photoyellowed under 350nm UV light for 24 hours, and the yellowness indices measured. These are presented in Table 5.13, and were very disappointing Very litle stabilization appeared to have been achieved at all, but rather worse, every wool sample was yellowed by the application of these triazines. In the cases of bleached and fluorescently whitened wool, quite marked yellowing (10-12 Y.I. units) occurred, such that any protective effect was totally lost. It would probably be reasonable to infer that the improvements in photostability (Δ Y.I.) observed were the result only of the increased initial yellowness of the wool. This method of application was thus considered to be of no value for this treatment.

Table 5.13 Treatment of Wool with Thiouronium Triazines by Exhaustion

WOOL SAMPLE	Initial Y.I.	Y.I. After Treatment	Y.I.After Photoyellowing	Δ Y.I.
Untreated Wool (control)	26.8	-	31.6	4.8
Untreated Wool + 1M	-	28.8	33.7	4.9
Untreated Wool + 2M	-	27.2	33.2	6.0
Untreated Wool + 3M	-	27.3	32.7	5.4
Peroxide Bleached Wool (control)	16.4	-	29.3	12.9
Bleached Wool + 1M	-	25.1	32.2	7.1
Bleached Wool + 2M	-	24.5	33.3	8.8
Bleached Wool + 3M	-	24.0	33.1	9.1
Fluorescently Whitened Wool (control)	0.7	-	29.9	29.2
FWA Wool + 1M	-	12.3	31.7	19.4
FWA Wool + 2M	-	13.3	31.6	18.3
FWA Wool + 3M	-	12.1	31.9	19.8

Application by the Pad/Dry Method

The purpose of the application methods was to incorporate the triazines within the fibre. It may have been that the conditions of the exhaustion process had contributed to the loss in whiteness of the wool samples, and hence it was decided to try the pad/dry approach. Instead of the wool being immersed in liquor for a long period of time, padding involves passing the wool through the solution at a constant rate, after which it is fed between two rollers. The gap between the rollers is so adjusted that the wool picks up its own weight of solution (100% pick up). Subsequent to this, samples are either rolled up and stored in the dark (pad/batch process) or dried in air at room temperature (pad/dry method). In the current problem, there was no requirement for the triazine to covalently bond to the wool protein, so the pad/dry approach was adequate.

Solutions (1 litre) of the three thiouronium triazines were prepared ($20g/dm^3$) and 2ml of Lissapol N (ICI) added. These were padded onto two samples each of untreated, bleached and fluorescently whitened wool to 100% pick up. Each sample was dried without rinsing.

At this stage there were two wool samples for each of the nine treatments. One of each of these was immersed in 1% ammonium hydroxide for half and hour at room temperature to convert the thiouronium salt to the thiol. These were then redried. The photostabilities of all samples were measured, and the results shown in Table 5.14 and 5.15.

This test again gave discouraging results. Most of the treated wool samples were yellowed by this treatment as much as by the exhaustion process, suggesting that it was the thiouronium salts which were causing the discolouration, rather than the method of application. An exception to this is seen in Table 5.14 for the fluorescently whitened fabric, where the extent of yellowing accrued from the pad/dry process is approximately half that ssen with the exhaustion treatment. However, the photoyellowing was then worse, and the colour of the photodegraded samples was almost as yellow as those from the other processes.

The only conclusions that could be drawn from this set of results were that the application of these materials to wool, whether in the thiouronium

Table 5.14 The Effect of Treating Wool with Thiouronium Triazines by the pad/dry Method

WOOL SAMPLE	Initial Y.I.	Y.I. After Treatment	Y.I. After Photoyellowing	Δ Y.I.
Untreated Wool (control)	26.6	-	31.3	4.7
Untreated Wool + 1M	-	27.3	33.0	5.7
Untreated Wool + 2M	-	27.2	32.3	5.1
Untreated Wool + 3M	-	28.1	36.3	8.2
Peroxide Bleached Wool (control)	16.5	-	29.1	12.6
Bleached Wool + 1M	-	24.7	30.9	6.2
Bleached Wool + 2M	-	24.8	31.7	6.9
Bleached Wool + 3M	-	24.0	31.2	7.2
Fluroescently Whitened Wool (control)	0.2	-	29.8	29.6
FWA Wool + 1M	-	7.1	28.2	21.1
FWA Wool + 2M	-	6.9	28.3	21.4
FWA Wool + 3M	-	6.3	29.2	22.9

Table 5.15 Treatment of Wool with Triazine Thiols by the pad/dry Method with Subsequent Immersion in Ammonium Hydroxide

WOOL SAMPLE	Initial Y.I.	Y.I. After Treatment	Y.I. After Photoyellowing	Δ Y.I.
Untreated Wool (control)	26.6	-	31.3	4.7
Untreated Wool + 1M Thiol	-	26.7	31.6	4.9
Untreated Wool + 2M Thiol	-	26.1	30.8	4.7
Untreated Wool + 3M Thiol	-	27.1	33.9	6.8
Peroxide Bleached Wool (control)	16.5	-	29.1	12.6
Bleached Wool + 1M Thiol	-	23.9	31.7	7.8
Bleached Wool + 2M Thiol	-	22.8	31.3	8.5
Bleached Wool + 3M Thiol	-	22.7	31.1	8.4
Fluorescently Whitened Wool (control)	0.2	-	29.8	29.6
FWA Wool + 1M Thiol	-	12.4	30.8	18.4
FWA Wool + 2M Thiol	-	12.6	31.1	18.5
FWA Wool + 3M Thiol	-	12.4	30.3	17.9

form or as the thiols, offered nothing to the stabilization of the fibre to photodegradation, and that their very presence on wool led to adverse discolouration.

5.7 Summary

The work described in this chapter is really a catalogue of the approaches to the stabilization of wool which failed to provide the desired effect. In some cases they were totally new ideas, such as the application of Tinuvin and the impregnation of wool with free amino acids. Others, such as the use of DABCO and thiols to protect wool, were extensions of ideas previously reported, albeit studying them from a different standpoint. However, it is unfortunately true that wool is not protected by any of these methods.

REFERENCES - Chapter 5

1) D.R.Graham & K.W.Statham, J.S.D.C., 1956, 72, 434

2) L.A.Holt, B.Milligan & L.J.Wolfram, Text.Res.J., 1974, 44, 846

3) N.A.Evans, D.E.Rivett & P.J.Waters, Text.Res.J., 1976, 46, 214

4) B.Milligan & L.A.Holt, Aust.J.Chem., 1974, 27, 195

5) L.J.Wolfram, I.W.S., P.D. Report 159, September 1973

6) A.S.Inglis & R.J.Simpson, Text.Res.J., 1968, 38, 109

7) I.H.Leaver, Text.Res.J., 1968, 38, 729

8) L.C.Gruen & A.S.Inglis, Text.Res.J., 1968, 38, 497

9) L.C.Gruen & A.S.Inglis, Text.Res.J., 1968, 38, 509

10) A.Kirkpatrick & J.A.Maclaren, Text.Res.J., 1967, 37, 510

11) J.E.Tucker, Text. Res. J., 1969, 39, 830

12) K.Roper, Private Communication to Dr.D.M.Lewis, IWS.

13) D.M.Lewis, Private communication (letter of 17/7/73, circulated to IWS personnel).

14) B.Milligan, L.A.Holt & I.H.Leaver, Int.Wool Text.Res.Conf., Aachen, 1975, III, 607

15) L.A.Holt, B.Milligan & L.J.Wolfram, Text.Res.J., 1975, 45, 257

16) L.A.Holt, I.H.Leaver & B.Milligan, Text.Res.J., 1976, 46, 539

17) D.M.Lewis & M.T.Pailthorpe, J.S.D.C., 1983, 99, 354

18) W.H.Koenig, Text.Res.J., 1965, 35, 708

19) B.Milligan & L.J.Wolfram, J.Text.Inst., 1973, 64, 170

20) B.Milligan & L.J.Wolfram, J.Text.Inst., 1972, 63, 515

21) N.H.Leon, Textile Progress, 1975, 7 (I) 1.

22) N.S.Allen, Polymer Photochem., 1981, 1, 243

23) A.B.Abdullah & L.W.E.Miles, Text.Res.J., 1984, 54, 415

24) I.H.Leaver, Photochem.Photobiol., 1978, 27, 439

25) C.H.Nicholls & M.T.Pailthorpe, J.Text.Inst., 1976, 67, 397

26) C.H.Nicholls, Developments in Polymer Photochemistry, 1980, Ed.N.S. Allen., P130

27) L.I.Grossweiner, Current Eye Research, 1984, 3, 137

28) D.King, Ph.D.Thesis, The City University, London. 1984, P174

29) J.I.Dunlop & C.H.Nicholls, Photochem.Photobiol., 1966, 5, 227

30) E.G.Bendit, J.Text.Inst., 1960, 51, 544

31) J.I.Dunlop & C.H.Nicholls, Nature, 1965, 205, 1310

32) McKellar & Allen, 1979, Photochemistry of Man-made fibres, Applied Science Publ., London

33) B.Milligan & D.J.Tucker, Text.Res.J., 1964, 34, 681

34) G.N.Evans & B.Milligan, Text.Res.J., 1966, 36, 927

35) W.E.Savige, Text.Res.J., 1968, 38, 101

36) L.C.Gruen & A.S.Inglis, Text.Res.J., 1968, 38, 1062

37) M.T.Pailthorpe, D.M.Lewis & M.Davies, I.W.S. PD Report CCD-108

38) D.M.Lewis & M.T.Pailthorpe, J.S.D.C., 1984, 100, 56

39) D.M.Lewis & I.Seltzer, J.S.D.C., 1968, 84, 501

40) D.M.Lewis, J.S.D.C., 1982, 98, 165

41) J.T.Thurston, J.R.Dudley, D.W.Kaiser, I.Hechenbleikner, F.C.Schaefer & D.Holm-Hansen, J.AM.Chem.Soc., 1951, 73, 2981

42) J.A.Maclaren & B.Milligan, "Wool Science", 1981, Science Press P167

43) D.M.Lewis, Private Communication, (IWS)

44) K.Mori & Y.Nakamura, J.Appl. Polym.Sci., 1983, 28, 691

45) K.Mori & Y.Nakamura, J.Polym.Sci.Chem.Ed., 1978, 16, 2055

46) K.Mori, N.A.Sabe & J.Nakamura, J.Polym.Sci.Polym.Lett.Ed., 1982, 20, 321

47) D.M.Lewis, Private Communication (IWS)

48) W.A.Keckle, Spectrochim.Acta., 1961, 17, 600

Conclusions

The work contained in this project is most significant, because it has showed that it is possible to chemically modify the amino acids in wool in such a way as to change the direction of the photodegradation pathway from the production of coloured products to primarily colourless ones. Starting with the essentially pragmatic approach of determining a technique which achieved the retardation the photoyellowing process, the chemical reactions involved have been elucidated to a large extent, enabling a reaction scheme to be proposed. In consequence, a method is now known which effectively and markedly increases the photostability of wool, not only in its natural state, but also when bleached and fluorescently whitened.

This process has the present limitation, in that in its current state, it is too costly to be economically viable on an industrial scale. However, the prinicple still remains, and the opportunity is presented for further research to develop a purely chemical means of applying the treatment, without the expensive need for ultraviolet light.

In the study of fluorescent whitening agent decay on wool, using diffuse reflectance spectroscopy, this work has shown that the older and accepted method of extracting residual FWA with dilute ammonia gives rise to misleading results. The new method allows the FWA to be monitored only on the surface, where the discolouration occurs, thereby providing a higher degree of sensitivity than previously observed, and avoiding false impressions of photostability arising from the undegraded FWA, protected within the bulk of the fibre. A number of the commercially available FWA's which are used with wool have been reassessed by this method, and the results compared with the older procedure, thus highlighting the differences and advantages of diffuse reflectance spectroscopy for this purpose.

Many other chemical treatments have been tested unsuccessfully, in the attempt to find further methods of retarding photoyellowing. These have emphasized the conclusion, reached by so many others in past years, that the photoyellowing of wool is a highly complex process, and is almost certainly the result of a diversity of individual reactions. To be successful,

a treatment has to either interact individually with most, if not all, of these, or to inhibit the supply of UV light to the fibre, and in every case tried, the yellowing reactions could not be inhibited without unacceptably changing the handle of the wool, or preventing the FWA from fluorescing.

Attempts to develop and apply a photostable FWA to wool have been reported. These have been unsuccessful, but a general conclusion may be drawn from these results and the work of others. It has been observed that even when an FWA is found which is stable to UV light in solution, once it is applied to wool it markedly increases the rate of yellowing. Nicholls & Pailthorpe, among others, have concluded that the increase in yellowing observed for fluorescently whitened wool is due to a sensitization of the wool by the FWA to the wavelengths around 350nm, which are abundant in natural sunlight, possibly by an energy transfer mechanism. It therefore seems reasonable to conclude that any FWA which absorbs in this region of the spectrum (which indeed they must, to produce the blue fluorescence emission required) is likely to sensitize wool yellowing, no matter how photostable or otherwise the FWA itself may be.

The following pages list three series of publications in Textile Research Journal on the photoyellowing of wool.

APPENDIX

"STUDIES IN WOOL YELLOWING"

Part

1) Not found in Text.Res.J. 1960, 1961 or 1962

2) Not found in Text.Res.J. 1960, 1961 or 1962

3) Milligan & Tucker TRJ, 1962, 32, 634. Sunlight Yellowing.

4) Inglis & Lennox, TRJ, 1963, 33, 431. Changes in AA composition due to hν.

5) Milligan & Tucker, TRJ, 1963, 33, 487. Effect of buffer pretreatment on sunlight yellowing of wool.

6) Maclaren, TRJ, 1963, 33, 773. Changes in AA composition due to yellowing

7) Milligan & Tucker, TRJ, 1964, 34, 681. Processes for retarding yellowing by sunlight.

8) Kirkpatrick & Maclaren, TRJ, 1964, 34, 1082. Yellowing of modified wool.

9) Inglis & Lennox, TRJ, 1965, 35, 104. Irradiation with different UV waveband.

10) Leach & Holt, TRJ, 1965, 35, 380. Effect of sunlight hν in presence of water

11) Not found in Text.Res.J. 1965.

12) Downes, Till & Lennox, TRJ, 1965, 35, 772. Quenching effect in liquid scintillation counting.

13) Lennox, Inglis & Holt, TRJ., 1966, 36, 837. Partial bleaching with visible light.

14) Evans & Milligan, TRJ, 1966, 36, 927. Attempts to inhibit sunlight yellowing.

15) Milligan, TRJ., 1966, 36, 1012. Yellowing of shrink-resistant wool under dyebath conditions.

16) Holt, Inglis & Lennox, 1967, 37, 490. Repeated alternate yellowing and peroxide bleaching.

17) Kirkpatrick & Maclaren, 1967, 37, 510. A survey for inhibitors of sunlight yellowing.

18) Savige, TRJ, 1968, 38, 101. Processes for inhibition of sun yellowing of FWA wool.

19) Inglis & Simpson, TRJ, 1968, 38, 109. Improved stability of FWA fabrics to simulated light.

20) Gruen & Inglis, TRJ, 1968, 38, 497. Changes in fluorescence of brightened wool upon irradiation.

21) Gruen & Inglis, TRJ, 1968, 38, 509. Further studies on wool treated with FWA's and thiourea HCl.

22) Leaver ,TRJ, 1968, 38, 729. ESR investigations of the action of UV radiation.

23) Lennox & King ,TRJ, 1968, 38, 754. UV yellowing and blue light bleaching of different wools.

24) King & Ramsay ,TRJ, 1969, 39, 198. Standards for the assesment of yellowing of FWA wools.

25) Lennox ,TRJ, 1969, 39, 700. UV sensitization to hydrothermal yellowing.

26) Leaver & Ramsay, TRJ, 1969, 39, 722. Photoyellowing of FWA'S

27) Leaver & Ramsay ,TRJ, 1969, 39, 730. The role of water in photoyellowing of FWA wool.

28) J.B.Caldwell & B.Milligan, TRJ. 1970, 40, 194. Treatment with Methyl Isothiocyanate.

29) G.C.Ramsay & L.J.Stephens, TRJ, 1971, 41, 80. Effect of OBA's in wash liquors.

30) M.G.King & G.C.Ramsay ,TRJ, 1971, 41, 81. Effect of wash liquors on photoyellowing.

" EFFECT OF LIGHT UPON WOOL "

Part

1) Launer, TRJ., 1963, 33, 258. Greening & Yellowing by Germicidal U.V.

2) Launer, TRJ., 1963, 33, 351. Comparative Reflectance as a Measure of Colour Change.

3) Launer, TRJ., 1963, 33, 910. Post Irradiation loss of Colour in the dark after Germicidal U.V.

4) Launer, TRJ., 1965, 35, 395. Bleaching & Yellowing by Sunlight.

5) Launer, TRJ., 1965, 35, 813. Yellowing & Bleaching by UV and Visible light.

6) Launer, TRJ., 1966, 36, 606. Spectral Conformity in Photobleaching on Wool.

" FLUORESCENT WHITENING AGENTS "

Part

1) Not found

2) Not found

3) Holt & Milligan, TRJ., 1974, 44, 181. Photodecomposition of

4) Holt & Miligan, TRJ., 1974, 44 452. Sensitization of Photoyellowing of Wool by Aromatic Aldehydes.

5) Holt, Milligan & Wolfram, TRJ., 1974, 44, 846. Use of Reducing Agents to Retard Yellowing of FWA Wool by Sunlight.

6) Holt, Milligan & Wolfram. TRJ., 1975, 45, 257. Whitening of Wool by Surface Application of Whitener/Polymer.

7) Not found

8) Holt, Leaver & Milligan, TRJ., 1976, 46, 539. Photochem. behaviour of fluorescently whitened wool.