5
Wool chemistry

W S SIMPSON

5.1 General introduction

This chapter describes the effects of radiation and heat on wool and its response to acids, alkalis, oxidants, reductants, followed by a brief treatment of specific reactions of its amino acid side chains. Chapter 7 is basically concerned with chemical modifications of wool designed to enhance one or more textile attributes such as flame-resistance.

Over the full range of wool types, fibre diameters vary between about 10 and 80 μm. Diffusion of reactant chemicals from an immersion solvent, which most commonly is water, may be slow. In addition to fibre diameter variations, diffusion is also moderated by the hydrophobic epicuticle on the external face of wool scale cells, with the path of least resistance usually along the junctions between these cells and the interior cortical cells. Within each cell there are more variations in protein organisation, resulting in micro-heterogeneous regions of both hydrophobic and hydrophilic character. A direct result of these complex variations in wool morphology is that kinetics of diffusion and polarity of the reactants may be at least as important as inherent reactivity. For example, highly reactive chlorine water preferentially attacks the surface of wool fibres within seconds, whereas immersion in an excess of cold dilute acid could take an hour or more to achieve an equilibrium uptake.

Provided the constraints to diffusion are taken into account, the chemical reactions of amino acid sidechains of wool proteins are consistent with those of proteins in general. Cystine (CYS) crosslinkages are a special feature of keratin fibres. Reactions of CYS with oxidants and reductants are of major importance, being an integral part of the chemistry involved in isolating wool proteins, in the physical behaviour of wool, and in technical processes such as shrinkproofing and bleaching. CYS is also one of the amino acids principally affected by radiation and heat, as described in Section 5.3.

5.2 Chemical composition

Wool is one of a large group of animal fibres that are almost entirely composed of a family of proteins generally known as α-keratins. Although there are some chemical variations between species, there are also remarkable similarities of structure and composition between comparable types of proteins in animal fibres. The physical form of keratin fibres is as diverse as porcupine quills, various horns and antlers, and extremely soft fine coats of small mammals such as the mouse.

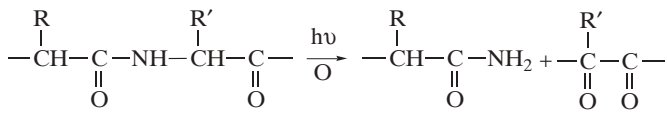
The overall amino acid composition of keratins depends, to a large part, on the relative proportions of the two major types of protein, namely the high- and low-sulphur proteins (see Chapter 3). Merino wool, for example, has a notably higher cystine (CYS) content than coarse wools as a result of having a larger proportion of high-sulphur proteins.

One of the earliest reliable amino acid analyses was that of Corfield and Robson.[1] It remains an adequate description of wool composition and is reproduced in Table 5.1.

5.3 Degradation by radiation and heat

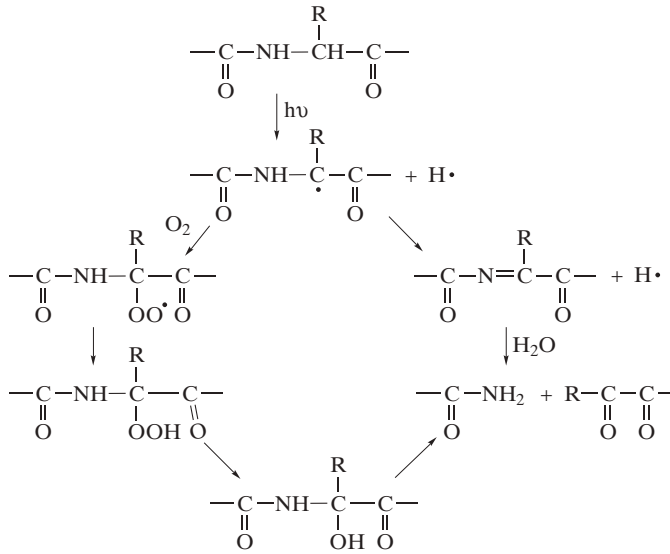
Most of the radiation chemistry of wool is concerned with the effects of exposure to sunlight. Weathering during wool growth varies greatly according to the type of fleece. A dense, fine wool Merino fleece is damaged almost exclusively at staple tips, with heavy secretions of woolgrease and suint contributing to the protection. Coarse open fleeces are more generally exposed to sunlight. Severe weathering implies brittle fibre tips, loss of staple strength and dull or yellow discolouration.

Photo-oxidation of CYS is the most damaging reaction, weakening the fibre. Other residues extensively damaged by prolonged exposure to sunlight are TYR, TRP, PHE, THR, MET, ILEU, LEU, PRO and HIS. Additional carbonyl and amide groups are formed following cleavage of the peptide N–C^{α} bond, as shown in equation [5.1].



[5.1]

This reaction is implicated both with fibre strength loss and subsequent susceptibility to hydrothermal yellowing (Section 7.3). Meybeck and Meybeck[2] suggested that aromatic amino acids absorb UV light and, after energy transfer to glycine and alanine, there is a photochemical conversion to glyoxylyl and pyruvyl peptides according to the mechanism of equation [5.2].



[5.2]

The intermediate structures on the left-hand side arise from further H atom transfers with additional wool protein chains. Minor amounts of other α-ketoacids are detectable in wool following irradiation in the dry state. Few carbonyl groups are detectable when wool is irradiated in water, despite pronounced yellowing. Photochemical damage also increases the susceptibility of wool to discolouration when subsequently exposed to dry heat. Even undamaged wool will develop brownish discolouration when exposed to temperatures over 110 °C for a prolonged period. Just as the dry heat yellowing of silk has been attributed to a dehydration reaction of the SER side chain, similar dehydrations leading to unsaturated carbon bonds have been suggested as the principal source of dry heat discolouration of wool.[3]

5.4 Photobleaching and photoyellowing

In Section 5.3, photochemical degradation has been implicated in sensitising wool to subsequent discolouration when boiled, as in dyeing, or exposed to dry heat. Depending on the conditions of exposure, sunlight may either yellow wool or bleach it. Irradiation of wool in ambient conditions with 254 nm UV light, causes wool to appear green, due to formation of two chromophores, which fade rapidly in post-irradiation reactions to leave a residual yellow colour.[4] Irradiation of wool with different UV wavebands selected from sunlight or artificial sources[5] demonstrated that UV below 331 nm increases wool yellowness, whereas visible light above 398 nm induces photobleaching. In the transition range, neither effect is markedly dominant

Table 5.1 Amino acid composition of wool [From: Corfield and Robson[1]]

Amino acid	Abbreviation	N as % of total N of wool
Alanine	ALA	4.12
Amide N		6.73
Arginine	ARG	19.1
Aspartic acid	ASP	4.38
Cystine	CYS	7.3
Glutamic acid	GLU	8.48
Glycine	GLY	6.29
Histidine	HIS	1.91
iso-Leucine	ILEU	2.44
Leucine	LEU	5.85
Lysine	LYS	3.92
Methionine	MET	0.32
Phenylalanine	PHE	2.12*
Proline	PRO	5.05
Serine	SER	8.66*
Threonine	THR	5.12*
Tryptophan	TRY	0.82
Tyrosine	TYR	2.62
Valine	VAL	4.16
Total		99.39

* Corrected by the factors 1.02, 1.10, 1.09 for PHE, SER and THR respectively for loss during hydrolysis.

although physical damage to the wool is strongly promoted by these intermediate wavelengths. Window glass absorbs radiation shorter than 305 nm so that wool carpets and furnishings exposed to transmitted sunlight noticeably whiten and brighten due to photobleaching of the wool substrate. Control measures for shade changes are described in Section 7.8.

Photobleaching by visible light has been considered as an alternative to chemical bleaching,[6] and this idea has been extended to evaluating blue-light sources for wool bleaching. Wet wool bleaches more rapidly than in the air-dry state, and King showed that thioglycollic acid enhanced still further the rate of photobleaching,[7] but the chemical residues are troublesome. More dramatic photobleaching results from blue-light irradiation of wool soaked in a dilute hydrogen peroxide solution. If the solution is also made alkaline, extraordinary levels of whiteness can be attained within a minute or so.[8] The results were sufficiently encouraging to develop a prototype bleaching machine for continuous treatment of wool sliver. Indus-

trial take-up of this technology has not occurred, quite probably because manufacturers would prefer raw material improvements to be the responsibility of wool suppliers, particularly as in this instance a substantial investment is required for equipment that, in turn, requires specially trained operators.

Major research efforts have been devoted to understanding the chemistry underlying photoyellowing. The summary of much of this work[9] highlights the problems of assigning yellowness to specific chromophores. TRP was long regarded as the most reactive aromatic absorber, with some known yellow decomposition products. One of the earliest correlations was found to be between the TRP content of a range of keratins and their rate of yellowing when irradiated at 310 nm in the dry state.[10]

More direct evidence was derived by radiolabelling TRP in wool, prior to UV irradiation. After solubilising the wool by reduction, alkylation and proteolytic digestion, a radioactive yellow fraction was isolated by ion-exchange chromatography.[11] Recent attempts to isolate and identify the yellow components of wool, however, exclude the possibility that TRP decomposition products are the main contributors.[9] The principal reason is that most of the known products of TRP degradation, including the major one, kynurenine, are colourless, and the minor coloured products such as N-formylkynurenine are very weak chromophores. Semi-quantitative calculations were sufficient to suggest they could not account for more than 20% of wool yellowness.

Isolation and characterisation of yellow wool components entails a difficult first step, i.e. solubilising wool in a manner which neither destroys them nor introduces new artifactual coloured compounds. Wool sufficiently damaged to be significantly yellow is inherently unstable, and dissolution with strong acid or alkaline treatments is visibly accompanied by substantial additional yellowing.[9] Simpson[9] found papain/bisulphite treatments were also questionable because a significant amount of enzyme is introduced as a contaminant. The pale yellow material in the solution of wool extracts is apparently destabilised by the reducing agent and the colour tends to bleach on standing and often disappears entirely within a day or so.

Simpson[9] considered the most satisfactory method was dilute acid hydrolysis at 60–80 °C for several days. Almost all the yellow colour was released in the first 5–10% of the wool to be solubilised, and, following a series of enzyme hydrolyses and chromatographic separations, the yellow fractions could be substantially purified. The main fraction was hydrophobic and was eluted from a reverse-phase chromatographic column as three components of very similar amino acid composition, optical density profile and very weak fluorescence. They appeared to be different levels of polymerisation of peptides, notable for very high proline content, about 22 mol %, and low in aromatic amino acid content.

Small proline-rich proteins (sprs) have been found in the cornified cell envelope of keratins and in the epidermis of many mammalian species,[12] and are notable for very low amounts of the aromatic acids TRP, TYR and PHE. These proteins are claimed to be particularly associated with wool epicuticle (Section 3.3.1). A major portion of wool yellowness thus appears to be derived from a minor wool protein. The method of extraction and purification results in a kind of hydrophobic complex, resistant to complete enzymatic hydrolysis. In pure aqueous solutions for example, the coloured material condenses into dark brown treacle-like droplets. A yellow-brown solution is restored by addition of methanol but alcoholic solutions are incompatible with further enzymatic hydrolyses.

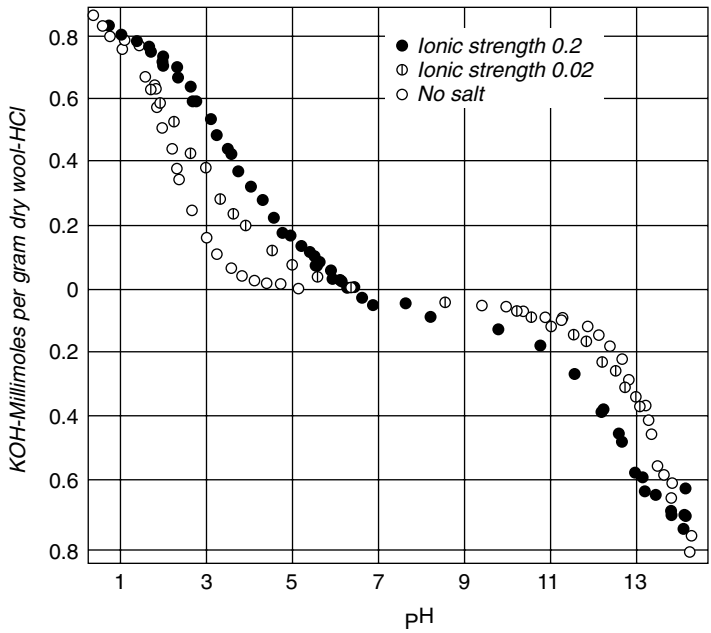
Another aspect of interest in this investigation[9] was the chromatographic separation of the high-proline fractions from a high-TYR/GLY fraction that was completely colourless. A clear, yellow component representing perhaps 10% of the total colour was also retrieved, which had fluorescence and optical density properties suggestive of a TRP degradation product incorporated in a mixture of chromatographically similar peptides.

5.5 Absorption of acids

At room temperature, immersion of wool in aqueous solutions of strong acids or alkalis is essentially an ion-exchange titration of the 0.8 mM/g of both carboxyl and amino groups on amino acid sidechains. Ionic interactions between —COO^- and —NH_4^+ groups play an important part in stabilising the protein structure of wool in the vicinity of its isolectronic point at about pH 6.5. Equilibration in an excess of either acidic or alkaline solution is slow, particularly in the pH 3–10 range, where a time of the order of an hour is required even with regular agitation of the solution. Equilibrium rates are slow as a consequence of an electronic barrier formed at fibre surfaces, following an initial absorption, and can be greatly reduced by addition of a neutral salt. Figure 5.1 illustrates the effect of adding KCl on the shape of the titration curve of wool with HCl and KOH.[13]

There has been considerable interest and effort devoted to providing a theoretical understanding of the acid/base behaviour of wool fibres. Essentially, wool is a fine example of a highly consolidated protein whose behaviour might usefully be compared to soluble proteins of similar acidic and basic amino acid content. The latter have titration characteristics more akin to simple acids and bases.

The two principal theoretical models for absorption of acids and bases by wool are those of Gilbert and Rideal,[17] and of Donnan. Both have been critically examined in several textbooks.[14–16] In the light of current knowledge, the former has been the most successful in terms of prediction of wool titration behaviour, and an abbreviated account of its application follows.



5.1 Combination of wool with hydrochloric acid and with potassium hydroxide as a function of pH. The influence of a salt on shifting the acidic side of the curve is shown. [*From Steinhardt and Harris.*[13]]

Gilbert and Rideal[17] made these assumptions:

i) All positively charged groups (mainly amines) have identical properties.
ii) All carboxylic groups have similar affinities for protons.
iii) Absorbed anions may occupy any positively charged site.

Thus, the anions and protons in an aqueous solution can be regarded as being independently absorbed by immersed wool samples.

The only other formal requirement of the theory is that electrical neutrality of the fibre must be maintained. The thermodynamic analysis that followed culminated in their well-known equation for titration of wool with hydrochloric acid.

$$\log_{10}\frac{\theta_H}{1-\theta_H} = -\mathrm{pH} - \frac{(\Delta\mu_H + \Delta\mu_{Cl})}{4\cdot 6\mathrm{RT}} \qquad [5.3]$$

(θ_H is the fraction of sites filled by protons, and the final term incorporates changes in the chemical potential of hydrogen and chloride ions respectively).

Steinhardt and Harris[13] were able to confirm that the equation did fit the experimental data for the titration of wool with hydrochloric acid. More

significantly still, if sodium chloride is added so that the titration is carried out at essentially constant ionic strength, Gilbert and Rideal derived the secondary equation

$$pH_{0.5} = \log_{10}[Cl] - \frac{\Delta\mu_H + \Delta\mu_{Cl}}{2{\cdot}3RT} \qquad [5.4]$$

The subscript 0.5 refers to an equilibrium pH, when half the proton binding sites are filled. When the chloride concentration is increased, so also is the pH at the point of half saturation. Therefore, less acid is required to achieve a given equilibrium pH when the chloride concentration is increased. It becomes difficult to extrapolate this result in a quantitative fashion for more complex wool–acid–salt combinations, but this basic result is a fundamental factor in devising good conditions for processes such as wool dyeing. Stated in the simplest terms, it affords a scientific basis for the advantages of adding salts in order to raise the ionic strength of dyebaths. This approach is taken further in Section 5.6.

Long exposure at room temperature to an excess of strong acid will hydrolyse some of the amide groups in wool, releasing ammonia and creating additional carboxylate groups, thereby increasing the measured acid uptake of wool. At pH values greater than 11, alkaline degradation reactions of cystine and peptide bond hydrolysis become increasingly likely for even brief exposure at room temperature.

Acids with more complex anions considerably alter the position of the titration curve as compared to HCl, but not its general shape, the changes reflecting a greater affinity of organic anions for wool compared to the chloride ion. For example, several dyestuff-like anions, such as diphenylbenzenesulphonate, are taken up as strongly at pH 4 as chloride ion at pH 2. An extreme example of supra-stoichiometric absorption results from immersion of wool in pure formic acid, which grossly inflates the fibres to several times their original diameters. After rinsing well with water they return to their initial state. The textbook by Alexander and Hudson[14] provides an extensive review of every aspect of absorption of both weak and strong acids. A majority of the original papers to which they refer were written as long ago as 1930–1950 but, for the most part, they have not been materially updated by more recent research.

5.6 Absorption of alkalis

Titration data for absorption of alkalis by wool mirrors, in many respects, absorption of acids but with one substantial difference. Alkalis degrade proteins at low concentrations and temperatures, at a significant rate, so that it is very difficult to attempt reversible alkaline titrations beyond a very restricted range by comparison with acids.

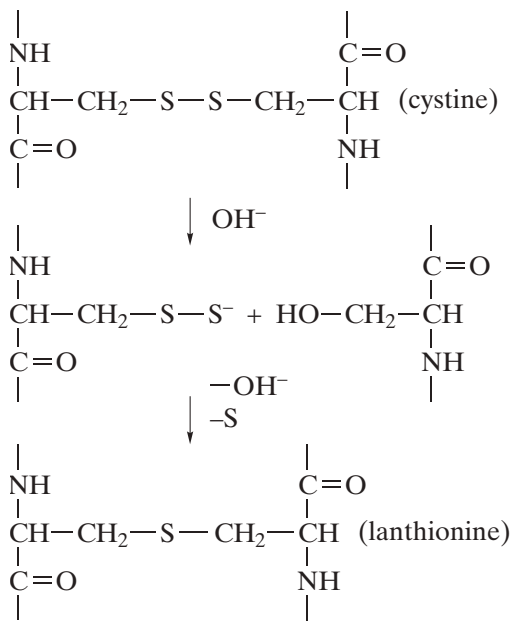
Chemical groups in wool that alter their state of ionisation in increasingly alkaline conditions are listed below. Their approximate concentration in most wool types, in milliequivalents/kg, and their normal titration range vary widely.

- HIS, (68), pH 5.5–7.0
- Terminal amino groups, (25), pH 7.5–8.5
- TYR, (260), pH 9.8–10.4
- LYS, (190), pH 9.5–10.5
- ARG, (600), pH 11.5–12.5

Addition of salts in an alkaline titration of wool has a similar effect in displacing the uptake curve as with acids, so that more alkali is taken up at less extreme pH values when neutral salts are added.

The reactions of cystine and other disulphides in alkaline conditions have attracted a great deal of research attention. Maclaren and Milligan list over 40 references yet conclude that there is still room for some disagreement about the mechanism.[18] In the case of combined cystine in wool, attack at the β-carbon offers a plausible mechanism for the degradation products that have been identified.

The principal reaction steps are illustrated in equation [5.5] (R = wool protein chain).



[5.5]

Lanthionine is one of the main products of alkaline degradation, and the selection of reaction steps shown also account for the appearance of some

free sulphur. Dehydroalanine is also formed as a minor product when some of the intermediate product sidechain HO—CH_2 loses water. The latter product is also known to be capable of reacting with lysine in alkaline conditions to form lysinoalanine. These examples are just indicative of the sequential and complex variations of degradative reactions possible when wool is exposed to strongly alkaline solutions.

5.7 Dyeing with acid dyestuffs

5.7.1 General

The theoretical background of absorption of low molecular weight mineral and organic acids is the same as that of the absorption of high molecular weight acids. However, as might be expected, the coulombic forces which substantially explain absorption of hydrochloric acid by wool, and the influence of salts added to the aqueous acid, fall short of explaining all the features of absorption of high molecular weight dyes. For these larger molecules, the principles of fixation in wool fibres is decidedly more complex. The morphological structure of wool is itself a mediating factor, as discussed in the next section. The detailed technology and practical aspects of wool dying are described in Chapter 8.

5.7.2 Morphological structure of wool and its influence on dyeing

The three steps in dye transfer from an aqueous dyebath are:

i) Diffusion of dye to fibre surfaces
ii) Dye transfer across the surfaces
iii) Diffusion of dye through wool fibre structures

Good dyeing equipment, affording thorough liquor circulation, should eliminate the delays and uneven access to dyes implied in the first step. For the second step, the wool epicuticle offers a significant resistance to dye penetration. It is now accepted that dyes gain access to undamaged wool fibres mainly via junctions between wool cuticle scales.[19] Lipids are present to varying degree at the surface and in intercellular interstices, and they present an obstacle to entry of dyes into wool fibres. Wool that has previously been scoured in the normal aqueous fashion, when extracted with a good solvent for lipids or alternatively stripped with anhydrous sodium *tert*-butoxide, responds with faster rates of dye uptake and better uniformity of dyeing.[20] Wools with surface damage, or degraded by sunlight, chlorination or carbonising treatments for example, will respond differently when exposed to dyebath conditions.

Dyestuffs, which first penetrate into wool between cuticle cell junctions as mentioned, are now known to next diffuse throughout all the non-keratinous regions, and also the endocuticle and intermacrofibrillar material regions of the cell membrane complex.[21,22] The final stages of dyestuff diffusion inside wool fibres entails a progressive transfer of dye into the sulphur-rich matrix proteins surrounding microfibrils in cortical cells.

Even when dye exhaustion is virtually complete, the internal fibre–dye equilibration process is not, and in practical situations an industrial dyeing process may justify an extended dyeing time. A further curious but informative observation from optical and electron microscope studies of dye location in wool fibres is that the initial favoured entry of dye through the cuticle scale junctions is also followed by dye transfer into the adjacent exocuticle and A-layer. The non-keratinous regions, which are so important in the early part of the dye cycle, are found to be almost totally devoid of dye at the completion of the dyeing process.

5.7.3 Dye uptake chemistry

The Gilbert and Rideal model was briefly described in the context of acid–base equilibria when wool is immersed in an aqueous solution (Section 5.5). Both this theory and the alternative approach based on the Donnan theory have been tested to find the extent to which they explain dye absorption. Lewis[23] concluded that neither theory is particularly successful, especially for dyes with a highly hydrophobic character. However, some of the concepts of the ionic attractions driving absorption of simple acid dyes are a logical extension of titration of wool with common organic acids.

A good example of this is the uptake of a fairly simple acid dye. Experimentally it could be demonstrated[24] that, from a dyebath containing just Crystal Ponceau and hydrochloric acid, there is an initial rapid absorption of both hydrogen and chloride ions. Over time, the slower diffusing dye anions displace chloride in the fibre, as demonstrated by first a rapid fall in chloride concentration in the bath followed by a more gradual rise again.

The influence of a salt on shifting the acidic side of the titration curve of wool is shown in Fig 5.1. Basically, at higher ionic strengths, hydrogen ions are more readily absorbed because the repulsive electrical charge which develops at the surface boundary of the fibres is substantially masked. Exactly the same effects explain why salts, commonly sodium acetate or sulphate, are to be found in most acid dyebaths. Less acid is required for a given uptake of hydrogen ions. This facilitates dye anion absorption at less extremely acid conditions and conveniently also reduces the risk of acid damage to the wool substrate.

Zollinger[25] expanded on these ionic aspects of dyestuff affinity for wool and distinguished between coulombic, van der Waals and hydrophobic binding forces. For three carefully selected acid dyes, there was a clear indication of the hydrophobic bonding interaction of an aliphatic sidechain.

These studies were based on comparisons of dyeing behaviour between normal and modified wools, notably by blocking ionic sites. Meybeck and Galafassi[26] contributed considerably to an understanding of the nature of acid dyestuff binding on wool. They showed that few salt anions (chloride) are released from wool in the course of dyeing compared to the amount of dye absorbed. For slightly hydrophobic dyes, of the order of 10% of bound chloride ions are released, while for more strongly hydrophobic dyes, release of chloride ions is negligible. This result, along with a variety of contributory studies,[23,pp68–72] added further weight to their propositions.

On the basis of these results, Meybeck and Galafassi formed three principles in relation to wool dyeing:

i) Hydrogen bonds are not formed between the dye and the fibre.
ii) Coulombic forces play a part in attracting dye anions into the wool fibres but then they locate to hydrophobic sites where they then become strongly fixed.
iii) To have a high affinity for the wool, anionic dyes must have a hydrophobic character. The structure of the dye molecule must be such that the hydrophobic substituents are situated some distance from the polar groups.

5.8 Acid, alkali and enzymic hydrolysis

5.8.1 Acid hydrolysis

Complete acid hydrolysis is the most common method of preparing solutions of amino acids from wool and other proteins for quantitative analysis. Evacuated, sealed tubes containing the sample and 6 M HCl are typically treated at 105 °C for 24 hours. TRP is destroyed and correction factors are applied to calculate SER, THR and TYR contents (see Table 5.1). This analytical routine is questionable for detection of amino acids modified by exposure of wool to sunlight. Partial oxidation products of cystine disproportionate to cysteic acid and cystine, and quite certainly unstable carbonyl groups (Section 5.3) notably glyoxylyl and pyruvyl either react with other side chains or simply decompose. Amides of GLU and ASP are hydrolysed and the ammonia released is also measured. The whole procedure is, of course, now a routine one for proteins in general. Amino acids and ammonia react with ninhydrin to form blue derivatives, which are quantified as they emerge from a chromatographic column.

Partial acid hydrolysis has been evaluated as a general method for selectively producing wool peptides suitable for gaining amino acid sequence information.[27] Maclaren and Milligan[18] summarised all the results, including hydrolysis in more dilute solutions and lower temperatures, and concluded that this approach was not useful for extensive sequencing of wool proteins. In weaker acid hydrolytic conditions, the amides of GLU and ASP are the most readily hydrolysed and a standard method has been developed for the analytical determination of amide nitrogen as ammonia and other amino groups so released. The peptide bond adjacent to ASP is the most labile, with GLY and SER the next most easily released amino acids.

Mild acid hydrolysis for the purpose of providing an analytical determination of amide groups in wool has been specifically developed because the drastic conditions employed for complete acid hydrolysis of wool and wool protein fractions (i.e. the preliminary step before amino analysis, typically 6 M HCl at 105 °C) compromises the measurement. The reason is that a significant fraction of other amino acids, notably SER and THR, decompose as well and, in so doing, release additional ammonia.

Acid hydrolysis aimed more specifically at wool amide groups, therefore, should be sufficiently mild to avoid these complications. Inglis *et al*. judged that a treatment with 2 M HCl at 100 °C for 14 hours in sealed evacuated tubes is adequate for the release of ammonia from all amide groups in wool (and silk), with no significant hydrolysis of other potential contaminants.[18]

Attempts to isolate yellow compounds from wool, as discussed in Section 5.4, seem likely now to employ variations of conditions for mild acid hydrolysis to advance the prospects for complete chemical characterisation. In particular, the emphasis should be on keeping the temperature low, possibly at the expense of longer reaction times of the order of several days, in order to avoid the loss of unstable compounds.

5.8.2 Alkaline hydrolysis and alkali solubility

Alkaline hydrolysis of proteins, including wool, destroys much of the CYS and lesser amounts of ARG, HIS and SER, but unlike acid hydrolysis it conserves the TRP content. In the context of wool chemistry, the most useful application of alkaline treatments is the alkali solubility test, the UK version being BS 3568:1988. This relatively mild treatment with 0.1 M NaOH at 65 °C for 1 hour is a standard test for assessing wool damage. It is particularly useful for detecting loss of CYS crosslinkages, acid damage and sunlight degradation, all of which increase solubility beyond about 10%, which is a figure typical for undamaged wool. Oxidative bleaching processes for example (Section 7.2) must be carefully managed to avoid

excessive oxidation of CYS (see also Sections 5.9 and 5.10) and the alkali solubility test is very commonly used as a quality control check.

Conversely the test is very sensitive to the introduction of new stable crosslinkages, which reduce the amount of extractable peptides. A mild alkaline treatment will itself convert some CYS to lanthionine crosslinkages and lower the amount extractable in a subsequent alkali solubility test.

5.8.3 Enzymic hydrolysis

The solid, crosslinked structure of wool is resistant to proteolytic enzymes. In the general introduction (Section 5.1) attention was drawn to the difficulties of large molecules permeating the wool structure. Trypsin does diffuse slowly along cell membranes and after several days incubation the fibres readily disintegrate into a dispersion of cortical and scale cells.[28] However, if wool is first reduced and alkylated, it is much more accessible to enzymes. Because enzymes afford a relatively mild form of digestion, novel crosslinkages or amino acid derivatives unstable to acid hydrolysis can be released and characterised.[29] In this way the reaction of phenylisocyanate with LYS, SER, THR and GLU residues could be detected[30] and the unusual peptide bond ε-(γ-glutamyl) lysine could be isolated and identified.[31,32]

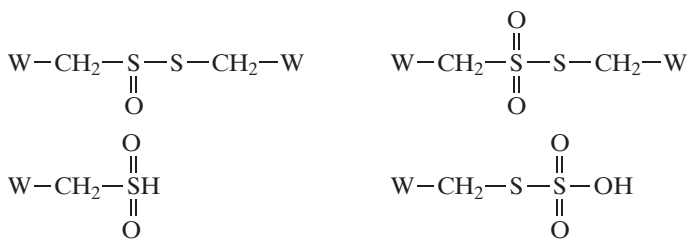
At least three proteolytic enzymes are necessary for near-complete hydrolysis following the reduction/alkylation step.[33] Typically, wide-spectrum enzymes such as papain, pepsin and pronase, used in combinations or sequential digestions, produce peptide mixtures that are ultimately cleaved to form a solution of individual amino acids. Prolidase is required to break proline bonds and amino peptidase removes N-terminal residues in step-wise fashion. Whilst these more complex procedures have identified some relatively unstable modified amino acids, they are not a universal panacea for resolution of all the questions about wool degradation. Essentially, the proteolytic enzymes are inactive when introduced to the solutions of yellow extracts obtained as described in Section 5.4. It is worth re-emphasizing that enzymes are effective in normal aqueous media but that they are unable to penetrate and degrade hydrophobic aggregates of complex wool peptides.

5.9 Oxidation with peracids

TRY, CYS, and cysteine (CYSH) are the amino acids most susceptible to oxidation. Because of its importance in crosslinking wool proteins and directly influencing the strength and elasticity of wool fibres, the detailed chemistry of CYS oxidation has been very extensively studied.

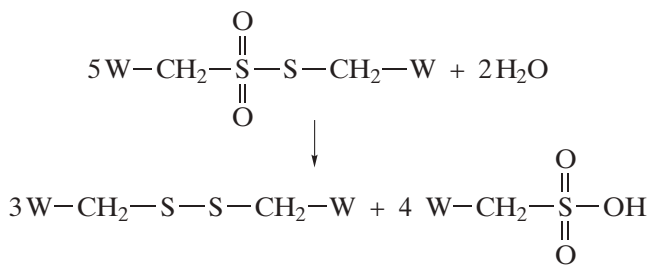
Complete oxidation of CYS to cysteic acid (CYSA) in wool is best achieved with either peracetic or, better still, performic acid. This method forms the basis for post-oxidation procedures beginning with ammonia extraction followed by successive precipitations and purifications in order to prepare well differentiated fractions of soluble wool proteins. The products of these separations have been described as α-, β- and γ-keratoses.[34] Although both the oxidising acids are likely to rupture some peptide bonds and possibly slowly attack other side chains, O'Donnell and Woods[34] found peracetic acid was the more likely of the two to have non-specific effects, possibly because there is a significant amount of hydrogen peroxide in its aqueous solutions. Prolonged oxidation with performic acid does, however, convert MET to its sulphoxide, TRP is converted to N-formyl kynurenine and other products, and some SER, THR and TYR may be affected by vigorous treatments.[18]

The principal present use of peracetic acid is as a component in the peracetic/ammonia solubility test. Under carefully controlled conditions, the test detects the presence of crosslinkages remaining in wool after cleavage of disulphide bonds.[27] When a sufficient excess of oxidant is present, the end product of oxidation of CYS is combined CYSA, i.e. W—CH_2—SO_3H, where W represents a wool protein chain. Exposure to lesser amounts of chemical oxidant, or photo-oxidising sunlight for that matter (see Section 5.3), results in formation of intermediate products. Chemical structures of those believed to be present[1] are shown in equation [5.6]



[5.6]

Treatment of partially oxidised wool with strong acids leads to disproportionation reactions of intermediate sulphoxides. A typical stoichiometric relationship is given by equation [5.7].



[5.7]

5.10 Chlorine-based oxidation

Partial oxidation of wool is commonly observed as a result of industrial processes for bleaching (see Section 7.2) and shrinkproofing wool (see Section 7.5). Hydrogen peroxide is a favoured bleaching agent, applied using conditions that limit damage to CYS within acceptable values.

Present-day technology essentially confines the industrial use of chlorine and its oxidative derivatives to acidic processes that principally modify wool surface properties. Therefore, they are primarily pretreatments for shrinkproofing, with a second-stage application of polymers (Section 7.5.7).

There is a very extensive literature describing oxidation of wool with gaseous chlorine, chlorine water and numerous chlorine-release compounds. One of the earliest observations was that of von Allwörden.[35] Wool fibres immersed in either chlorine- or bromine-water develop sacs on individual scales of the fibre surfaces. Osmotic pressure can develop due to oxidation and dissolution of some protein components of cuticle scale cells, provided the epicuticle is undamaged and prevents their diffusion into the immersion liquid. This reaction is therefore the basis of a simple laboratory test using a microscope to detect surface damage of immersed wool fibres.

Chlorine derived from hypochlorite solutions has a markedly different reaction with wool depending on the pH of the treatment. This is because the active agent in a solution of calcium hypochlorite, commonly known as bleaching powder, is the hypochlorous ion, ClO^- at pH values above 8.5, mainly undissociated HClO in near neutral conditions, while at pH 2 free chlorine accounts for about 70% of the total active chlorine in a dilute solution. Alkaline conditions result in high CYSA levels in the cuticle, whereas acidic treatments promote extraction of soluble acidic peptides from cuticle cells. The alkaline treatment results in a resist to acid dye uptake, whereas the acid method enhances it compared to untreated wool. These differences have been exploited in patterned fabrics to afford cross-dyed effects.[36]

5.11 Reduction

Wool keratins are structurally stabilised by the disulphide crosslinkages of cystine. Reductive cleavage of cystine, followed by structural reshaping of a fabric and then reforming the bonds, is the basis of chemical setting of pleats and creases. The same principles apply to the permanent waving of human hair.

Complete reduction entails two successive nucleophilic displacement reactions – see equation [5.8] (W = wool protein chain and RS^- is the reductant thiol).

$$\begin{aligned} &W-CH_2-S-S-CH_2-W + RS^- \rightleftharpoons W-CH_2-S-S-R + W-CH_2-S^- \\ &W-CH_2-S-S-R + RS^- \rightleftharpoons R-S-S-R + W-CH_2-S^- \end{aligned} \quad [5.8]$$

A high concentration of a thiol, such as thioglycollic acid, would be required to achieve full reduction in acid conditions. By adjusting to pH 9–10, which is the pK value for thiol groups, adding a proportion of an organic solvent such as n-propanol, and using a high concentration of a protein disaggregating medium such as urea, total reduction of cystine is readily attained.

Maclaren and Milligan[18] emphasise the importance of pure reagents. For example, freshly distilled thioglycollic acid avoids acylation of amino groups by thiolactone impurities, and urea should be free of ammonium cyanate, likely to form carbamoyl derivatives with amino groups. Sodium borohydride is another reductant offering high yields of thiol but there is also some peptide bond hydrolysis.[37] Extensive reduction of wool cystine may also be achieved with tetrakis (hydroxymethyl) phosphonium chloride (THPC) (see equation [5.9]). The active agent is the phosphine derivative formed by dissociation of THPC in aqueous solution.[38]

$$W-CH_2-S-S-CH_2-W + R-\underset{R}{\overset{R}{P}} + H_2O \longrightarrow 2\,W-CH_2-SH + R-\underset{R}{\overset{R}{P}}=O \quad [5.9]$$

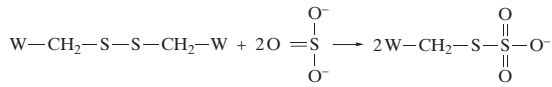
(R = hydroxymethyl group)

By way of contrast with the reversible equilibrium reduction characteristics of thiol solutions, the reaction with THPC is irreversible and requires but a small excess of reagent. Because phosphines react slowly with alkylating agents, it is also possible to carry out alkylation of thiol groups derived from cystine with chloroacetate in the same solution.[39] Some undesirable modifications of TYR and cysteine can occur[38] with THPC, whereas near-quantitative reduction can be achieved with tri-n-butyl phosphine, without unwanted side reactions.[40]

5.12 Sulphitolysis

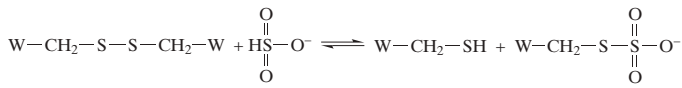
Sulphitolysis of cystine in wool is of major industrial importance in processes such as the setting of yarns and fabrics, mild bleaching methods, and after-treatments following oxidative shrinkproofing and bleaching processes.

Sulphite (SO_3^{2-}), bisulphite (HSO_3^-) and disulphite ($S_2O_5^{2-}$) exist in equilibrium in aqueous solution. HSO_3^- is predominant in acid conditions and SO_3^{2-} is the main species above pH 7. At pH >9 sulphitolysis of cystine is a reversible bimolecular displacement reaction,[41] see equation [5.10]



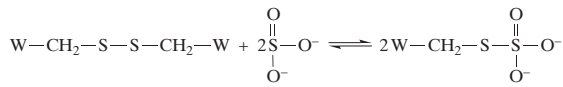
[5.10]

Below pH 9 the reaction is more complex, that with bisulphite forming thiol and S-sulphonate anions (equation [5.11]).



[5.11]

Oxidative sulphitolysis is also possible (equation [5.12])



[5.12]

Sulphitolysis reactions reverse readily with rinsing in water but reversal is slower in acidic conditions. There have been long-standing speculations about wool cystine being divisible into four fractions of different reactivity, but Maclaren and Milligan[18] conclude, after reviewing all the evidence, that there is not a sound case for this proposition.

5.13 Metal salts

Chrome dyeing is by far the most widely used process involving metal complexes in wool and is dealt with in Chapter 8. More recently, zirconium and titanium salts have featured in the development of flame-retardant wool products (see Section 7.7). Wool does, however, interact strongly with a wide variety of metal salts useful in more limited applications.

As might be anticipated, the principal binding sites are carboxyl and sulphydryl groups for metal cations. Sometimes, more elaborate metal complexes with amino, guanidine and imidazole groups have been proposed in order to account for large uptakes. Maclaren and Milligan[18] provide a comprehensive description of metal–wool reactions. The principal studies and applications are summarised here.

5.13.1 Mercury

Methyl mercuric iodide is generally used in quantitative analysis of thiol and disulphide groups in wool.[42] Mercury salts bind in large amounts, e.g. over 2000 μmol g^{-1}, of the chloride.[43,44] However, Hg binding is suppressed by excess chloride, which obviates the use of wool as a sorbent for mercury salts in sea water. Mercuric salts are well known for their ability to form

complexes with nitrogen compounds such as amines. Extended multinuclear complexes are likely in the water-swollen matrix proteins of wool, and these would account for the supra-stoichiometric absorption observed with mercuric chloride solutions. Thiol groups would react readily and completely with mercuric ions.

5.13.2 Silver

Silver ion is taken up in greater amount as the pH is increased, and is reversibly bound on carboxyl groups with a small amount irreversibly reacting with all the thiol groups present to form a mercaptide.[45]

In alkaline conditions, silver ions appear to catalyse degradation of cystine, resulting in H_2S and additional thiol groups being formed, which in turn convert to mercaptides. This conclusion was supported by evidence for a stoichiometric release of hydrogen ions into solution as silver was absorbed in acidic conditions, where decomposition of cystine by silver salts is negligible. There is close to complete binding of Ag^+ ion on carboxyl groups at pH 6, this falling to about 22% at pH 1.5.[45]

The additional uptake of silver in alkaline conditions due to extra mercaptide formation has led to some useful applications. There has been some exploitation of silver ion in histochemistry, notably as a heavy-metal stain useful as a marker in electron microscopy. Weight increases of the order of 16% are achieved with treatments of wool in ammoniacal silver nitrate solutions at pH 10.5.[46] Electron micrographs of wool sections subsequently show dense crystallites of silver sulphide in sulphur-rich regions of the fine structure of wool, presumably formed by reactions involving hydrogen sulphide released by alkaline hydrolysis of cystine. Similar deposits of lead sulphide were observed following alkaline sodium plumbite treatments of wool.[47]

5.13.3 Copper

About 280 μmol g^{-1} Cu(II) is taken up by wool from a perchlorate solution,[48] forming a green complex with carboxyl groups. Treatment with cuprammonium hydroxide in concentrated ammonia results in a 40% weight increase, contraction of the fibres, and loss of the normal keratin structure.[49] A brief treatment is fully reversible by rinsing in dilute acid. These two widely different outcomes, the first essentially reflecting the affinity of cupric ions for carboxyl groups in mildly acidic conditions, and the second the supra-stoichiometric absorption possible in strongly alkaline conditions, favour cupric ion interaction with presumably amino and other nitrogen-containing side chains.

Copper salts are well known for their ability to form complex amines, the affinity being markedly stronger than is the case for silver. On the other hand, the susceptibility of cystine to degradation in alkaline solutions containing silver ion is much greater than for comparable solutions containing copper complexes. Provided the treatment of wool in ammoniacal copper is not too prolonged, as mentioned earlier, the absorbed salts can be washed out and the fibres return to their normal state without a residual amount of copper sulphide or combined copper mercaptide. Supercontraction is the historic description of the structural collapse, and in this respect the treatment is an excellent demonstration of the dependence of wool proteins on hydrogen bonding and amino–carboxyl salt linkages in order to maintain a stable structural form. Interactions which alter the structural stability of wool are described in detail in Chapter 4.

5.13.4 Aluminium

Aluminium ions bind on carboxyl groups, and also on sulphonic acid groups produced by oxidative bleaching processes which modify a proportion of the cystine. The rate and extent of binding is increased by adding n-propanol to the solution, with a pronounced effect on the mechanical properties of the fibres. Basically the treatments reduce the stress required to produce an initial strain, or, in other words, the wool is more easily extended yet loses nothing in ultimate strength or recovery from extension. There are some subtleties of Al/alcohol treatments such that the ultimate effects are sensitive to the percentage of water in the ethanol, propanol or butanol mixture, and the actual quantity of Al absorbed appear to be less important than the absorption locations opened up by the particular solvent mixture.[50]

Unlike Ag^{+} and Cu^{+2}, Al^{+3} has a very low affinity for nitrogenous groups. Just as the ammoniacal copper treatment of wool (Section 5.13.3) offers an example of structural destabilisation due to very extensive destruction of crosslinking hydrogen bonds, so, too, the more easily extendable wools treated in some Al/water/organic solvent mixtures highlights another structural feature. In such solutions, the Al^{+3} ion is sufficiently small to be transported into hydrophobic parts of the wool structure opened up by the organic solvent. Interference with the hydrogen bond cross linkages in these regions is therefore demonstrably a significant factor in the physical properties of wool.

Since Al ions hydrolyse at pH values greater than 4, the optimum pH for wool treatments is in the pH 3–4 range, where the rate of hydrolysis is low and wool has sufficient negative character to strongly absorb Al cations.

5.13.5 Other metals

Wool modified by polymer grafting of methacrylic acid to increase its carboxylic acid content has been evaluated for removing iron, and potentially other metal ion pollutants, present in water. Heavy metal complexes, including uranyl acetate, phosphotungstic acid and osmic acid have been used for examination of wool by X-ray diffraction and electron microscopy.[18] There does not appear to be much specificity attached to these treatments. Following some initial absorption, which may well have a specific type of binding site, what appears to follow is a time-dependent accretion of more heavy metal ions on the original binding template. These accretions build up most readily in the most accessible regions of the fibre structure and clearly delineate cell boundaries and other features readily observable in electron micrographs.

5.14 Miscellaneous reactions

5.14.1 Iodination

Iodine dissolved in alcohol or potassium iodide reacts with wool in two ways. There is formation of a complex with amino groups which is reversible[51] and irreversible di-substitution in the TYR side chain. Richards and Speakman[52] found the latter reaction converted up to 96% of TYR to 2:4 diiodotyrosine, from treatments in alcoholic solutions of iodine.

5.14.2 Ninhydrin

Ninhydrin has a well-known reaction with the amino groups of amino acids in solution, forming a strong blue colour used in quantitative analysis of the amino acids in hydrolysed proteins. Wool boiled in ninhydrin takes up sufficient for a weight increase of 15%, and this results in changes in fibre mechanical properties.[53] The main physical effect is a large increase in the work needed to extend the fibres in water. At first, this was thought to be due to ninhydrin forming additional crosslinkages, but a more likely reason is the lower absorption of water by the treated wool when immersed. The weight change, however, indicates that reactions additional to those with the free amino groups in intact wool must occur, as they would account only for a theoretical 3% weight increase. This is most likely a parallel situation to the accumulation of heavy metals such as osmium (Section 5.13.5) in wool, owing to successive reactions or condensations of more reagent upon an original group-specific template of reactions with wool fibres.

5.14.3 Diazonium salts and staining tests

Pauly[54] first demonstrated the reaction of diazotised sulphanilic acid with TYR and HIS residues of soluble proteins. Depending on pH and other reaction conditions, other residues are also modified.[18] The principal interest in this reaction in wool chemistry is its adaptation as a visual test of fibre damage. Undamaged fibres remain colourless under conditions where damaged fibres rapidly develop red-brown colouration. Glynn[55] preferred 2-nitroaniline as the base of a diazonium salt for this test. Allied with a microscope, this very simple test reaction is an extremely useful one in wool quality control.

Staining tests can be very informative regarding the sites and extent of damage sustained by wool fibres and fabrics. Simpson and Page[56] found it was possible to mix a selected high-molecular weight acid dye and a low-molecular weight basic dye to form a staining solution that showed no tendency to co-precipitate. This proved to be very useful in delineating progressive damage to wool fabrics exposed to sunlight degradation behind window glass.

The standard staining solution was as follows:

- 0.06% CI Acid Red 52
- 0.025% CI Basic Blue 3
- 0.12% acetic acid

Application conditions were 15 minutes at 20 °C followed by 1 minute rinsing.

The colours of surface and shielded fibres showed every variation from red, pink, mauve to blue according to changes in ionic character and permeability.

5.14.4 Esterification

Alexander *et al.* demonstrated that wool treated with boiling methanol (65 °C), n-propanol (97 °C), and n-butanol (117 °C) for 6 hours with 0.1 M HCl as catalyst, resulted in 60, 50, 35% esterification respectively of the carboxyl sidechains.[57] Some amide groups are converted to esters and some susceptible peptide bonds may succumb to N- and O-methylation.[58]

5.15 Crosslinking

5.15.1 Effects of crosslinking

Potential practical applications of crosslinking reactions include the restoration of physical properties such as depletion of natural cystine crosslinking

due to degradation by sunlight. Industrial processes such as oxidative bleaching also damage cystine and suggest that some repair mechanism may be helpful.

A treatment which utilises a bifunctional reagent reactive with wool protein sidechains is the obvious approach to take. In many instances, however, there is no discernable effect on fibre properties because only one functional group reacts with a pendant group on a protein chain or alternatively the predominant bifunctional reactions are with the same protein chain.

A long chain molecule with reactive end-groups may be more successful at crosslinking than rigid aromatic bifunctional reagents,[59] which require more precise positioning of reactive groups on wool proteins to effect a cross-linking reaction.

Definitive proof of crosslinking requires isolation and characterisation of the relevant amino acid–reagent linkages. A majority of potential crosslinkages would hydrolyse should the wool be solubilised. Isolation by following a reduction/alkylation route followed by extraction and several steps of enzymic hydrolysis is always a lengthy and difficult procedure with no certainty of eventual success.

Changes in fibre solubility (in various reagents), swelling, setting, tensile behaviour, abrasion resistance and supercontraction are alternative indicators, which most commonly can be reasonably reliable measures of crosslinking. Their disadvantage, of course, is that they do not identify the particular fibre protein end-groups involved in crosslinking.

Of the several recognised solubility tests, the performic acid/ammonia test is probably the best in most circumstances.[60] The first step is oxidation of all the cystine with performic acid, followed by an extraction of soluble proteins with dilute ammonia. New crosslinkages stable to oxidation reduce the amount of extractable material.

Concentrated urea solutions are well-known swelling and disaggregating solvents for proteins, in combination with a reductant, such as urea/thioglycollate[61] or urea/bisulphite.[62]

The alkali solubility test[63] has the longest history and is a standard method still (0.1 M sodium hydroxide at 65 °C for 1 hour) but can be difficult to interpret. Urea/reductant methods are valuable when crosslinkages are likely to be unstable to oxidising agents or alkalis.

5.15.2 Formaldehyde

Formaldehyde reactions with wool have been the subject of numerous studies, in large part because formaldehyde is capable of introducing

crosslinkages in wool, as well as being cheap and being a small molecule that diffuses rapidly into wool fibres. Although formaldehyde appears to confer no measurable improvements to fibre strength or fabric abrasion resistance when used either as a pre-treatment or included in dyebaths and bleaching processes,[18] it can be successfully used to replace a dye carrier when wool/polyester blends are dyed at high temperature.[64]

There is a considerable history of formaldehyde usage with wool products that do not involve crosslinking, but depend essentially on disinfection. Some examples include dusting greasy wool bales with paraformaldehyde to inhibit growth of microorganisms in storage, disinfection of blankets during laundering, and sterilising Indian wool suspected of containing Anthrax spores.[18]

Sidechains of ARG, LYS, TYR, TRP, HIS, cysteine, and the amide derivatives of ASP and GLU are known to be capable of reaction with formaldehyde. Some of these reactions can be bi-functional as well as mono-functional, so that new wool protein crosslinkages are likely. In addition to simple —CH_2—, i.e. methylene crosslinkages, formaldehyde has a known propensity for self-condensation so that —CH_2—$(OCH_2)_n$— or oxymethylene crosslinkages are feasible.

Verifying the sites and extent of formaldehyde reactions with wool has proved to be difficult since most of the modified amino acid sidechains are unstable under the hydrolytic conditions required to release them for analysis.

McPhee[65] did, however, conclude that ARG residues are extensively modified under all conditions, whereas the amide derivatives of ASP and GLU react only in acid or in alkaline solutions. Whereas formaldehyde treatments of wool in acid conditions did not improve resistance to attack by insects, McPhee found that treatments at pH 12 modified most of the TRP and roughly half of the ARG and primary amino and amide groups. The treated wool, after neutralisation, showed good resistance to clothes moth larvae and newly hatched carpet beetles. Table 5.2, reproduced by Maclaren and Milligan[18] from the primary data of McPhee[65] and Reddie and Nicholls,[66] illustrates the complexity of the formaldehyde reactions with wool under different conditions.

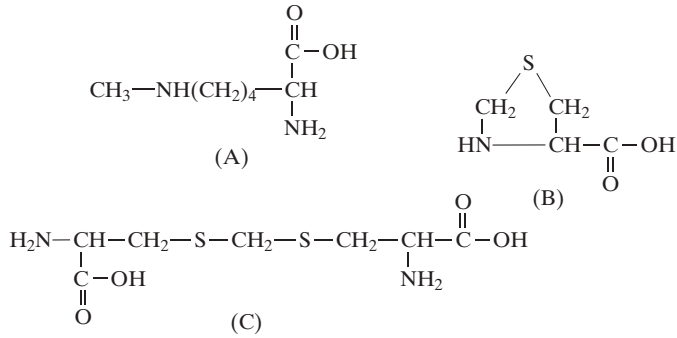
Maclaren and Milligan[18] discuss in detail the experimental difficulties in identifying amino acid modifications caused by formaldehyde treatments, essentially because the hydrolytic reagents needed to release individual amino acids may in themselves induce additional modifications.

Modified amino acids isolated from acid hydrolysates of formaldehyde-treated wool include N^{ε}-methyllysine (A), thiazolidine-4-carboxylic acid (B) and djenkolic acid (C), the latter being the only example of a crosslinkage so far identified (Equation [5.13]).[66–68]

Table 5.2 The extent of reaction of formaldehyde with various residues in wool

Conditions of treatment				Amount of HCHO bound ($\mu mol.g^{-1}$)	Groups modified ($\mu mol.g^{-1}$)[a]					
Conc. (M)	pH	Temp. (°C)	Time (h)		AMIDE[b]	LYS	ARG[e]	TYR	TRP	Ref.
0.33	0.5	35	20	770	300	0[c]	400	50[f]	50[g]	45
2.7	7.0	20	48	450	50	150[c]	300	0	50	45
2.7	12.2	20	0.5	1200	400	50[c]	300	0	50	45
0.35	0.1	60	2	1270	—	80[d]	—	55[d]	40[h]	46
0.35	6.7	60	2	870	—	140[d]	—	25[d]	25[h]	46
3.5	3.0	98	24	—	—	210[d]	—	160[d]	40[h]	46

[a] The initial amide, lysine, arginine, tyrosine, and tryptophan contents found by McPhee[65] were 670, 210, 600, 350, and 54 $\mu mol.g^{-1}$, respectively; the initial lysine, tyrosine, and tryptophan contents found by Reddie and Nicholls[66] were 240, 420, and 40 $\mu mol.g^{-1}$ respectively.
[b] By ammonia determination after partial hydrolysis.
[c] By colorimetry after ninhydrin treatment.
[d] By indirect analysis after treatment with 1-fluoro-2,4-dinitrobenzene.
[e] By reflectance spectrophotometry after applying Sakaguchi reagent.
[f] By colorimetry after applying Pauly reagent and dissolution in alkali.
[g] By reflectance spectrophotometry after applying Ehrlich reagent.
[h] By colourimetry (Spies and Chambers method).



[5.13]

5.15.3 Dialdehydes

Dialdehydes have attracted research and practical interest because of their obvious potential for crosslinking proteins in general. Glutaraldehyde is clearly the most successful. It has been applied in treatments of woolly sheepskins, where it has a dual role in tanning the leather and protecting the wool from felting during laundering.[69]

Glutaraldehyde reacts predominantly with LYS residues, although there is an unresolved debate about the nature of the crosslinkages.[18,p192] The main disadvantage of the process is that it imparts a golden colour to the wool, although this can be mitigated to some extent by adding bisulphite to the treatment bath.

5.15.4 Other bifunctional reagents

One of the early papers describing a crosslinking process for wool employed various alkyl dibromide solutions applied in a one-step treatment which included dithionite. This reducing agent opened up the fibre by cleavage of some cystine, allowing the dibromide to reform this crosslink and add a variety of other bifunctional reactions.[70]

Acyl and aryl dihalides, dimaleimides and bifunctional acid chlorides, isocyanates and active esters have all been reacted with wool from both aqueous and organic solvents. An example of this type of treatment is that of cyanuric chloride (2,4,6 trichloro-s-triazine), which is a relatively cheap industrial reagent, applied from an aqueous acetone solution at room temperature.[71] About 300 μmol/g of wool of the reagent is incorporated and there is reduced solubility in all the regular alkali, urea-bisulphite and acid solubility tests, which is strong circumstantial evidence for crosslinking.

Apart from the commercial use of glutaraldehyde as a tanning and fibre stabilisation process (Section 5.15.3) and the regular re-discovery of useful applications for formaldehyde treatments, there has been no

sustained development of crosslinking processes, presumably as any product performance advantages are insufficient to justify their industrial development.

References

1 Corfield M C and Robson A, 'The amino acid composition of wool', *Proc. Int. Wool Text. Res. Conf.*, Australia, 1955, C79–86.
2 Meybeck A and Meybeck J, 'Photo-oxidation of the peptide group, I. Fibrous Proteins', *Photochem. Photobiol.*, 1967, **6**, 355–63.
3 Hoare J L, 'Chemical aspects of wool yellowing', *WRONZ Communication* ***2***, 1968.
4 Launer H F, Effect of light upon wool, 'Part I, Greening and yellowing by germicidal ultraviolet.' 'Part II, Post-irradiation loss of colour in the dark after germicidal ultraviolet', *Text. Res. J.*, 1963, **33,** 258–63, 910–18: 'Part IV, Bleaching and yellowing by sunlight.' 'Part V, Yellowing and bleaching by ultraviolet and visible arc light', *Text. Res. J.*, 1965, **35**, 395–400, 813–19.
5 Inglis A S and Lennox F G, 'Studies in wool yellowing, *Part IX*, Irradiation with different UV wavebands', *Text. Res. J.*, 1965, **35**, 104–9.
6 Launer H F, 'Rapid bleaching of wool with extremely intense visible light', *Text. Res. J.*, 1971, **41**, 311–14.
7 King M G, 'The effects of reducing agents on the photobleaching and photoyellowing of wool', *J. Text. Inst.*, 1971, **62**, 251–60.
8 Simpson W S, 'Comparison of chemical and photochemical bleaches for wool', *Proc. 8th Int. Wool Text. Res. Conf.*, Christchurch, NZ, **IV**, 279–87, 1990.
9 Simpson W S, 'Physics and chemistry of wool yellowing', *WRONZ Report R217*, 1999.
10 Lennox F G and Rowlands R J, 'Photochemical degradation of proteins', *Photochem. Photobiol.*, 1969, **9**, 359–67.
11 Holt L A and Milligan B, 'Application of enzymic hydrolysis and tritium labelling to a study of the modification of tryptophyl residues in proteins', *Aust. J. Biol. Sci.*, 1973, **26**, 871–6.
12 Steinert P M, Kartasova T and Marekov L N, 'Biochemical evidence that small proline-rich proteins and trichohyalin function in epithelia by modulation of the biochemical properties of their cornified cell envelopes', *J. Biol. Chem.*, 1998, **273**, 11758–69.
13 Steinhardt J and Harris M, 'Combination of wool protein with acid and base – HCl and KOH', *J. Res. Natl. Bur. Standards*, 1940, **24,** 335–67.
14 Alexander P and Hudson R F, *Wool: Its Chemistry and Physics*, London, Chapman and Hall, 1954.
15 Vickerstaff T, *The Physical Chemistry of Dyeing*, London, Oliver and Boyd, 1954.
16 Peters R H, *Textile Chemistry – The Physical Chemistry of Dyeing, Vol. 3*, Amsterdam, Elsevier, 1975.
17 Gilbert G A and Rideal E K, 'The combination of fibrous proteins with acids', *Proc. Roy. Soc.*, 1944, **A182**, 335–46.
18 Maclaren J A and Milligan B, *Wool Science. The Chemical Reactivity of the Wool Fibre*. NSW Science Press, 1981.

19 Hall R O, 'Fibre structure in relation to dyeing', *J. Soc. Dyers and Col.*, 1937, **53**, 341–4.
20 Joko K, Koga J and Koroki N, 'The interaction of dyes with wool keratin – the effect of solvent treatment on dyeing behaviour', *Proc. 7th Int. Wool Text. Conf.*, Vol. 5, 1985, 23–32.
21 Leeder J D, Rippon J A and Rivett D E, 'Modification of the surface properties of wool by treatment with anhydrous alkali', *ibid*, Vol. 4, 312–21.
22 Leeder J D, Rippon J A, Rothery F E and Stapleton I W, 'Use of the transmission microscope to study dyeing and diffusion processes', *ibid*, Vol. 5, 99–108.
23 Lewis D M, *Wool dyeing*, *Soc. Dyers and Col.*, Bradford, 1992.
24 Elod E, 'Theory of the dyeing process. Influence of acid dyes on animal fibres', *Trans. Farad. Soc.*, 1933, 327–47.
25 Zollinger H, 'The dye and the substrate: The role of hydrophobic bonding in dyeing processes', *J. Soc. Dyers and Col.*, 1965, **81**, 345–50.
26 Meybeck J and Galafassi P, 'The effects of hydrocarbon substituents in azo dyes on wool dyeing', *4th Int. Wool Text. Res. Conf.*, Berkeley, Calif, *App. Polymer Symp.*, No. 18, Part 1, 463–72, 1971.
27 Consden R and Gordon A H, 'The peptides of cystine in partial hydrolysates of wool', *Biochem. J.*, 1950, **46**, 8–20.
28 Burgess R, 'The use of trypsin for the determination of the resistance of wool fibres to bacterial disintegration', *J. Text. Inst.*, 1934, **25**, T289–94.
29 Holt L A, Milligan B and Roxburgh C M, 'ASP, ASN, GLU and GLN contents of wool and two derived protein fractions', *Aust. J. Biol. Sci.*, 1971, **24**, 509–14.
30 Caldwell J B, Milligan B and Roxburgh C M, 'The sites of reaction of phenylisocyanate with wool', *J. Text. Inst.*, 1973, **64**, 461–7.
31 Asquith R S, Otterburn M S, Buchanan J H, Cole M, Fletcher J C and Gardner K L, 'Identification of εN (γ-glutamyl) lysine crosslinks in native wool keratins', *Biochim. Biophys. Acta*, 1970, **221**, 342–8.
32 Milligan B, Holt L A and Caldwell J B, 'The enzymic hydrolysis of wool for amino acid analysis', *4th Int. Wool Text. Res. Conf.*, Berkeley, Calif., *Appl. Polymer Symp.*, No. 18, Part 1, 1971, 113–25.
33 Schmitz I, Baumann H and Zahn H, 'Ein Beitrag zur enzymatschen Totalhydrolyse von Wollkeratin', *Proc. 5th Int. Wool Text. Res. Conf.*, Aachen, **II**, 313–25, 1975.
34 O'Donnell I J, and Woods E F, 'The preparation of wool protein solutions', *Proc. Int. Wool Text. Res. Conf.*, Australia, CSIRO, 1955, **Vol. B**, 48–55.
35 von Allwörden K, 'Properties of wool – detection of damaged wool by chemical means', *Z. Angewandte Chem.*, 1916, **29**, 27–32.
36 Simpson W S, 'Effect of chlorination under different conditions on the dyeing of wool', *WRONZ Communication **47***, 1976.
37 Gillespie J M, O'Donnell I J, Thompson E O P and Woods E F, 'Preparation and properties of wool proteins', *J. Text. Inst.*, 1960, **51**, T703–9.
38 Wolfram L K, 'The reaction of tris(hydroxymethyl) phosphine with keratin', *Proc. 3rd Int. Wool Text. Res. Conf.*, Paris, 1965, **II**, 505–12.
39 Maclaren J A, 'Quantitative reduction and alkylation of wool', *Text. Res. J.*, 1971, **41**, 713–14.
40 Maclaren J A and Sweetman B J, 'Preparation of reduced wool and S-alkylated wool keratins using tri-butylphosphine', *Austr. J. Chem.*, 1966, **19**, 2355–60.

41 Cecil R in *The Proteins*, ed. H Neurath. Academic Press, NY, Vol. 1, 1963, p. 438.
42 Leach S J, Meachers A and Springell P H, 'Micro- and submicro methods for the estimation of thiol and disulphide groups in insoluble proteins using radioactive mercurials', *Anal. Biochem.*, 1966, **15**, 18–30.
43 Friedman M and Mavri M S, 'Sorption behaviour of mercuric salts on chemically modified wools and polyamino acids', *J. Appl. Polymer Sci.*, 1973, **17**, 2183–90.
44 Friedman M, Harrison C S, Ward W H and Lundgren H P, 'Sorption behaviour of mercuric and methylmercuric salts on wool', *J. Appl. Polymer Sci.*, 1973, **17**, 337–90.
45 Simpson W S and Mason P C R, 'Absorption of silver ions by wool', *Text. Res. J.*, 1969, **39**, 434–41.
46 Kassenbeck P and Hagege R, 'Development Des Methods D. Analyses Histochemique', *3rd Int. Congr. Wool Textile Res.*, Paris, 1965, **I**, 245–58.
47 Sikorski J and Simpson W S, 'Studies of the reactivity of keratin with heavy metals', *J. Roy. Microscopical Soc.*, 1959, **68**, 35–40.
48 Guthrie R E and Laurie S H, 'Binding of copper II to mohair keratin', *Austr. J. Chem.*, 1968, **21**, 2437–43.
49 Whewell C S and Woods H J, 'A reversible contraction phenomenon in animal hairs', *Nature*, 1944, **54**, 546–9.
50 Edgar J S and Simpson W S, 'The effect of aluminium on the load – extension characteristics of some wool yarns' and 'The absorption of aluminium by wool from water alcohol mixtures', *Text. Res. J.*, 1975, **45**, 809–11 and 281–4.
51 Blackburn S and Phillips H, 'The action of iodine on wool', *J. Soc. Dyers and Col.*, 1945, **61**, 100–3.
52 Richards H R and Speakman J B, 'The iodination of wool', *J. Soc. Dyers and Col.*, 1955, **71**, 537–44.
53 Cockburn R and Speakman J B, 'Cross-linking reactions in keratin III. The action of ninhydrin on wool', *Proc. Int. Wool Text. Res. Conf.*, Australia, 1955, C315–39.
54 Pauly H, 'The diazo reactions of proteins', *Z. Physiol. Chem.*, 1915, **94**, 284–90.
55 Glynn M V, 'Diazo compounds in the determination of wool damage', *J. Soc. Dyers Col.*, 1952, **68**, 16–20.
56 Simpson W S and Page C T, 'The effect of light on wool and the inhibition of light tendering', *WRONZ Report **No 60***, 1979, pp. 3–5.
57 Alexander P, Carter D, Earland C and Ford O E, 'Esterification of the carboxyl groups in wool', *Biochem. J.*, 1951, **48**, 629–32.
58 Holt L A and Milligan B, 'Esterification of wool', *Austr. J. Biol. Sci.*, 1970, **23**, 165–73.
59 Hinton E H Jnr, 'A survey and critique of the literature on crosslinking agents and mechanisms as related to wool keratin', *Text. Res. J.*, 1974, **44**, 233–92.
60 Caldwell J B, Leach S J and Milligan B, 'Solubility as a criterion of crosslinking in wool', *Text. Res. J.*, 1966, **36**, 1091–5.
61 Gillespie J M, 'The isolation and properties of some soluble proteins from wool', *Austr. J. Biol. Sci.*, 1964, **17**, 282–300.
62 Lees K, Peryman R V and Elsworth F F, 'The solubility of wool in urea-bisulphite solutions and its use as a measure of fibre modification – Part II', *J. Text. Inst.*, 1960, **51**, T717–32.
63 Harris M and Smith A L, 'Oxidation of wool IV. Alkali solubility test for determining the extent of oxidation', *Amer. Dyest. Rep.*, 1936, **25**, 542–5.

64 Baumann H, Muller H, Mochel L and Spiegelmacher P, 'Chemische veränderungen und schutz der Wolle beim HT-Färben von Polyester/wolle – Stückware, *Melliand Textilber.*, 1977, **58**, 420–2.
65 McPhee J R, 'The reaction of formaldehyde with wool and its effect on digestion by insects', *Text. Res. J.*, 1958, **28**, 303–14.
66 Reddie R N and Nicholls C H, 'Some reactions between wool and formaldehyde', *Text. Res. J.*, 1971, **41**, 841–52.
67 Trézl L, Heiszman J and Tyihák E, 'Changes in the thermal behaviour of wool due to pretreatments. Formation of heat resistant crosslinkage', *5th Int. Wool Text. Res. Conf.*, Aachen, 1975, **II**, 488–98.
68 Middlebrook W R and Phillips H, 'The action of formaldehyde on the cystine disulphide linkages of wool', *Biochem. J.*, 1947, **41**, 218–23.
69 Happich W F, 'New process expands uses for woolskins', *4th Int. Wool Text. Res. Conf.*, Berkeley, Calif., *Appl. Polymer Symp.*, **No**. **18**, 1971, Part 2, 1483–90.
70 Marzona M, Di Modica G and Marzona M, 'Crosslinking of wool keratin with bifunctional aldehydes', *Text. Res. J.*, 1971, **41**, 701–5.
71 Harris M and Brown A E, 'New developments in the chemical modification of wool', *Amer. Dyest. Rep.*, 1947, **36**, 316–19.