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Non-Conventional Reactive Dye Systems for Attaching Chromophores to Wool, Protein and Polyamide Substrates

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Reactive dyes, which form a covalent bond to the substrate onto which they are applied, have been used in textile applications for many years, and various reactive systems have been researched and commercialised. Reactive dyes also find use in biochemical and biotechnology areas, in particular as biolabels and tracers. This review examines reactive textile dye systems which have not achieved commercial acceptance, and also considers reactive systems that have been proposed solely for biochemical application to proteins.

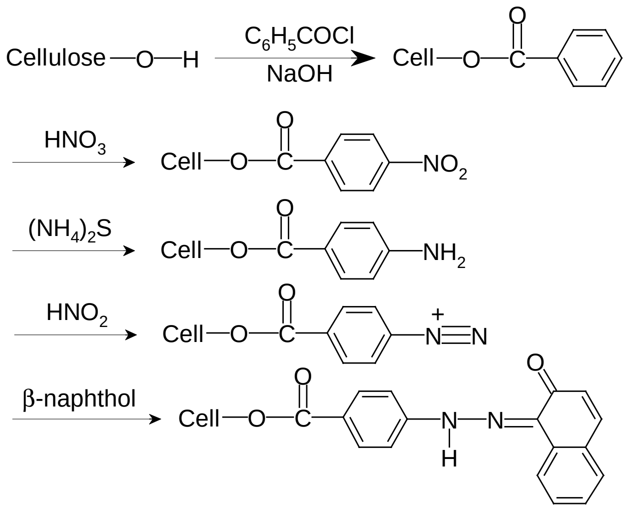
Keywords: reactive dyes, biochemical labelling, polyamides, proteins

INTRODUCTION

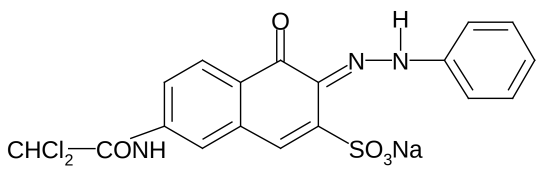
Textile dyes are generally retained on their substrate by physical forces of attraction, mechanical retention, or by covalent bond formation. Reactive dyes constitute the last type, and are characterised by having a group capable of forming a covalent bond between a carbon atom (or, rarely, a phosphorus atom) in the dye molecule and an oxygen, nitrogen or sulphur atom in the substrate. Thus hydroxylic groups in cellulose and protein fibres, amino groups in polyamide and protein fibres, and mercapto groups in protein fibres provide the appropriate substrate heteroatom [1]. Because of the strength of the resultant covalent bonds, reactive dyeings show a very high degree of fastness to washing and wet treatments. In addition, reactive dyes may provide a wide range of bright colours, as dye-fibre reaction allows the use of small dye molecules which are fixed on the fibre without recourse to aggregation [2,3].

The idea of a chemical reaction between dye and substrate to form a covalent bond goes back to the last century [4]. Cross and Bevan suggested in 1895 modifying cellulose by benzoylation and nitration, followed by reduction and finally diazotisation and coupling with β-naphthol (Scheme 1). This process had no practical application because the carboxylic ester group is relatively sensitive to alkaline treatments.

Scheme 1



Further attempts were made but it was not until 1956 and following the work of Rattee and Stephen, that the first recognised range of reactive dyes, the Procion dichloro-s-triazine dyes, were introduced by ICI for use on cellulose fibres. Surprisingly, the first reactive dye, Supramine Orange R **1**, was actually introduced in 1932 by IG Farben for dyeing wool with high wet fastness, but the reactivity of the ω-chlorine atom of the acetamido group was not recognised at the time.



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After Rattee's discovery, the textile reactive dye field developed rapidly and in the last twenty years the concept of substrate-reactive dyes has also been increasingly extended into biochemical and biotechnology areas [5,6]. For example, fluorescent dyes are commonly used as tracers for qualitative and quantitative analysis of biomolecules using highly sensitive analytical methods. For these applications it is generally necessary to chemically link the dye to a biologically active ligand, such as a cell membrane, protein, antibody, enzyme, nucleic acid or other biomolecule to make a conjugate with fluorescence properties which can be detected or measured. Clinical and research applications include the localisation of biological structures by fluorescence microscopy, flow cytometric analysis of cells, measurement of the physiology of cells, determination of DNA sequences and detection and quantification of amines, thiols and phenols in proteins or polymers.

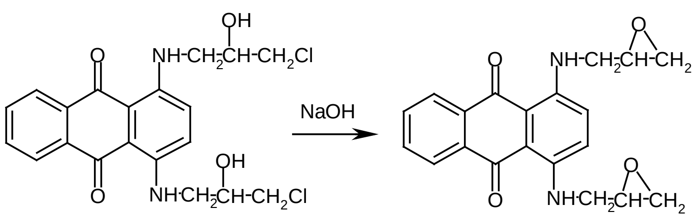
Since their introduction on the market, reactive dyes have been intensively studied and a significant number of new reactive systems have been patented. Commercial reactive groups for wool reactive dyes have been reviewed by Lewis [7-9]. It is the object of this review to examine other reactive textile dye systems which have been proposed for dyeing wool and other polyamide fibres, but have not achieved commercial acceptance, and also to consider reactive systems that have been proposed solely for biochemical application to proteins. For convenience these are all referred to as non-conventional reactive dye systems.

NON-CONVENTIONAL REACTIVE SYSTEMS

Chorohydrin and alkyl halide dyes

These dyes react through a mechanism of nucleophilic substitution [10] and the chlorohydrin group is converted during the process of application (alkaline conditions) to an epoxide reactive group. An example is the dye C.I. Reactive Blue 6, which was at one time commercially available (Scheme 2).

Scheme 2



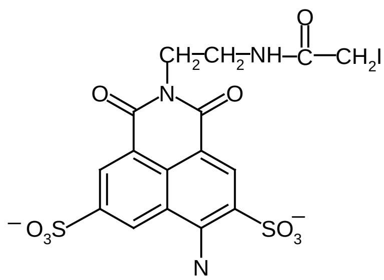
The chromophores mostly used with this system were anthraquinones, phthalocyanines (in some cases having two reactive groups) and azo dyes. In the last, the reactive group could be attached to the coupling component or the diazo component. The reactivity of these dyes could be increased by replacing the chlorine atom by bromine [11] and such dyes were applied to wool, nylon, cotton and polyester as substrates [12-15]. In the case of polyester, the dyes were behaving as conventional disperse dyes.

In a somewhat different approach, reactive halides have been used as pretreatments of wool to improve its dyeing properties. Thus a water soluble cationic polyamide resin (Hercosett 57) has been suggested [16]. During the process of treating the wool with Hercosett 57 (an adipic acid-diethylenetriamine-epichlorohydrin copolymer), an azetidinium cation is formed, which reacts with nucleophilic groups in the wool and also with further secondary and tertiary amino groups in the polymer itself to form a resin with a high percentage of crosslinks with the wool fibre.

Cegarra and Riva demonstrated that wool treated with Hercosett 57 allows a rapid absorption of reactive dyes, even at low temperatures [17], and Shirasawa et al. were able to obtain dyeings with excellent levelling on polyamides using mixtures of epichlorohydrin compounds and dichloropropanol [18]. Fries observed the formation of dye-fibre covalent bonds during the dyeing of Hercosett-treated wool with reactive dyes of the difluorochloropyrimidine and vinylsulphone types [19].

Lucifer yellow iodoacetamide (**2**), a water-soluble dye, is readily conjugated to thiols of low-density lipoproteins (LDL) and has been used in a flow cytometry assay of accessible thiols on the cell surface [20].

Several reactive halide groups have been used in biolabelling. Bimane dyes (**3**, **4**) having a bromomethyl group are essentially nonfluorescent until conjugated with thiols including glutathione [21-23], plasma thiols [24] and peptides [25]. The dyes absorb at 390-400 nm and after conjugation, they emit in the range 475-485 nm [26].



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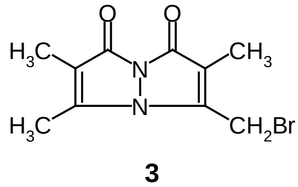

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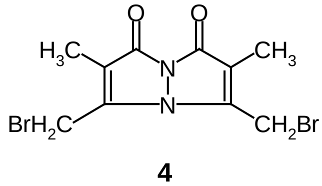

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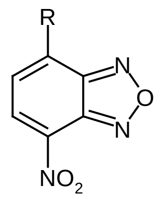
The dibromobimane (**4**) is a crosslinking reagent for proteins and has been used to crosslink thiols in haemoglobin [27] and myosin [28,29].

Aryl halides such as 4-chloro-7-nitrobenzo-furazan (**5**) and 4-fluoro-7-nitrobenzofurazan (**6**) are useful reagents for fluorogenic derivatization of amines [30]. They absorb in the U.V. range (around 330 nm) but after conjugation with primary and secondary aliphatic amines, they absorb at 465 nm and 485 nm, respectively. Examples include the reaction with lysine [31], glycine [32], proline and hydroxyproline [33,34]. These reagents have found application in the chromatographic analysis of amino acids [35] and the enantiomeric separation of D, L-amino acids on a chiral column [36].

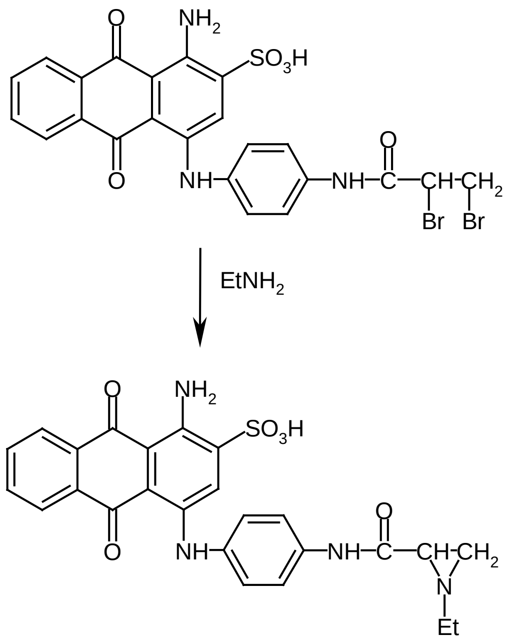


5 (R = Cl) **6** (R = F)

Aziridine dyes

This class of dye is based on the aziridine group as a reactive system and has been proposed for application to wool and polyamides [37,38]. The synthesis of the aziridine group involves the reaction of a 2,3-dibromopropanamide derivative with ethylamine in aqueous medium at 80 °C (Scheme 3).

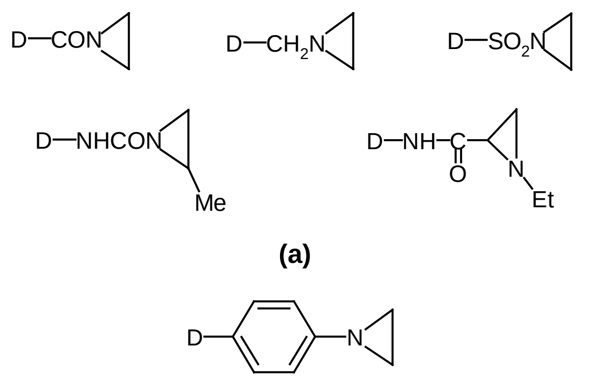
Scheme 3



Several reactive dyes containing aziridine groups attached to a dye molecule by various bridging groups (Figure 1a) have been described, and recently Burkinshaw and Hallas patented dyes in which this group is directly connected to the conjugated system [39] (Figure 1b).

These authors studied the application of these dyes on various substrates (from an aqueous dyebath) and found that the wash fastness on nylon 6.6 was superior to that obtained from conventional disperse dyes of related structure.

Figure 1 (D = chromophore)



(a)

(b)

Azide dyes

Reactive dyes containing a sulfonylazide group were initially applied to cellulose through a nucleophilic substitution reaction (Scheme 4).

Scheme 4

$$\text{Cel}-\text{O}^- + \text{Dye}-\text{SO}_2\text{N}_3 \longrightarrow \text{Cel}-\text{O}-\text{SO}_2-\text{Dye} + \text{N}_3^-$$

Subsequently it was observed that this reactive group could show a different behaviour under the effect of UV light or high temperatures. Under these conditions this group decomposes generating nitrogen and a highly reactive nitrene species that takes part in various chemical reactions, and was utilised for reactive dyeing of nylon [40,41], propylene and polyester [42,43]. In 1974, Griffiths et al. patented a range of reactive dyes based on the sulfonylazide group for dyeing nylon, polyamide and polyester [44].

Griffiths and McDarmaid studied the application of these dyes to nylon, having suggested that the sulphonylazide group acts through two different mechanisms, depending on the application conditions [45,46]. Thus, at temperatures up to 120 °C the azide ion can be easily replaced by a nucleophilic species, and in the case of polyamide substrates this results in attachment of the dye to the substrate by a sulphonamide linkage (Scheme 5). However, at higher temperatures (ca. 170 °C) or under the influence of UV light, cleavage of the azide group can occur to give a reactive nitrene which undergoes insertion into C-H bonds of the polymer (Scheme 6). Both mechanisms result in the formation of a sulphonamide bridge between the dye and the substrate.

Scheme 5

$$\text{Nylon}-NH_2 + \text{Dye}-SO_2N_3 \longrightarrow \text{Nylon}-NH-SO_2-\text{Dye} + N_3^-$$

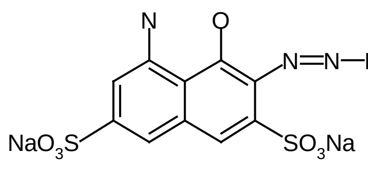
Scheme 6

$$\text{Dye}-SO_2N_3 \xrightarrow[h\nu]{200\ °C} \text{Dye}-SO_2-N + N_2$$

$$\text{Dye}-SO_2-N + R-H \longrightarrow \text{Dye}-SO_2-NH-R$$

This type of reactive group is of special interest due to the fact that all polymeric textiles have C-H bonds and so should be capable of providing a higher percentage of dye-fibre fixation in comparison with conventional reactive systems.

Milligan and Holt studied the application of reactive azo dyes containing azidophenyl **7a**, azidosulphonyl **7b** and diazidotriazinyl **7c** substituents [47]. It was observed that only the azidophenyl dyes reacted photochemically (or by heating at 200 °C) with wool fibres, but in low yields only. In some cases the process was accompanied by a change in colour which is technically undesirable. The dyes **7b** and **7c** undergo chemical reaction during the preliminary application process, and consequently post-dyeing irradiation or heating causes little additional fixation.



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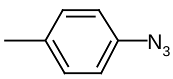
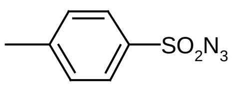
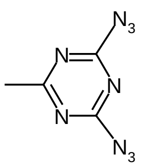
R = 

7a

R = 

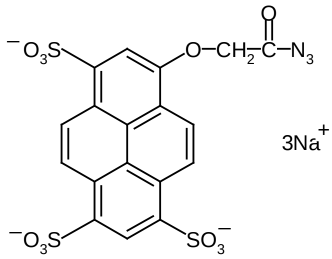
7b

R = 

7c

The temperatures at which thermolysis of the sulphonylazide group takes place in reactive dyes have been investigated and it was found that there was no correlation between the dye structure and the decomposition temperature, which occurred over a range of 25 °C [48].

In general, such short-wavelength photoreactive groups are not recommended in amine reactive fluorophores for preparing bioconjugates, as UV light causes photodegradation of the fluorophore. This is not so problematical with blue fluorescers, and for example, Cascade Blue acetyl azide (**8**) is a reactive dye for preparing blue fluorescent bioconjugates and has been suggested for identifying proteins located on extracellular cell surfaces [49,50]. Blue fluorescent probes provide a contrasting colour that can be distinguished from the orange or red fluorescence of longer-wavelength probes.



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Isothiouronium and isothiocyanate dyes

Guise and Stapleton studied the application of reactive dyes containing the isothiouronium group (Thiolan dyes) to wool [51,52]. These dyes are easily synthesised (Scheme 7) by reaction of an alkyl halide (R-X) with thiourea, but do tend to hydrolyse rapidly in alkaline solution to give a thiol.

Scheme 7

$$R-X + S{=}C(NH_2)_2 \longrightarrow R-S{=}C(NH_2)_2^+\ X^-$$

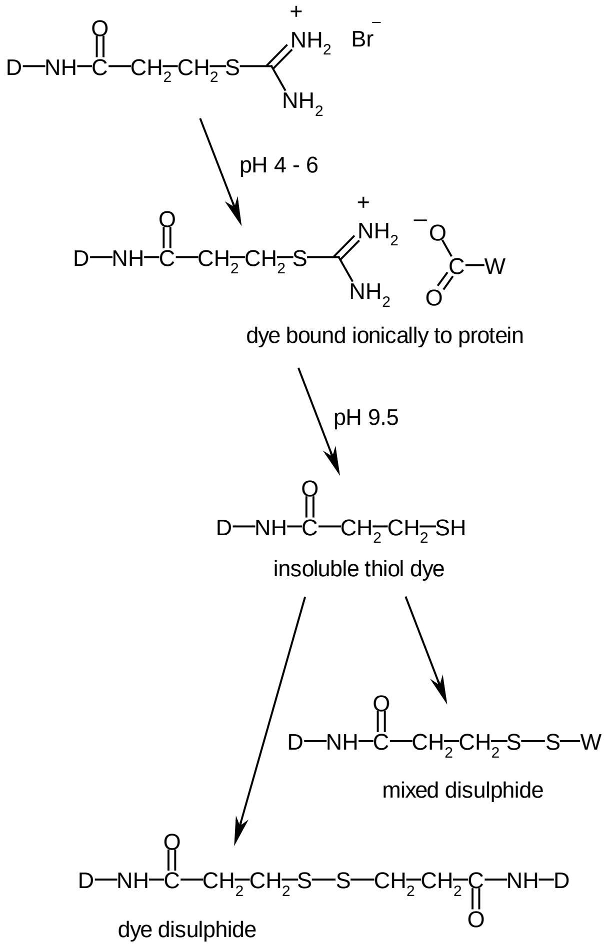
$$\xrightarrow{OH^-} RSH$$

Reactive dyes of this type had previously been patented as printing dyes for cotton (Alcian dyes, ICI). Their application to wool was impractical though, due to their decomposition during the dyeing process, producing insoluble products. This behaviour was attributed to the fact that Alcian dyes were benzylisothiouronium salts (with a benzylic bridge between the reactive group and the chromophore). Thus SN1 hydrolysis could occur readily due to resonance stabilisation of the "benzylic" cation.

With the objective of applying these dyes to wool, Stapleton suggested the incorporation of the propionamido bridging group ($-NHCOCH_2CH_2-$) in order to establish a bridge between the reactive group and the chromophore which was able to resist hydrolysis during typical wool dyeing conditions (boiling; pH<7).

In the application of these dyes to wool (Scheme 8), at pH 4–6, the isothiouronium group has a protecting effect on the wool thiol groups and it also helps to solubilise the dye thus permitting good dye diffusion in the fibre. Under basic conditions (pH 9.5) cleavage of the isothiouronium group occurs, producing an insoluble thiol which is able to react covalently with the disulphide groups in wool.

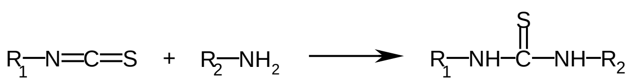
Scheme 8 (D = dye, W = wool)



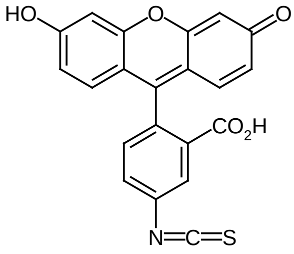
Although the aim was to develop a process involving dye-fibre fixation through –S–S– bonds, Stapleton concluded that the extent of this reaction was minimal (20%), and that the good wash fastness of this type of dyes (Thiolan) was essentially due to their insolubilisation inside the fibre after hydrolysis of the isothiouronium group.

Isothiocyanates (R–NCS) can react with amine residues of proteins forming thioureas which are moderately stable (Scheme 9).

Scheme 9



Isothiocyanate dyes, such as fluorescein-5-isothiocyanate (FITC) (**9**) and tetramethylrhodamine-5-isothiocyanate (**10**), are the most widely used fluorescent labelling reagents. Some applications of FITC include its conjugation to oligonucleotides as hybridisation probes [53], its use in amino acid sequencing [54], and in the separation of amino acids by capillary electrophoresis [55,56].



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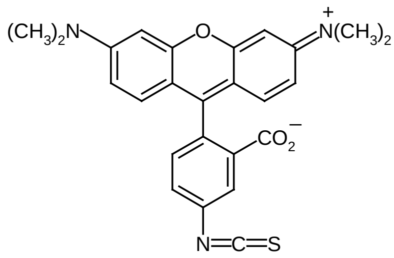

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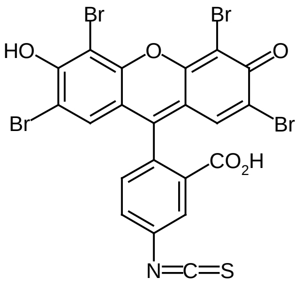
Other chromophores with isothiocyanate reactive groups have been suggested as amine-reactive probes for preparing immunoreagents. Examples include eosin-5-isothiocyanate (**11**) and erythrosin-5-isothiocyanate (**12**) to study protein and virus particles in solution and in membranes [57-60], rhodamine-6-isothiocyanate (**13**) for DNA sequencing applications [61], and malachite green isothiocyanate (**14**) for antibody conjugation [62].



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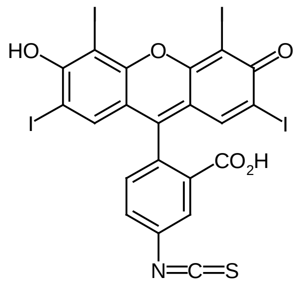

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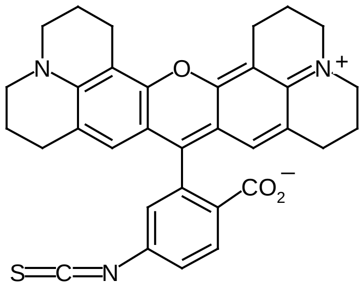

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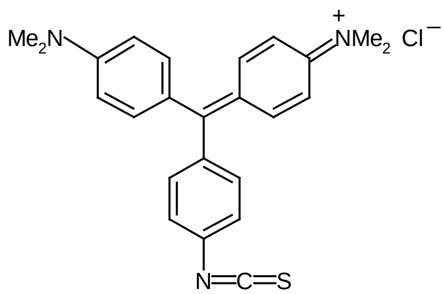

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Methylol dyes

A good reactive dye should produce a dye-substrate bond which has a high degree of resistance to hydrolysis. However many of the conventional reactive dyes used for the continuous dyeing of polyester/cotton blends are unstable under the conditions needed to promote the dye-fibre reaction (alkaline medium). The appearance of the first reactive dyes containing methylol groups gave an alternative to conventional reactive dyes because they could be applied to cellulose under slightly acidic conditions. These dyes (Calcobond) contained N-methylol end groups and were applied to cellulose at pH 5–6 under dry-heat conditions [63] (Scheme 10).

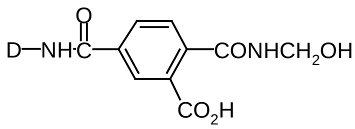
Scheme 10 (D = dye, Cel = cellulose)

$$D{-}NH{-}CH_2OH + H_3O^+ \rightleftharpoons D{-}NH{-}CH_2^+ + 2\,H_2O$$

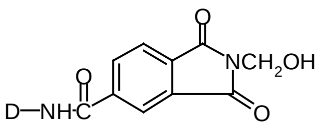
$$D{-}NH{-}CH_2^+ + H_2O + Cel{-}OH \rightleftharpoons D{-}NH{-}CH_2{-}O{-}Cel + H_3O^+$$

Subsequently, reactive dyes containing methylol carbamoyl groups (Figure 2a) for polyester/cotton blends were patented [64]. In spite of the high dye-fibre fixation the resultant ether bonds were hydrolysed easily in acidic media thus inhibiting commercial application of these dyes. In 1974 Yamamoto claimed the application of reactive dyes based on N-hydroxymethylol groups linked to a phthalimide nucleus (Figure 2b) for dyeing polyamide, wool and silk [65].

Figure 2 (D = chromophore)



(a)



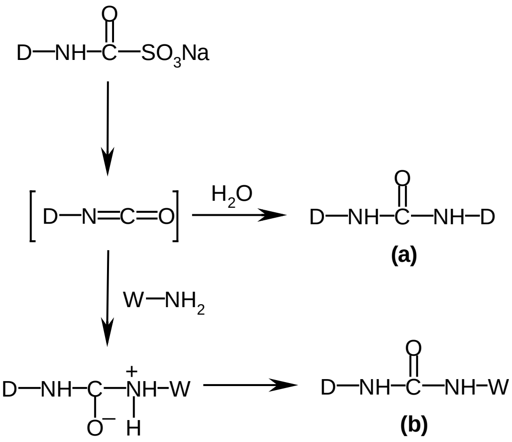
(b)

Carbamoyl dyes

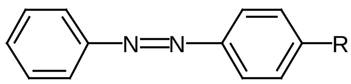
In the field of non-conventional reactive dyes, acylating compounds have been used, particularly acyl halides [66], mixed anhydrides [67] and isocyanates, in order to induce the establishment of covalent bonds with nucleophilic groups in the textile fibres. Unfortunately, in the majority of cases these reactive intermediates are easily hydrolysed, which of course compromises their commercial application.

Kirkpatrick and MacLaren applied reactive dyes with carbamoyl sulphonate groups to wool, and obtained fixation to the fibre [68-70]. The carbamoyl sulphonate group can be considered as a protected isocyanate group and it was suggested that the reaction occurred through the formation of a urea with the amino groups in wool (Scheme 11b). Simultaneously, an insoluble urea linked dimeric dye is formed through the interaction of two molecules of the dye (Scheme 11a).

Scheme 11 (D = chromophore, W = wool)



Subsequently, the same authors studied the application of similar dyes containing azobenzene, anthraquinone and pyrazolone chromophores which generate the same reactive group when applied to the fibre [71] (**15a** and **15b**). Dyeing on wool was followed by radiotracer techniques with ^{14}C and good fixation yields were observed (wash fastness rating 3-4; ISO3) in contrast to similar dyes (**15c**) which lacked the reactive group (wash fastness rating 1; ISO3).



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15a R = $-NHCOSCH_2CO_2H$

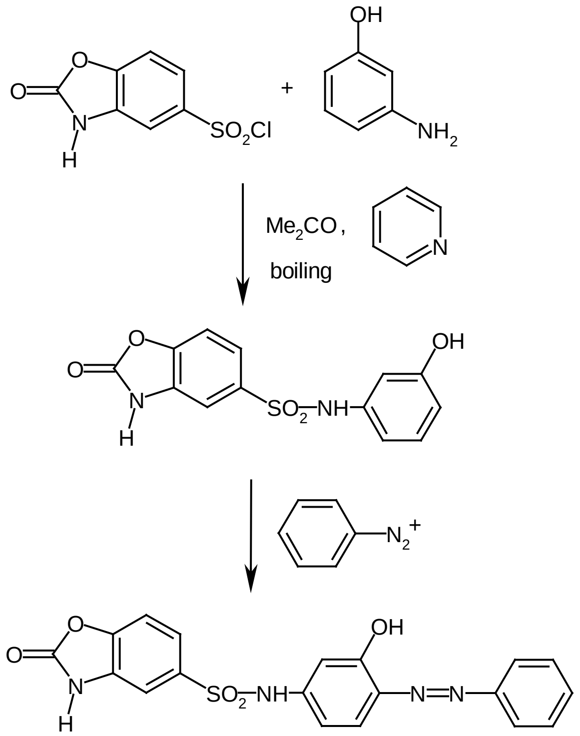
15b R = $-NHCOSCH_2CH_2SO_3H$

15c R = $-CO_2H$

Oxazolone dyes

Simov and Kalcheva synthesised a series of reactive dyes containing oxazolone groups [72] (Scheme 12) which were applied to nylon and wool under slightly acidic conditions. The dyed fibres were submitted to extraction with acetone and the results indicated the establishment of covalent bonds between the dye and the substrate. According to the authors, in the case of wool the reaction occurred through the opening of the oxazolone ring and reaction with protein amino groups leading to the formation of a urea linkage between the dye and wool fibre.

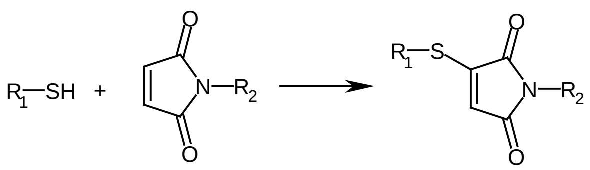
Scheme 12



Maleimide dyes

Zahan and Altenhofen described a synthetic route to dyes for wool containing maleimide groups as the reactive system [73]. In fact, the addition of a thiol group to the activated double bond of maleimides (Scheme 13) is a known reaction, and is used for the quantitative determination of thiol groups in proteins [74-78].

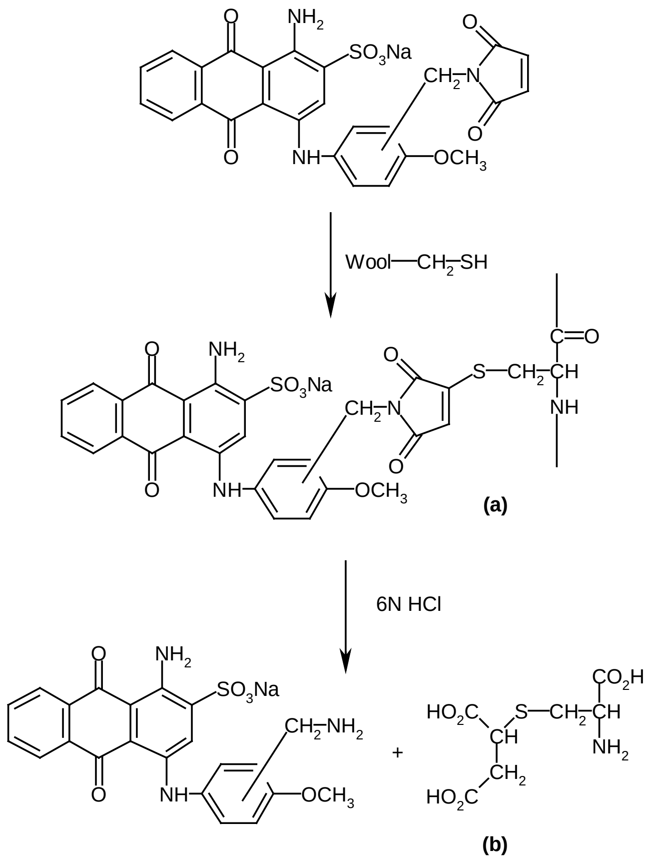
Scheme 13



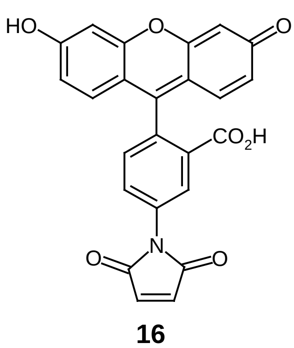
Finnimore demonstrated that dyes containing substituted maleimide residues (Scheme 14a) could react with the cysteine residues of wool at 80 °C in slightly acidic aqueous media with good fixation yields [79]. The decrease in thiol group content of the wool and the identification of *S*-succinylcysteine (Scheme 14b) in the wool hydrolysate proved that reaction occurred between the dye and the cysteine residues.

This author also suggested that the maleimide group could react with the amine groups of wool, particularly, with the ε-NH_2 of lysine, based on the fact that good fixation was also observed on nylon fibres. However, this suggestion needs further confirmation.

Scheme 14



Thiol-reactive dyes with maleimide groups are used to prepare fluorescent peptides for probing biological structures. Examples include fluorescein-5-maleimide (**16**) [80] and tetramethylrhodamine maleimide (**17**) [81,82], which have been suggested for preparing fluorescent thiol conjugates of biomolecules. Eosin-5-maleimide (**18**) [80] has been reported to react selectively with a lysine residue of band-3-protein in human erythrocytes, inhibiting anion exchange in these cells [83,84]. The coumarin maleimide dye (**19**) has been used in a flow cytometry assay for nucleolar protein staining in order to distinguish highly proliferating cancer cells [85], and also to determine the thiol content of proteins, cells and plasma [86-89].



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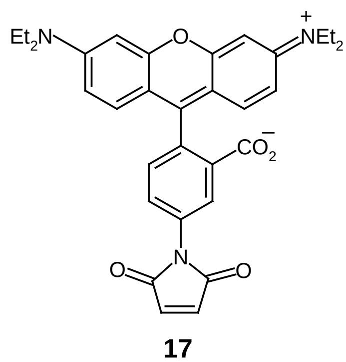

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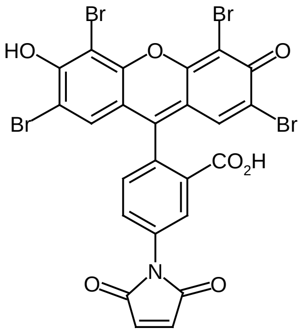

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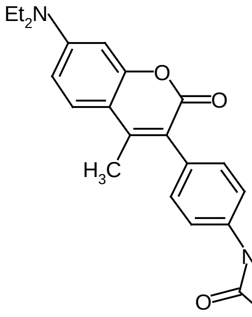


19

Thiosulphate dyes

Dyes with thiosulphate reactive groups react with wool cysteine residues to produce mixed disulphides (Scheme 15a) which can undergo partial disproportionation to symmetrical disulphides [90] (Scheme 15b). The dye "Wool Fast Turquoise Blue SW" (Hoechst), now withdrawn from the market, is one example of this type of dye and was based on the sodium N-ethylthiosulphate derivative of the sulphonamide group ($–SO_2–NH–CH_2–CH_2–S–SO_3Na$) as the reactive system. The sodium bisulphite formed in the first reaction (Scheme 15a) reduces the cystine residues, so generating new cysteine residues available to react with the dye. As a consequence the dyeings show high wash fastness.

Scheme 15 (D = chromophore)

$$\mathrm{D{-}SSO_3Na} + \mathrm{HS{-}Wool} \longrightarrow \mathrm{D{-}S{-}S{-}Wool} + \mathrm{NaHSO_3}$$

(a)

$$2\ \mathrm{D{-}S{-}S{-}Wool} \longrightarrow \mathrm{D{-}S{-}S{-}D} + \mathrm{Wool{-}S{-}S{-}Wool}$$

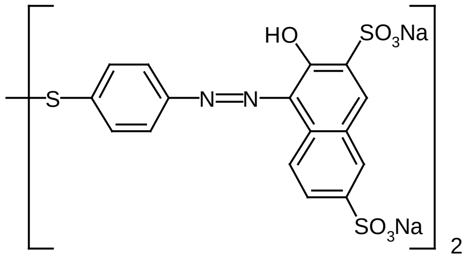
(b)

Disulphide dyes

Asquith et al. studied the application of reactive dyes containing disulphide groups to wool (**20**, **21**) and suggested that formation of mixed disulphides between the dye and the cysteine residues was occurring [91]. To ensure efficient reduction of cystine to cysteine residues, the method used thioglycollic acid ($HSCH_2CO_2H$) in the dyeing bath (1 hour at 60 °C).

Studies using different concentrations of the dye suggested that two mechanisms were involved: when the thiol concentration is in excess, the disulphide dye is completely reduced by the thiol, so driving the reaction to the right (Scheme 16a); when the two reactants are present in roughly equal amounts, a mixed disulphide between the dye and –SH groups of cysteine residues is formed (Scheme 16b).

However, the authors recognised that the disulphide dyes underwent disulphide exchange under the conditions used only at high concentrations of dye, and even then, only slight covalent bonding was achieved.



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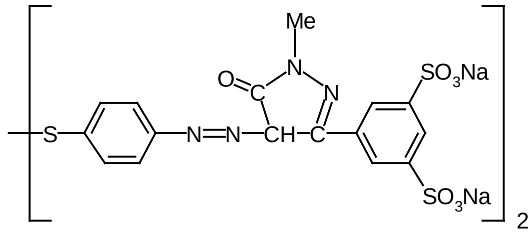

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Scheme 16 (D = chromophore)

$$\mathrm{D{-}S{-}S{-}D} + 2\ \mathrm{HS{-}Wool} \longrightarrow 2\ \mathrm{D{-}SH} + \mathrm{Wool{-}S{-}S{-}Wool}$$

(a)

$$\mathrm{D{-}S{-}S{-}D} + \mathrm{HS{-}Wool} \longrightarrow \mathrm{D{-}SH} + \mathrm{D{-}S{-}S{-}Wool}$$

(b)

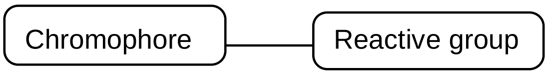
Dyes which react by amide formation

Reactive dyes containing phosphonic acid groups for cellulose fibres were discovered in 1970 by the Stanford Research Institute, California, USA, and their application was developed in subsequent years [92]. In 1978, ICI introduced this class of dye to the market [93] (previously as Procion T dyes, later as Procilene dyes) which were mixtures of water soluble phosphonic acid – substituted dyes and conventional disperse dyes, to be used on polyester/cellulose blends. The thermofixation process (200 °C) involved the use of cyanamide and dicyanamide to promote the dye-fibre reaction. In 1979, Burlington Industries patented dyes with phosphonic groups containing a sulphonamide bridge between the chromophore and the reactive system to be used on rayon, nylon and wool [94]. In general, these dyes were azo dyes in which the phosphonic group was linked to the diazo component, or occasionally to the coupling component [95].

In 1978, a new class of reactive dyes was patented with carboxylic acid groups, for application to substrates containing hydroxy groups, especially cellulosics and organic polymers containing two or more hydroxy groups [96]. The authors claimed that fixation (200 °C; cyanamide) was also obtained on wool and nylon but the depth of shade was not as good as on rayon.

Compared to conventional reactive dyes, dyes with acid groups (phosphonic or carboxylic) have a simpler structure (Figure 3).

Figure 3

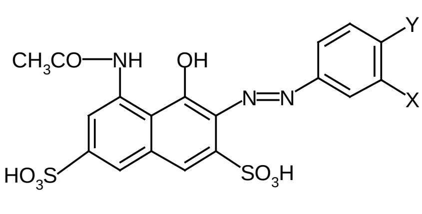


In practice, however, it is necessary to ensure the presence of additional groups to provide good solubility in water. The main advantages of these dyes are the low cost of their synthesis, their low molecular weight, the purity of shade and high fibre diffusion rate. As with conventional reactive dyes, these dyes may contain more than one reactive group. The phosphonic group ($–PO_3H_2$) may be substituted by a phosphoric group ($–OPO_3H_2$) as both are effective in the dye-fibre reaction.

The reaction between phosphonic and carboxylic acid dyes and cellulose and protein fibres has been studied by several authors. The use of carbodiimides as an aftertreatment of dyed wool to promote the dye-fibre reaction [97,98] and to improve the intrinsic cosmetic qualities of bleached human hair [99] have been described. Bello found the same results on nylon 6,6 suggesting the application of these dyes in the pH range of 4.5 – 7.4 to avoid hydrolysis [100]. He also concluded that the extent of dye-fibre reaction was strongly dependent on the time and the temperature used for the dyeing process.

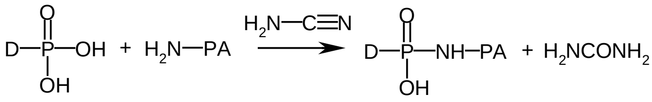
Nkeonye studied the application of these types of dyes to cellulose (acid dyeing, pH 5 – 6) and obtained results as good as for Procilene dyes [101,102]. The same author studied the fixation of these dyes on wool and nylon by thermofixation techniques (2 minutes at 195 °C) in the presence of cyanamide compounds [103] (Scheme 17a). He concluded that the fixation was more efficient on nylon and the high alkali-fastness of the nylon dyeings indicated covalent attachment of the dyes to the substrate (Scheme 17b and 17c). In the case of wool, the wash fastness of the dyeings exhibited only moderate resistance to alkali.

Scheme 17 (D = chromophore, PA = polyamide)

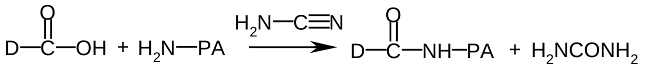


X	Y
$PO(OH)_2$	H
OH	CO_2H

(a)



(b)



(c)

Previously, Soiron had already suggested the used of carbodiimides in association with other additives to increase the fixation yield of trichloropyrimidine dyes on wool and nylon [104,105]. This process involves the aftertreatment (60-80 °C; pH 4.0 – 5.5) of the dyed fibres with mixtures of dicyandiamide, urea, $(ClCH_2CH_2)_2O$, $Me_2NCH_2CH_2N(Me)CH_2CH_2NMe_2$, NH_4Cl and HCHO, which fixed the unreacted residual dye on the fibres without injuring them. According to the author, this method allows more exact colour control than conventional methods and permits direct drying of the aftertreated fibres.

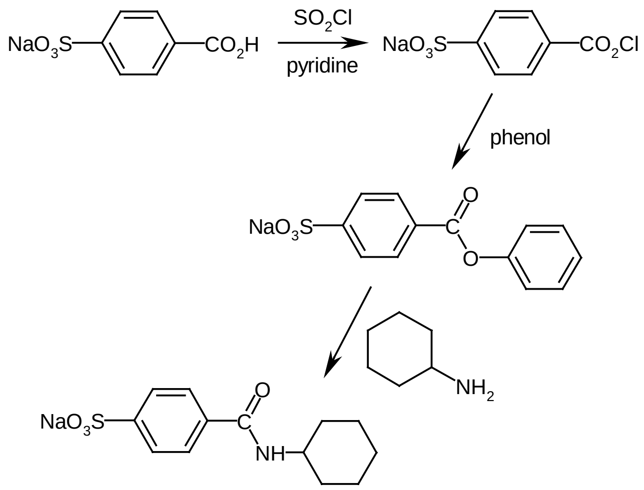
The light fastness of reactive dyeings with carboxylic and phosphonic acid dyes, on cellulose, is of the same order as that obtained with conventional reactive dyes containing similar chromophores [106, 107] and it would be of particular interest to study the application of these dyes in other substrates such as wool.

Reactive carboxylated dyes for cellulose are not available on the market. According to the dyeing process [96], the concentration of additives and reactants necessary to promote the dye-fibre reaction was inconsistent and changed from dye to dye. However, the main reason that prevented their commercial exploitation was the low alkali stability of the dye-cotton ester bond [103].

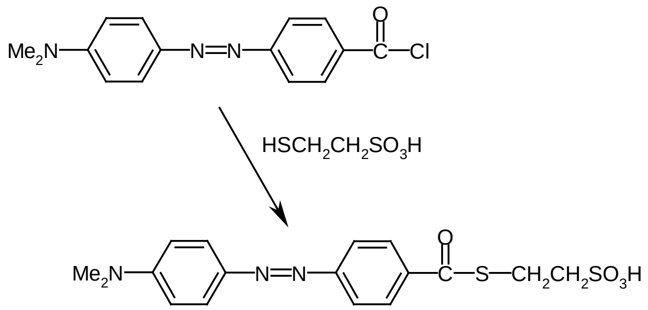
Compounds containing active esters are very popular in peptide synthesis for obtaining amides by reaction with amines. Generally, these reactions are carried out at or below room temperature under anhydrous conditions. Caldwell and Milligan studied the application of these compounds to wool and synthesised soluble carboxylic esters from acyl chlorides and phenol and obtained the corresponding amide by reaction with cyclohexylamine [66] (Scheme 18). The reaction of the active esters with wool (aqueous medium; boil) was unsatisfactory since most of the dye was lost by competitive hydrolysis in the dyebath.

It was subsequently found to be possible to obtain good results on wool with similar dyes using soluble active thioesters [71]. These compounds were obtained by reacting the acylating agent with the appropriate thiol, and were resistant to hydrolysis (Scheme 19).

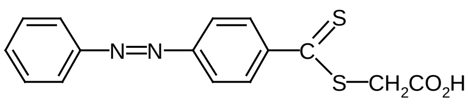
Scheme 18



Scheme 19



Leon and co-workers patented reactive dyes containing carboxyalkylcarbodithioates (**22**) as active esters for dyeing keratinous fibres [108]. The reactive dyes were used to dye hair and wool in fast shades by first reducing the substrate, or reducing it during dye application. Subsequently, Ciba patented similar dyes for wool by incorporating a halogenotriazinyl radical in the dye molecule [109] (**23**).



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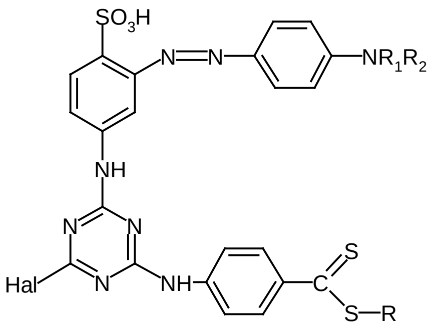


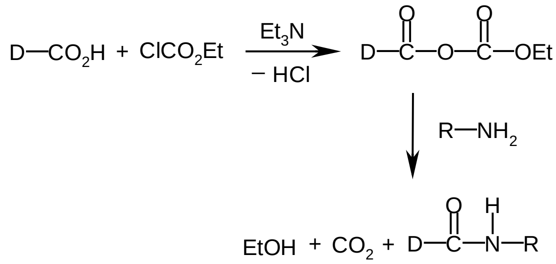
Hal = halogen

R = $C_nH_{2n}CO_2H$, $C_nH_{2n}CO_2Me$, CH_2CH_2OH

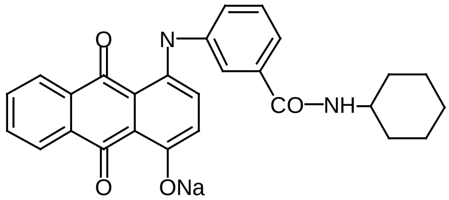
23

Dyes containing carboxylic acid groups have the potential to react with the amino groups of wool and nylon fibres by prior activation with ethyl chloroformate (forming a mixed anhydride). Recently, Gomes et al. prepared dyes containing carboxylic acid groups (Scheme 20) derived from J acid, H acid, pyrazolone and anthraquinone chromophores, and applied them to fibres by the mixed anhydride method [67].

Scheme 20 (D = chromophore, R = nylon, wool)



The scope of this reaction has also been examined in solution by reacting the carboxylic acid dyes with cyclohexylamine and lysine as model amine substrates. The expected amides (such as **24** and **25**) were formed in good yields and were isolated and characterised.



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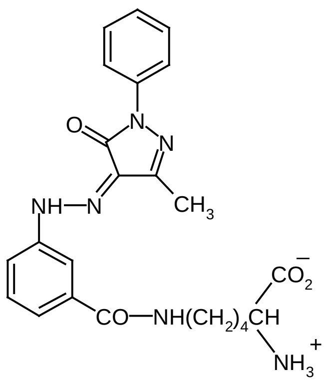

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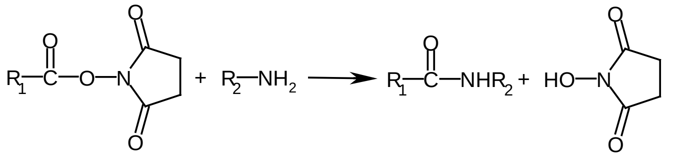
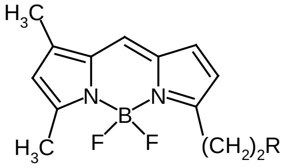
The mixed anhydrides were able to react with the nucleophilic groups in wool (aqueous medium; 100 °C), particularly amino groups, to form covalent bonds (amide link) between the dye and the substrate, which were resistant to alkaline hydrolysis (ISO 105 CO3 test). According to the authors, a pH value of 4 – 6 for fixation is an advantage, since it means that there is less wool damage.

Succinimidyl esters are important amine-reactive probes because the amide bonds they form with aliphatic amines are very stable (Scheme 21). There is a wide range of reactive dyes of this type available for use as immunoreagents, including, for example, the BODIPY dye (**26**) [110] and 5-carboxyfluorescein succinimidyl ester (**27**) for automated DNA sequencing [111-113], Rhodol Green (**28**) [114], Texas Red-X (**29**) [115] and 7-diethylaminocoumarin (**30**) [116] as derivatives for protein conjugates.

Scheme 21





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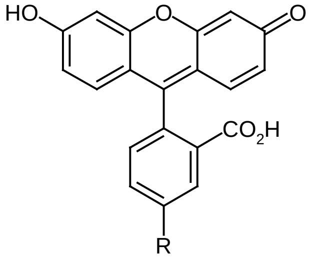

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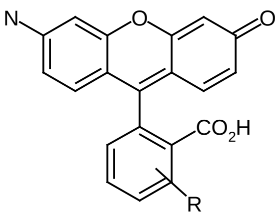

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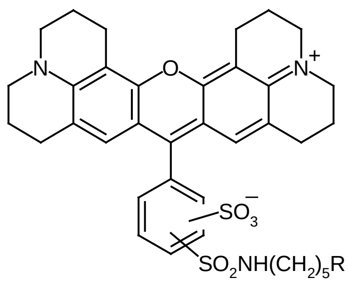

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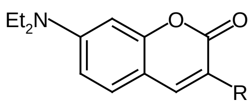

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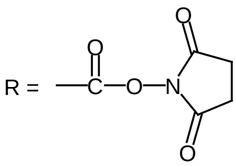
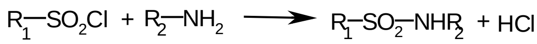
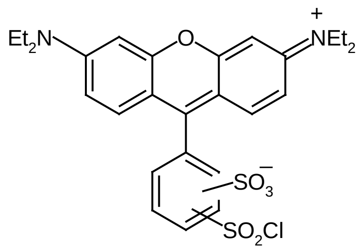


The sulfonyl chloride reactive group is also much used in dyes for protein labelling, although it is too water sensitive for textile applications. Biolabelling examples include long-wavelength fluorophores of the rhodamine type, such as Lissamine Rhodamine B sulfonyl chloride (**31**) and Texas Red sulfonyl chloride (**32**) [117]. Such dyes react with amino groups in proteins to form stable sulfonamides, and are used as reactive probes for preparing protein conjugates in fluorescence microscopy and flow cytometry. Isosulfan Blue sulfonyl chloride (**33**) is an example of a non-fluorescent dye of this type, useful for the preparation of photosensitising conjugates [118-a].

Dyes (**31**) – (**33**) are all used as their mixed isomeric sulfonyl chlorides.

Scheme 22





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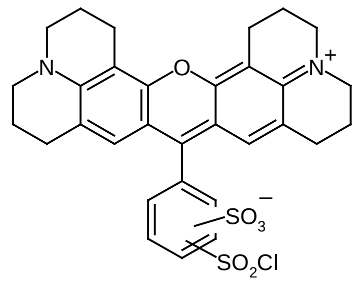

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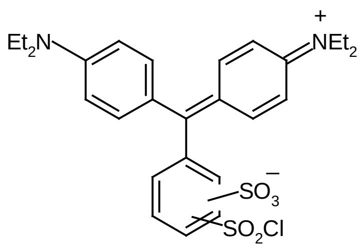
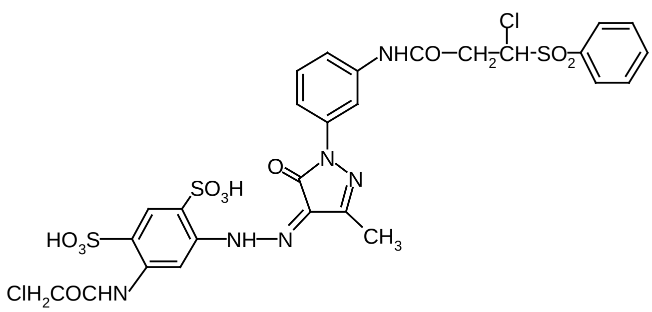


33

Polyfunctional reactive dyes

With the goal of improving the efficiency of dye-fibre fixation, bifunctional reactive dyes have been cited in the patent literature for many years. The use of dyes containing two reactive groups of either different or identical nature has been claimed for application to cellulose fibres [119-121]. For wool fibres there are on the market bifunctional reactive dyes from the Lanasol range, which contain two α-bromoacrylamido groups. Other reactive groups (34,35) have been suggested [122,123] but have had no commercial success.



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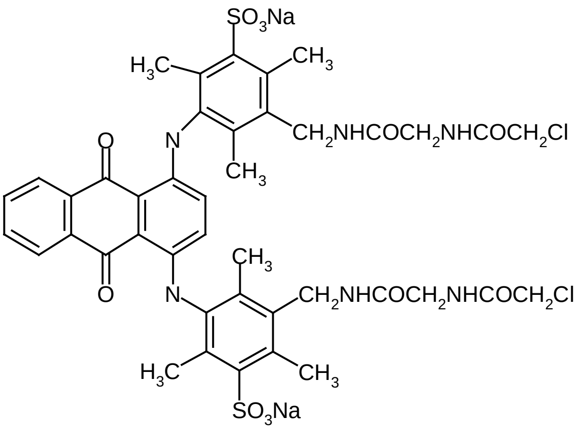
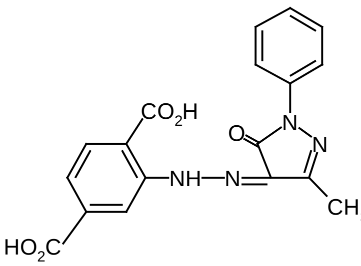


35

Recently, Moura et al. suggested the use of *bis*-carboxylic reactive dyes (**36**, **37**), based on 2-aminoterephthalic acid, for wool and polyamides [124]. The dyes were applied to the fibres by prior activation of the carboxylic group using the mixed anhydride method, and dye fixation was higher with the *bis*-reactive dyes when compared with the monocarboxylic derivatives.



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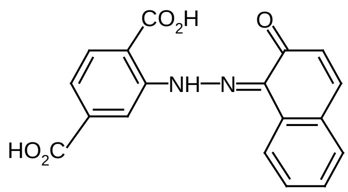
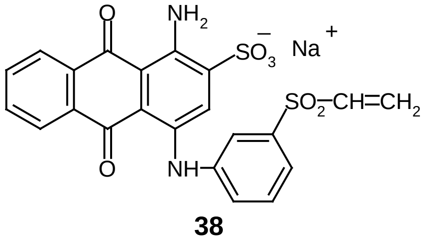


37

Vinylsulphone dyes

In 1952, Hoechst introduced their Remalan range of reactive dyes for wool, based on the vinylsulphone group or its β-sulfatoethylsulphone precursor. These dyes form very stable thioether adducts with proteins. The vinylsulphone reactive functionality has also been used in biolabelling dyes, examples being Uniblue A vinyl sulfone (**38**) [118-b] and the lithium salt of Lucifer Yellow VS [125], as water-soluble reagents for modifying thiols or amines.



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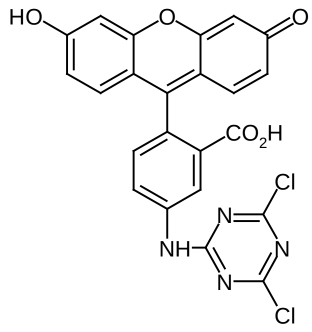
Dichloro-s-triazine dyes

Halo-s-triazines represent an important class of commercial reactive dyes for cellulose fibres, in which the labile halogen atom is displaced by a hydroxylic group of cellulose. This system is also highly reactive towards the amino groups of proteins and dichloro-s-triazinylaminofluoresceins (**39**) have been proposed for preparing protein conjugates [126-128].



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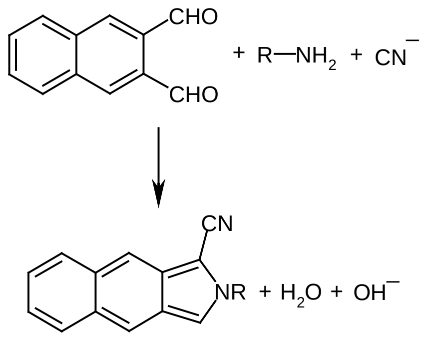
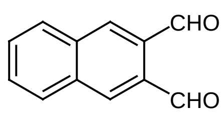
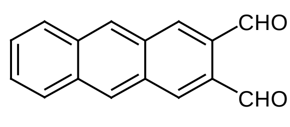
Aldehyde dyes

Aromatic aldehydes can be used as reagents for analysis of low molecular weight amines. Naphthalene-2,3-dicarboxaldehyde (**40**) [129], anthracene-2,3-dicarboxaldehyde (**41**) and 3-(4-carboxybenzoyl)-quinoline-2-caboxaldehyde (**42**) are non-fluorescent until they are reacted with a primary amine in the presence of cyanide or thiols to form highly fluorescent isoindoles.

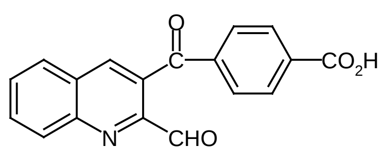
Scheme 23







40 **41**



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Applications include the derivatisation of amines separated by HPLC [130] or capillary electrophoresis [131,132], and the detection of amines in glycoproteins [133] and in human cerebrospinal fluid [134]. The determination of cyanide in blood and urine has been reported [135-138].

CONCLUSIONS

Reactive dyes share only a small portion of the wool market and wool is still dyed mainly with acid dyes and mordant dyes. The brightness and fastness qualities achievable with reactive dyes have to be set against their high cost and the care required to apply them. However, due to current pressures to minimise environmental problems (e.g. restriction of the use of chromium salts and premetallised dyes) there may be an increase in the consumption of reactive dyes in the future. The achievement of maximum levels of dye-fibre fixation is still a goal, and thus the importance of polyfunctional reactive dyes will also be increasingly recognised. In the biochemical area, the use of reactive groups to covalently link dyes, especially fluorescent dyes, to proteins, peptides and other biomolecules is of major importance and continues to grow. Covalent attachment of non-dye functional molecules to biomolecules is also of major significance (e.g. attaching toxins to cell-targeting peptides), and whilst colour is not involved, the reactive chemistry involved is identical to that used for reactive dyes. In such highly specialised applications cost is not a prime consideration, and thus a much wider range of functional groups can be envisaged than is feasible in textile dyes. Thus it is likely that new developments in reactive dye chemistry are most likely to arise in the areas of biochemistry, molecular biology and diagnostics.

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