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ORIGINAL ARTICLE

Diffuse reflectance spectroscopy of fibrous proteins

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Abstract UV–visible diffuse reflectance (DR) spectra of the fibrous proteins wool and feather keratin, silk fibroin and bovine skin collagen are presented. Natural wool contains much higher levels of visible chromophores across the whole visible range (700–400 nm) than the other proteins and only those above 450 nm are effectively removed by bleaching. Both oxidative and reductive bleaching are inefficient for removing yellow chromophores (450–400 nm absorbers) from wool. The DR spectra of the four UV-absorbing amino acids tryptophan, tyrosine, cystine and phenylalanine were recorded as finely ground powders. In contrast to their UV–visible spectra in aqueous solution where tryptophan and tyrosine are the major UV absorbing species, surprisingly the disulphide chromophore of solid cystine has the strongest UV absorbance measured using the DR remission function $F(R)_{\infty}$. The DR spectra of unpigmented feather and wool keratin appear to be dominated by cystine absorption near 290 nm, whereas silk fibroin appears similar to tyrosine. Because cystine has a flat reflectance spectrum in the visible region from 700 to 400 nm and the powder therefore appears white, cystine absorption does not contribute to the cream colour of wool despite the high concentration of cystine residues near the cuticle surface. The disulphide absorption of solid L-cystine in the DR spectrum at 290 nm is significantly red shifted by $\sim$40 nm relative to its wavelength in solution, whereas homocystine and lipoic acid showed smaller red shifts of 20 nm. The large red shift observed for cystine and the large difference in intensity of absorption in its UV–visible and DR spectra may be due to differences in the dihedral angle between the crystalline solid and the solvated molecules in solution.

Keywords Fibrous protein · Diffuse reflectance · Keratin · Silk · Collagen · Cystine

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Introduction

Transmission spectroscopy in the UV–visible region is routinely used to study the electronic transitions of molecules in solution that are responsible for colour. However, the technique is less useful for opaque or translucent solid materials, including polymers and fibres, biomaterials and complex biocomposites such as human skin. For these materials reflectance spectroscopy offers an alternative approach, where the light diffusely reflected from phase boundaries can be analysed. Reflectance spectroscopy is widely used commercially for colour measurement and colour matching in the textile and paper industries and for many other applications where coloration using dyes, inks, pigments or paints is important. The theory of diffuse reflectance spectrophotometry was originally developed by Kubelka and Munk 1931, (Kubelka 1948) and the practical applications have subsequently been described in detail (Kortum et al. 1963; Wendlandt and Hecht 1966; Kortum 1969).

One of our interests is the colour of undyed fibrous materials used to make textiles, which is an important commercial property. This is because the whiter the clean untreated fibre, the greater the range of bright shades that can be obtained after dyeing or bleaching, including brilliant whites and pastels that are essential for new growth markets in next-to-skin casual knitwear and sportswear. Wool struggles to compete with cotton and synthetics in

these markets because of its natural cream colour and inferior photostability (Millington 2009). Although a range of tryptophan and tyrosine oxidation products have recently been identified as yellow chromophores formed in wool following prolonged exposure to ultraviolet radiation or sunlight (Dyer et al. 2006a, b), the chromophores responsible for the natural cream colour of wool remain unknown.

Wool colour is normally defined in terms of the three tristimulus values (XYZ) derived from the reflectance spectrum in the visible region. Scoured wool colour is normally described in terms of two of these parameters, Y (brightness) and $Y - Z$ (yellowness). The colour of scoured Merino wool ranges from pale cream to deep yellow. Yellowness values $(Y - Z) < 8.5$ are considered good and command premium prices, whereas $Y - Z$ values >11 indicate highly yellowed wool and are discounted (Wood 2002). Studies on Collinsville Merinos showed that scoured wool colour is heritable and this was confirmed by studies on both New Zealand (Wuliji et al. 2001) and Australian Merinos using New South Wales (Smith and Purvis 2009) and South Australian (Hebart and Brien 2009) flocks. Heritability (h^2) values derived from these studies were in the range 0.25–0.54, showing that scoured wool colour is a moderate-to-high heritable trait and suggesting that it should respond fairly rapidly to selection. Improving the whiteness and photostability of Merino wool by selection is a strategy currently being investigated in projects supported by the *Cooperative Research Centre (CRC) for Sheep Industry Innovation* based in Armidale NSW. A more recent analysis of data from eight genetically well-characterised flocks created by this CRC in different regions of Australia totalling 5,000 ewes [the Information Nucleus (Fogarty et al. 2007)] has shown that yellowness has a higher heritability of 0.70, probably due to the increased genetic diversity of these flocks (Hatcher et al. 2010). The yellowness of wool $(Y - Z)$ increases with mean fibre diameter (MFD) (Fleet et al. 2009; Smith and Purvis 2009) and selection of wool for finer MFD results in some improvement in its colour properties. This effect is due to increased light scattering and decreased absorption as MFD decreases, and a model to eliminate the effects of MFD on the XYZ tristimulus values has recently been described (Wang et al. 2011).

Other fibrous proteins such as silk fibroin, feather keratin and collagen, are significantly whiter than wool and the influence of factors such as amino acid content and fibre morphology on the colour of fibrous proteins has not previously been reported. It is known that changing the surface properties of wool fibres by physically flattening (Bhoyro et al. 2001) or chemically removing the cuticle cells (Levene and Shakkour 1995) significantly affects their reflection and lustre properties. Although the UV–visible transmission spectra of solid horsehair keratin (Bendit and Ross 1961) and wool (Nicholls and Pailthorpe 1976) have previously been reported, it is the reflectance properties rather than transmittance that largely determine the perceived colour of materials.

This paper describes diffuse reflectance studies on fibrous proteins and solid amino acid powders in order to determine the extent to which the UV-absorbing amino acid residues tryptophan, tyrosine and cystine affect their reflectance properties, in particular for fibrous proteins in the UV region. The diffuse reflectance spectra of tryptophan, tyrosine and cystine are compared with conventional UV–visible spectra in aqueous solution, and the interesting reflectance properties of cystine are discussed and compared with the disulphide compounds homocystine and lipoic acid.

Experimental

Materials

Amino acids, homocystine, lipoic acid, magnesium oxide and cysteic acid were laboratory grade (Sigma Aldrich, Sydney, Australia) and used as received. A second sample of laboratory grade L-cystine was also obtained from a different source (BDH, Poole, UK). Cysteine free base was freshly prepared from a filtered solution of cysteine hydrochloride (4 g) in ethanol (50 cm^3). The solution was neutralised with aqueous ammonia (28%, $\sim$5 cm^3) and the precipitated cysteine was filtered and washed with ethanol.

Loom-state pure Merino wool 2/2 twill obtained from Aweave Textiles, Melbourne was given a minimum finish of a greasy blow for 15 s followed by open-width scouring. Oxidative bleaching of this fabric was carried out using alkaline hydrogen peroxide (50%, 15 ml/L) in the presence of tetrasodium pyrophosphate (6 g/L, pH 8.5, 60°C for 1 h). The fabric was rinsed well with water purified by reverse osmosis, hydroextracted and dried at room temperature to avoid thermal yellowing. Reductive bleaching of wool was carried out by generating sodium dithionite in situ from sodium borohydride and sodium metabisulphite. The bath was set at 40°C and sodium metabisulphite (8 g/L) was added followed by ColorClear™ WB (Rohm & Haas: a sodium borohydride formulation in alkaline solution, 2 g/L) and formic acid to set the pH to 4.0–4.5. The temperature was then raised to 60°C for 1 h before thorough rinsing, hydroextraction and drying at room temperature. Double bleaching involved peroxide bleaching and rinsing immediately followed by reductive bleaching as described. Undyed silk fabric for silk painting was obtained from a local craft shop, washed gently in warm water to remove any residual sericin and well rinsed. White feathers

from a royal spoonbill (*Platalea regia*) were gently washed in warm water containing a few drops of non-ionic surfactant, rinsed and dried. Reconstituted Type 1 bovine skin collagen was kindly provided by CSIRO Materials Science and Engineering, Melbourne, Australia.

Methods

Transmission spectra of amino acid solutions were obtained using a Cary 300 Bio UV–visible spectrophotometer (Varian) fitted with 1 cm square quartz cells. Diffuse reflectance spectra were obtained using the same instrument fitted with a 70-mm diameter integrating sphere (DRA-CA-301, Labsphere, New Hampshire, USA) machined from Spectralon©, a pure form of polytetrafluorethylene (PTFE). Spectra of textile fabrics were obtained by mounting the specimen directly over the sample port using a 0° wedge and a Spectralon© reference standard to record the baseline. Spectra of powdered materials and loose fibres were measured by placing ~2 g of material into small re-sealable polyethylene (PE) bags transparent to UV to 250 nm and placing the bag over the sample port using a 0° wedge. For amino acids ~2 g of powder is sufficient to provide sufficient sample thickness of 2–3 mm over the 15 mm diameter instrument sample port. Spectra of powders were referenced against powdered glycine that had been finely ground in a pestle and mortar and held in a similar PE bag. Both glycine and magnesium oxide powder in PE bags gave very similar diffuse reflectance baselines to Spectralon© between 850–250 nm. We found the use of re-sealable PE bags to be a highly convenient method of measuring the diffuse reflectance spectra of powdered samples that avoids the use of open powder cells, which may allow traces of powder to contaminate the sphere. The thin PE film has no observable effect on the diffuse reflectance spectrum.

Diffuse reflectance spectra measured in this study are presented as both reflectance (%R) and remission function $F(R)_\infty$ against wavelength. For an infinitely thick opaque layer (effectively a few millimetres for fine powders) the remission function is determined by the Kubelka–Munk equation according to:

$$F(R)_\infty = \frac{k}{s} = \frac{(1 - R_\infty)^2}{2R_\infty} \quad (1)$$

where, k is molar absorption coefficient, s scattering coefficient, R_∞ the relative diffuse reflectance:

$$R_\infty = \frac{\phi_{\text{sample}}}{\phi_{\text{standard}}} \quad (2)$$

which is the diffuse reflectance measured on the sample referred to a non-absorbing standard material, ϕ_{sample} and ϕ_{standard} representing the diffuse reflectance data for the sample and the standard reflector at a given wavelength. Spectra based on the remission function allow qualitative comparison with conventional UV–visible absorption spectra.

Colour tristimulus values (XYZ) and yellowness index (E313) were measured on a GretagMacbeth Color-Eye 7000A reflectance spectrophotometer using D65 standard illumination.

Results and discussion

The diffuse reflectance and remission function spectra of untreated and bleached wool fabrics are shown in Fig. 1, and colour data for the same fabrics are shown in Table 1. The Figure shows that a number of chromophores are initially present in untreated wool fabric and these absorb across the whole visible range (700–400 nm). Bleaching has the effect of removing most of the visible chromophores in the 700–450 nm range and flattening the reflectance spectrum significantly. Yellowness ($Y - Z$) values decrease accordingly after bleaching, but nevertheless significant absorption of blue and violet wavelengths (450–400 nm) still occurs so that even double-bleached wool remains noticeably cream coloured when compared against bleached polyester or cotton (Millington 2009; Millington et al. 2011). The inability to remove these yellow chromophores from wool remains an issue and a more efficient bleaching process would be commercially valuable.

Below 290 nm, the diffuse reflectance of wool is negligible and absorption [$F(R)_\infty$] is high, showing that strong UV-absorbing species are present that are largely unaffected by bleaching treatments.

Figure 2 compares the diffuse reflectance spectra of the four fibrous proteins. Wool has the lowest reflectance and was the yellowest of the four fibres even after double bleaching, as shown in Table 1. It is interesting that wool and feather keratin showed much higher absorption in the UV (400–250 nm) region than collagen and silk. It was thought this was probably due to differences in the content of the four UV-absorbing amino acids tryptophan, tyrosine, cystine and phenylalanine in these proteins. The absorption spectra of these four amino acids in phosphate buffer at pH 7.1 are shown in Fig. 3. These spectra agree closely with the previous work (Anson et al. 1952) and it is clear that tryptophan is the strongest absorber of the four. The molar absorption coefficients of aqueous tryptophan, tyrosine and cystine at 280 nm are routinely used by biochemists to determine the concentrations of proteins in solution from the numbers of residues present (Pace et al. 1995).

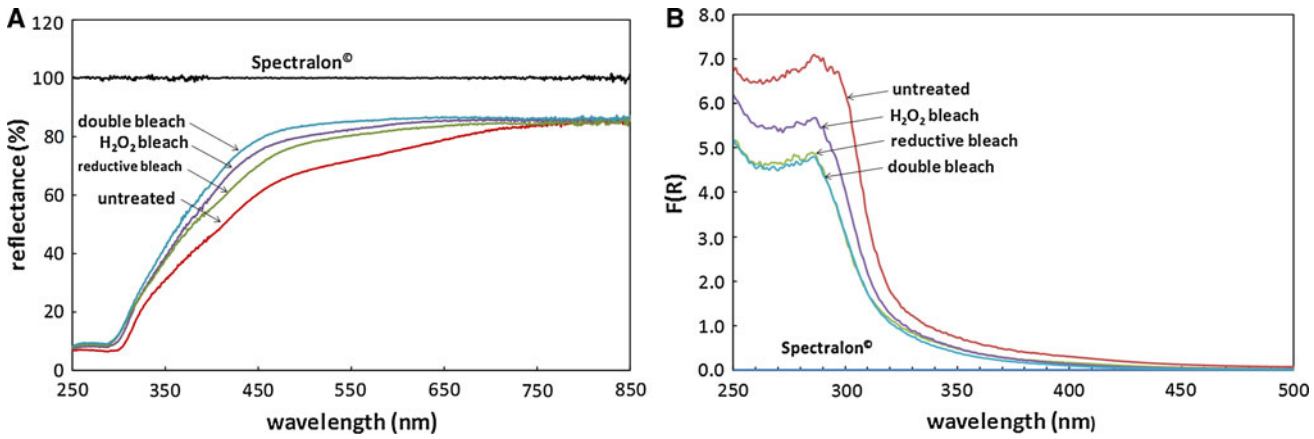

Fig. 1 Diffuse reflectance spectra of wool fabrics in (**a**) $\%R$ and (**b**) $F(R)_\infty$ format. The lower baseline reflectance of the fabrics compared to the Spectralon© standard is due to their composite nature, composed of woven yarns and individual fibres, rather than a perfect matt white diffusing surface

Table 1 Colour tristimulus and yellowness data for fibrous proteins

Sample	X	Y	Z	$Y - Z$	Yellowness index E313
Untreated wool	63.1	66.6	58.4	8.2	22.4
Reductive bleached wool	69.8	74.3	67.6	6.7	17.7
H_2O_2-bleached wool	71.3	75.5	71.6	3.8	13.8
Double-bleached wool	72.8	77.4	74.9	2.5	11.2
Royal spoonbill feather	73.6	78.1	77.0	1.0	9.3
Bovine collagen	87.0	91.8	92.2	−0.4	7.8
Silk	78.9	83.3	85.3	−1.9	5.7

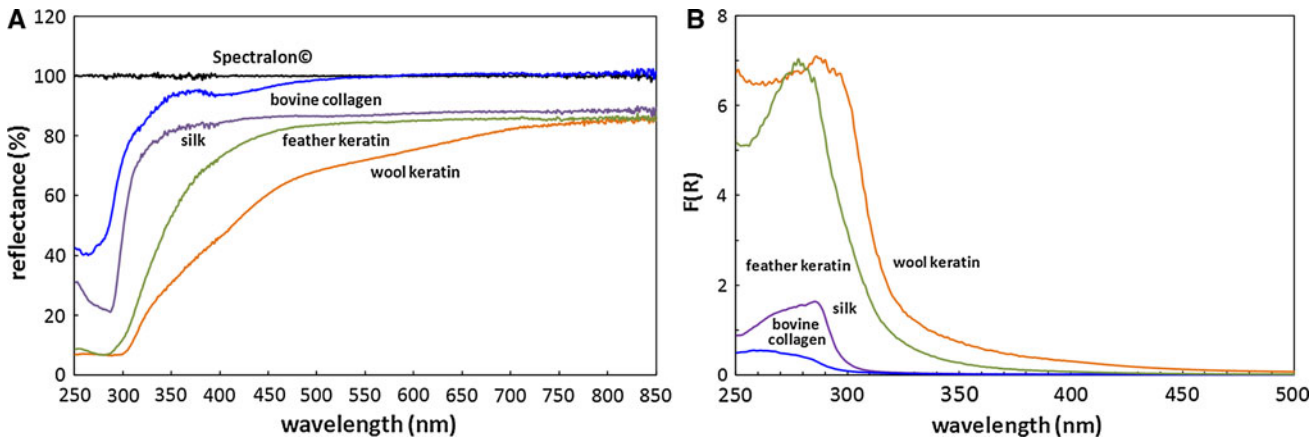

Fig. 2 Diffuse reflectance spectra of fibrous proteins in (**a**) $\%R$ and (**b**) $F(R)_\infty$ format. Reconstituted collagen provides an excellent white diffusing surface >550 nm, while the fabric structure of wool and silk and the morphology of feather keratin reduce their reflectance relative to Spectralon©

We carried out a diffuse reflectance study on these four amino acids. Their diffuse reflectance spectra after grinding the crystalline materials to fine powders in a pestle and mortar and placing in re-sealable PE bags are shown in Fig. 4.

Surprisingly, powdered tryptophan shows the weakest absorption in diffuse reflectance mode, in contrast to its strong absorption spectrum in solution shown in Fig. 3, and also shows higher reflectance than the standard material below 280 nm. The behaviour below 280 nm is due to the

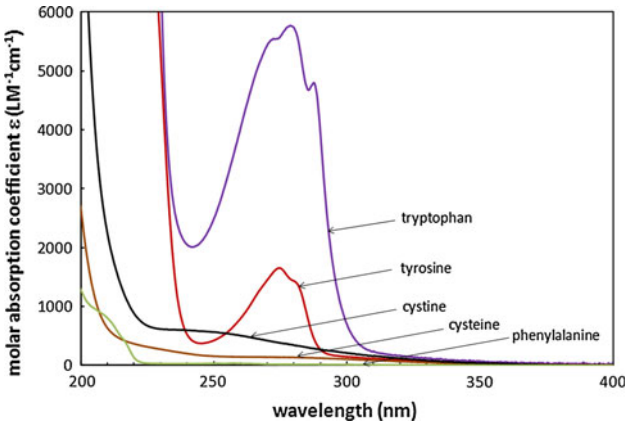

Fig. 3 UV–visible spectra of amino acids in aqueous solution at pH 7.1

fluorescence of tryptophan and a similar effect on the diffuse reflectance spectrum been reported previously (Anglin et al. 1972). Another surprising observation in Fig. 4 is the very strong absorption of solid cystine peaking at 290 nm, which would not have been expected from solution studies where only a weak inflexion point at 250 nm is observed.

Instrumental artefacts in the form of strongly absorbing peaks have previously been reported in the diffuse reflectance spectra of powdered salts in the UV region (Griffiths et al. 1959). Dilution of the substrate in the solid state with a highly reflective non-absorbing compound such as lithium fluoride or magnesium oxide can be used to identify these instrumental artefacts since such features do not show the expected decrease in absorption on dilution. To investigate whether this was the case for solid cystine a series of solid mixtures of cystine and glycine were prepared to determine the effect of dilution on the cystine S–S absorption, and their DR spectra are shown in Fig. 5.

Figure 5 confirms that the peak at 290 nm is not an instrumental artefact. A second sample of L-cystine obtained from a different source was also examined and it gave an identical diffuse reflectance spectrum. It is interesting to note that the absorption spectrum of solid L-cystine as a KBr disc also shows a strong peak near 282 nm

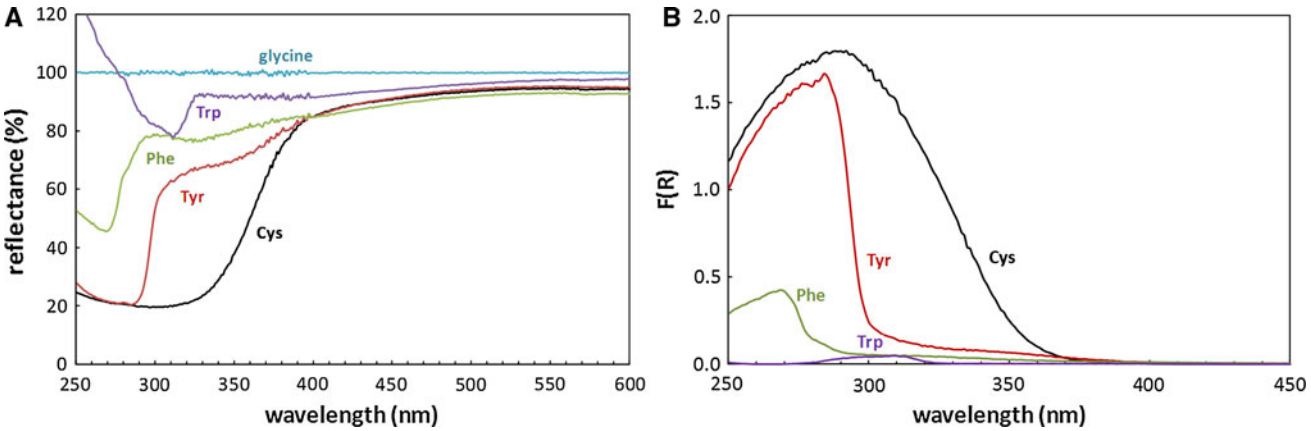

Fig. 4 Diffuse reflectance spectra of finely powdered amino acids in re-sealable polyethylene bags in (**a**) $\%R$ and (**b**) $F(R)_{\infty}$ format

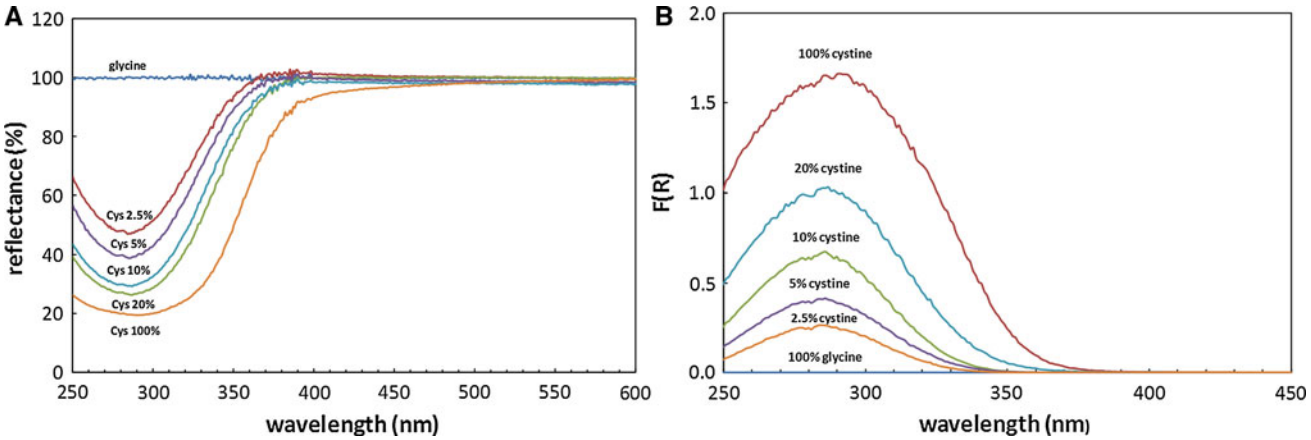

Fig. 5 Diffuse reflectance spectra of mixtures of solid cystine and glycine powders in re-sealable polyethylene bags in (**a**) $\%R$ and (**b**) $F(R)_{\infty}$ format

($e = 916$ L M^{-1} cm^{-1}) (Imanishi and Isemura 1969) rather than the weak inflection point at 249 nm ($e = 340$ L M^{-1} cm^{-1}) observed in solution (Anson et al. 1952).

Diffuse reflectance studies on solid amino acids have been reported previously in a study looking at how the spectra change after exposure to UV radiation (Anglin et al. 1972). In these studies, the amino acids were deposited onto the surface of aluminium mirrors as thick aqueous pastes and vibrated to form even deposits 1–2 mm thick. Preparations of different thickness (0.2–1 mm) were found to have little effect on the reflectance spectrum. The reported reflectance spectra of tryptophan, tyrosine, cystine and phenylalanine are very similar to those reported here, suggesting that the use of loose finely ground powders in PE bags as a sampling technique is much quicker and simpler method to obtain diffuse reflectance spectra.

Comparison of Figs. 2 and 4 shows that the diffuse reflectance spectra of unpigmented feather and wool keratin appear to be dominated by the cystine absorption at 280–290 nm, whereas silk fibroin appears very similar to tyrosine. This observation agrees with the UV-absorbing amino acid composition of fibrous proteins shown in Table 2. For feather keratin the cystine peak occurs at 280 nm and for wool keratin it is close to 290 nm, which may be due to differences in the dihedral angle χ(CSSC) of cystine residues in the different keratin proteins. The dihedral angle in disulphides has a profound effect on both the wavelength and oscillator strength of the S–S absorption. The wavelength range is very wide and has been reported to be from 250–370 nm (Boyd 1972).

The data in Table 2 for wool is obtained from amino acid analysis of the liquor obtained following hydrolysis of large numbers of wool fibres. However, wool fibres have a complex morphology consisting of cells, with flattened overlapping cuticle cells forming a protective sheath around the inner cortical cells. In fine wool, such as that obtained from Merino sheep, the cuticle is normally one cell thick (approximately 20 × 30 × 0.5 μm) and usually constitutes about 10% by weight of the total fibre. Sections of cuticle cells show an internal series of laminations, comprising outer sulphur-rich bands known as the exocuticle and inner regions of lower sulphur content called the endocuticle. On the exposed surface of cuticle cells, a thin proteinaceous band (the epicuticle, 2–4 nm thick) and a covalently bound lipid component (0.9 nm thick) form a resistant hydrophobic barrier. The UV absorption properties of saturated lipids are similar to the polyethylene film used to contain the powdered samples and it is unlikely that the surface lipid plays any significant role in diffuse reflectance from the fibre. However, the epicuticle protein contains much higher levels of sulphur than the whole fibre, and its half-cystine content has been estimated to be as high as 35% from XPS studies (Ward et al. 1993). This high cystine content near the fibre surface may well influence the diffuse reflectance spectrum.

Table 2 UV-absorbing amino acid composition of fibrous proteins

Protein	Amino acid composition (mol%)				Reference
	Cys (1/2)	Phe	Trp	Tyr	
Merino wool	9.9	2.8	0.5	3.5	Corfield and Robson (1955)
Chicken feather barbs	7.9	3.0	0.2	1.2	Harrap and Woods (1967)
Bombyx mori silk fibroin	0.1	0.6	0.2	4.9	Schroeder and Kay (1955)
Bovine skin collagen	–	1.3	–	0.4	Bowes et al. (1955)

Figure 1b shows that the intensity of the absorption at 290 nm is reduced following oxidative and reductive bleaching. This is expected since both bleaching processes reduce the Cys content of wool to form either oxidation products such as cysteic acid or reduced species such as cysteine. Figure 6 shows the diffuse reflectance spectra of the oxidation and reduction products of cystine as fine powders, confirming that they both have significantly lower absorption than cystine at 290 nm. The spectrum of free cysteine contains a weak absorption at 285 nm and this is probably due to partial oxidation to cystine during drying in air.

One question posed by these results is whether the cystine content of fibrous proteins, and wool in particular, has any influence on colour. The tristimulus and yellowness index values of powdered cystine and its simple oxidation and reduction products are shown in Table 3, which suggests that these compounds have little impact on colour. The powders appear white, all of them significantly whiter than wool, and they have similar reflectance spectra and XYZ tristimulus values. Unlike wool, cystine has flat reflectance spectra in the visible region from 750–400 nm as shown in Figs. 4 and 5. However, certain cystine products are known to influence colour during the alkali yellowing of wool due to formation of yellow polysulphides and lanthionine. It has been suggested by studies on model cystine compounds that alkali treatment of wool generates intensely yellow *N*-methylthiopyruvamide and yellow polysulphide chromophores which can decompose to form elemental sulphur (Hill and Ghadimi 1996).

Whether traces of coloured cystine-derived compounds are present in natural wool is unknown. The principal location of the natural cream chromophores of wool in terms of its morphology is also unknown, and two recent reports on the location of the chromophores generated by

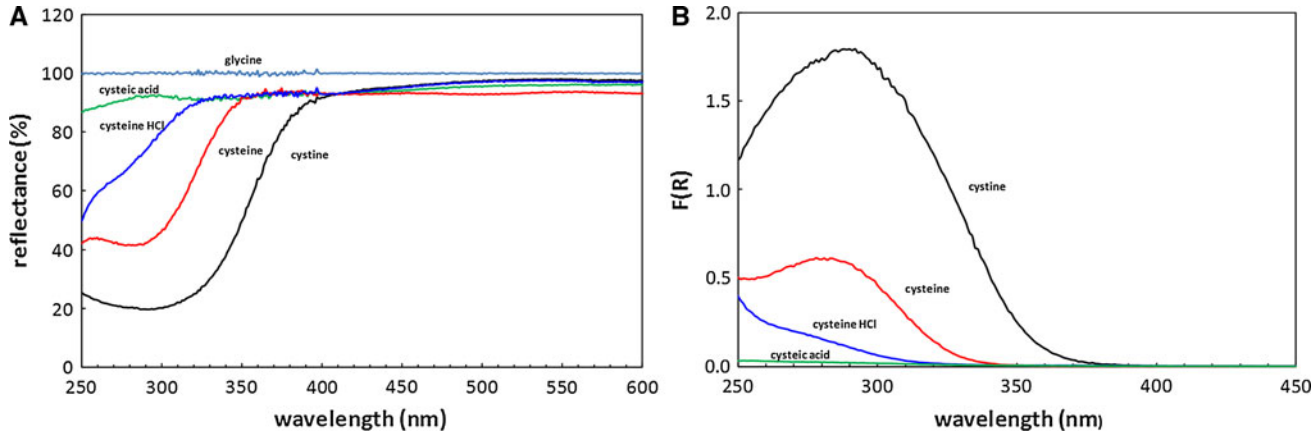

Fig. 6 Diffuse reflectance spectra of cystine, cysteic acid, cysteine and cysteine hydrochloride powders in re-sealable polyethylene bags in (**a**) $\%R$ and (**b**) $F(R)_{\infty}$ format

Table 3 Colour tristimulus and yellowness data for cystine and its oxidation and reduction products measured as finely ground powders in PE bags

Sample	X	Y	Z	$Y - Z$ (yellowness)	Yellowness index E313
Cystine	87.7	92.5	96.0	−3.5	4.1
Cysteine	86.2	91.0	96.2	−5.3	1.6
Cysteine HCl	85.4	90.2	93.7	−3.5	3.9
Cysteic acid	86.2	90.7	94.1	−3.4	4.5
Glycine	86.6	91.3	96.8	−5.5	1.4

UV exposure gave conflicting results. Studies where the morphology of the fibre had been scrambled to different levels by chopping and milling wool sliver into powders suggested that yellowing by UVB radiation occurs preferentially in the cuticle (Zhang et al. 2008). However, doubt was cast on this finding in a subsequent study where whole wool fibres were exposed to UVB, then mechanically separated into cuticle and cortical components and milled into powders. In this case, the data showed that the cortical material yellowed more than cuticle-enriched samples (Dyer et al. 2010). Further studies are required to determine whether coloured compounds derived from cystine play any role as natural or UV-initiated chromophores in wool.

The similarity between the DR spectra of wool and feather keratin which contain high levels of cystine residues and powdered cystine itself suggests that cystine may be more important as a primary UV absorber in keratins than previously thought. It is well known that cystine residues in wool keratin are oxidised during the exposure to sunlight, ultimately resulting in formation of cysteic acid. Until now it has been considered that cystine is only a minor direct absorber of UV radiation by comparing the absorption spectra of tryptophan, tyrosine, and cystine in solution (Fig. 3). The DR spectra in Fig. 4 strongly suggest the opposite, that cystine is in fact the primary absorber of wavelengths below 400 nm in the solid keratin proteins. It will be of some interest to determine the importance of this finding for other biomaterials, in particular the stratum corneum and epidermis of human skin which contain keratin intermediate filaments.

One of the interesting findings of this study is the different wavelength observed for the absorption of the cystine chromophore in the solid state and in aqueous solution. There are several possible reasons for this large red shift of ~40 nm. Both the wavelength and oscillator strength of the disulphide absorption depend upon the dihedral angle χ(CSSC) as described by Boyd (1972). Differences in dihedral angle between cystine in the solid state and in solution are possible if the crystal structure dictates a more strained structure. The literature suggests that there is some disagreement regarding the dihedral angle in hexagonal L-cystine; the angle reported in the crystal structure determination (Oughton and Harrison 1959) is 106° but later work by van Wart et al. suggests this structure is erroneous (van Wart et al. 1973) and a subsequent paper by this group (van Wart and Scheraga 1976) quotes a value of 74°. The more the value of χ(CSSC) deviates from 90°, the higher the red shift in absorption wavelength (Boyd 1972). It is interesting to note that dithianes having a dihedral angle of ~60° have an absorption peak at 280–290 nm (Boyd 1972).

We were interested in whether similar red shifts in diffuse reflectance peaks were observed for other disulphide compounds. The only other disulphide compounds available were homocystine, which has a similar structure to cystine except for having two extra CH_2 groups present, and lipoic acid which has a disulphide bond in a strained 5-membered dithiolane ring. Their DR spectra

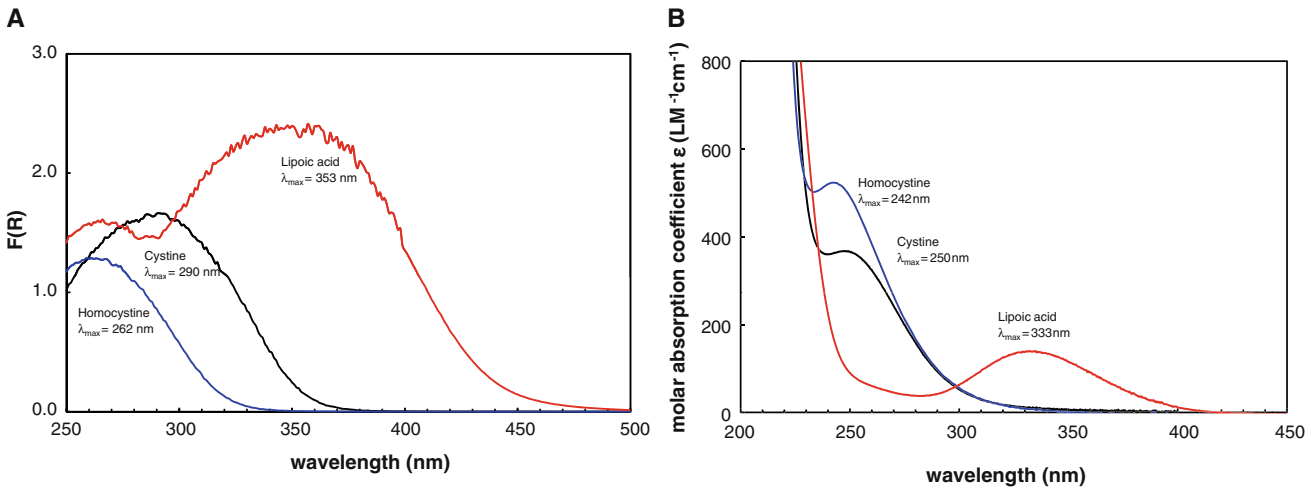

Fig. 7 **a** Diffuse reflectance spectra of cystine, homocystine and lipoic acid powders in re-sealable polyethylene bags in $F(R)_{\infty}$ format. **b** UV–visible spectra of the same compounds in aqueous solution. Lipoic acid was dissolved in 1:1 v/v ethanol: water

and UV–visible spectra in aqueous solution are shown in Fig. 7.

For solid homocystine the diffuse reflectance peak at 262 nm is also red shifted by 20 nm relative to that observed in aqueous solution (242 nm). Similarly lipoic acid also shows a 20-nm red shift from 333 nm in solution to 353 nm for the DR spectrum of the powder. The higher red shift of 40 nm observed for L-cystine may hence be due to a more strained conformation in the solid state.

Conclusions

UV–visible diffuse reflectance studies on finely ground tryptophan, tyrosine, phenylalanine and cystine powders have surprisingly shown that the disulphide chromophore of solid cystine has the strongest UV absorbance of the four measured using the remission function $F(R)_{\infty}$. This result contrasts strongly with studies in aqueous solution where it is well known that tryptophan and tyrosine have the highest molar extinction coefficients. The disulphide absorption of solid L-cystine in the DR spectrum at 290 nm is significantly red shifted by $\sim$40 nm relative to its wavelength in solution. The DR spectra of two other disulphide compounds, homocystine and lipoic acid, exhibited smaller red shifts of 20 nm from their absorption maxima in solution. The larger red shift and increase in intensity for the disulphide chromophore of solid L-cystine relative to aqueous solution may be due to differences in the dihedral angle χ(CSSC) imposed by the hexagonal crystal lattice.

It is normally accepted that tryptophan and tyrosine are the primary UV chromophores present in wool and feather keratin on the basis of the UV–visible absorption spectra of the free amino acids in aqueous solution. DR spectra of these fibrous proteins show a strong peak at 280–290 nm which is very similar to that observed for cystine powder, whereas silk fibroin, which contains very little cystine, and bovine skin collagen which is cystine-free, have quite different DR spectra. It seems likely therefore that the cystine residues provide an important primary UV-absorbing species in keratins, although cystine probably does not contribute to the natural cream colour of these proteins.

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