Effect of temperature and bath composition on the dyeing of cotton with catalase-treated bleaching effluent

Tzanko Tzanov,[a] Silgia Costa,[a] Georg M Guebitz[b] and Artur Cavaco-Paulo[a,*]

[a]*University of Minho, Textile Engineering Department, 4800 Guimaraes, Portugal*
Email: artur@eng.uminho.pt

[b]*Technical University of Graz, Dept of Environmental Biotechnology, 8010 Graz, Austria*

Coloration Technology

Society of Dyers and Colourists

Conventional rinsing to remove residual hydrogen peroxide after the bleaching of cotton fabric can be replaced by enzymatic cleaning using catalases. Our previous study showed that dyeing in such catalase-treated bleaching baths results in a shade change on the dyed fabrics. The aim of this present study was to investigate the reasons for the unacceptable colour differences of the dyed fabrics. Experiments were performed to study the possible temperature-dependent dye interactions in the standard dyebath, and in the dyebath prepared with bleaching effluent. Fluorescence emission spectra were used to detect changes in the structure of the catalase related to the thermal unfolding and denaturing of the protein. It was found that the colour difference on the dyed textiles could be attributed to the temperature-dependent dye–enzyme interaction and precipitation, as well as to the complexity of the bleaching bath composition.

Introduction

Hydrogen peroxide is currently the most commonly used bleaching agent for all natural fibres of plant or animal origin. However, the dyeing of fabric that has first been bleached with hydrogen peroxide requires high energy and high levels of water consumption. This is due to the necessary rinsing of the material in order to remove any peroxide residue that might be harmful to the applied oxidation-sensitive dyes [1,2]. Even this washing process cannot guarantee the complete removal of peroxide. Contemporary biotechnology offers the opportunity to reduce this large water consumption by the use of catalases for peroxide decomposition. The enzyme catalase is widely distributed in nature and well known for its ability to catalyse the conversion of hydrogen peroxide to water and gaseous oxygen [3,4].

There are two possible approaches for the application of this enzyme to the cleaning of residual bleaching baths prior to dyeing. One possibility is to drain off the bleaching bath and to refill it with fresh water containing the enzyme, and the second strategy is to add the enzyme directly to the bleaching liquor and then reuse it for dyeing. The first approach has been widely adopted in the textile industry and catalase-based commercial products are generally applied in that way [5]. The second approach represents a new, unconventional dyeing technique, e.g. dyeing in the bleaching bath, which may ensure efficient reduction of water and energy consumption, and process duration, avoiding the extensive washing process of cellulose fabrics after bleaching. In our previous study, comparing the two approaches for application of the enzyme, the direct treatment of the residual bleaching bath with catalase followed by dyeing in the same liquor, resulted in a higher and unacceptable colour differences of the dyed fabrics [6]. The aim of the present experimental work was to investigate the reasons for this colour failure.

Experimental

Bleaching and dyeing conditions

The monochlorotriazine dye CI Reactive Blue 198 (Evercion Blue HEGN, commercial grade dye obtained from Everlight) was applied in an 'all-in' dyeing process on previously bleached 100% cotton woven fabric. The fabric samples, 2.5 g each, were bleached at 90 °C for 60 min following the recipe: silicate (BDH), 3.5% owf; soda ash (Merck), 1% owf; sodium hydroxide (Merck), 1%; and 35% hydrogen peroxide (Aldrich), 4% owf.

The dyeing procedure that was performed was an 'all-in' process, e.g. all the dyeing chemicals and the textile material were added at the beginning of the process. The conditions were: 3% owf CI Reactive Blue 198 in the presence of Glauber's salt 60 g/l, soda ash 20 g/l, dyeing temperature 80 °C and dyeing time 60 min. This 'all-in' dyeing procedure was chosen in order to compare dyeings in the standard dyebath and in that prepared with catalase-treated bleaching effluent dyebath, since in the latter the alkali is already present in the bleaching effluent. Both dyeing and bleaching were carried out in an Ahiba Spectradye (Datacolor) dyeing apparatus at a liquor ratio 20:1. Each dyeing experiment was repeated three times. Dyed fabrics were washed-off at the same liquor ratio with non-ionic detergent Hostapal CV (Clariant) for 30 min at 90 °C to remove the unfixed dye.

Enzyme treatment

The bleaching bath was treated with 4 ml/l bovine liver catalase (EC 1.11.1.6 hydrogen peroxide oxidoreductase) (product number C-100 from Sigma, 38.5 mg/ml protein) for 15 min until no presence of hydrogen peroxide was detected with analytical test strips from Merck (Merckoquant Peroxide-Tests: 1.10011.0001, 1.10081.0001, 1.10337.0001). The treated liquor was used to prepare the dyebath with CI Reactive Blue 198.

Temperature/activity profile of the enzyme

The enzyme activity and stability were measured following the decomposition of hydrogen peroxide by UV spectrophotometric assay (Unicam Heλios UV–Vis spectrophotometer) [7] at temperatures ranging from 30 to 80 °C, after incubation times of 5 and 30 min, respectively.

Total colour difference

The colour difference (ΔE^*) of the dyed fabrics was determined using a reflectance measuring apparatus (Datacolor Spectraflash 600) according to the CIELAB colour difference concept at standard illuminant D_{65} (LAV/Spec. Excl., d/8, D_{65}/10°).

Thermal scanning of fluorescence

The temperature dependence of the intrinsic fluorescence emission of catalase was determined using a fluorescence spectrometer FLUOROLOG-2 (Spex Industries), provided with water-jacket sample cell holder. The temperature was varied using an external circulating water bath. Emission spectra were collected at intervals of 10° by setting the excitation monochromator at 285 nm and scanning the emission monochromator from 300 to 450 nm.

Rate of exhaustion and fixation

In order to define the rate of exhaustion and fixation, 1 ml samples were taken from the dyeing liquor at different times during the dyeing process. Samples were studied spectrophotometrically after adequate dilution (1:50) at 627 nm and the concentration of the dye was determined according to the previously set dye calibration curves in the corresponding solvent. The rate of fixation was determined after multiple washing at 90 °C, until no dye was removed from the dyed fabric. All the washing solutions were collected and the concentration of the fixed dye was determined from the absorbance at the maximum wavelength of the dye (627 nm). The percentage of exhaustion was determined as a proportion between the dye initially presented in the dyebath C_i and the dye remaining at the end of dyeing C_f (Eqn 1) and the fixation yield (Eqn 2) where C_w is the dye removed after multiple washing.

$$E(\%) = \left(1 - \frac{C_f}{C_i}\right) \times 100 \quad (1)$$

$$F(\%) = \left(1 - \frac{C_f + C_w}{C_i}\right) \times 100 \quad (2)$$

Results and Discussion

Dye exhaustion and fixation

The dye uptake of the fabric can be determined spectrophotometrically by detecting the amount of dye remaining in the dyebath after dyeing is completed. Common practice is that the concentration of the dye is determined according to its calibration curve in water since a linear relationship is usually found between absorbance and concentration. For the measurements to be reliable, the extinction coefficient of the dye, as determined from the slope of the Beer–Lambert calibration graph, should be constant, even though the composition and the temperature of the dye solution may vary. However, some reactive dyes give pronounced changes in extinction coefficient when the salt concentration or pH of the solution is changed or when the solution is boiled. Thus it is very difficult to obtain reliable data of dyebath exhaustion/fixation yield [8].

Spectrophotometric analysis for determining the concentration of dye solutions becomes much more complex when the dyeing process is performed in a catalase-treated bleaching bath because of the influence of all the bleaching chemicals and products extracted from the cellulose. In our experiments the dye concentration was calculated using calibration curves recorded with dyeing liquors prepared with the corresponding solvent systems: water; a typical dyeing composition without dye; and bleaching effluent with added salt and alkali (Figure 1).

The plots demonstrate linear relationships with high values of the correlation coefficients. The slopes determined by the linear regression slightly decreased, e.g. the extinction coefficient of the dye decreased in other than water solvents. According to these calibration curves the concentration of the dye during the dyeing process was monitored. From Figure 2 it can be seen that the rate of dye exhaustion in the dyebath prepared with bleaching effluent was lower overall than the standard dyeing procedure. After a dyeing period of 100 min the dye exhaustion of the bleaching effluent dyebath was 66% whilst the standard dyebath was 71% (a similar trend was obtained for dyebath fixation). These results suggest some interaction between the bleaching bath components and

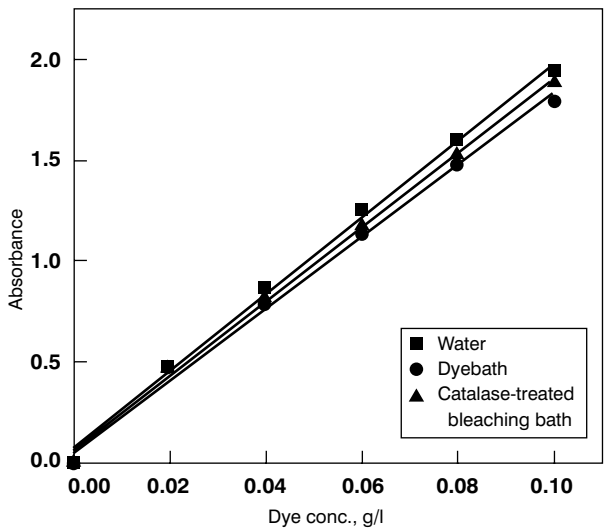

Figure 1 Beer's law plots for CI Reactive Blue 198 in different solvents.

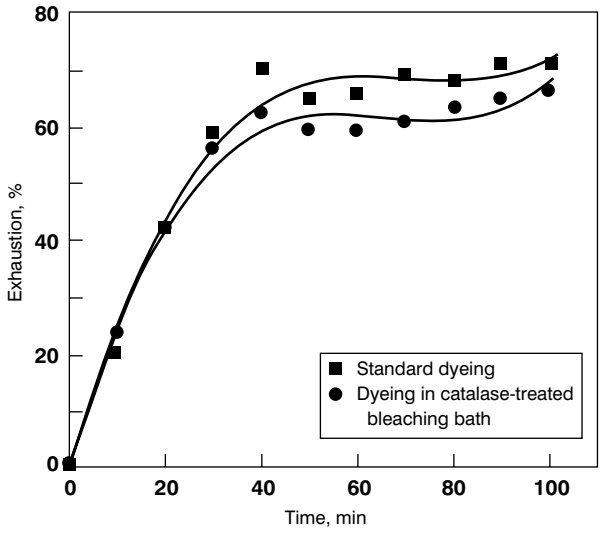

Figure 2 Rate of dyebath exhaustion for CI Reactive Blue 198

the dye, leading to possible dye aggregation and reduced substantivity toward the substrate.

The resulting total colour difference ΔE^* between fabrics dyed in a bleaching bath and those dyed in a standard dyebath was in the range of 2–3 CIELAB units [6] (and thus considered unacceptable [9–11]) and mainly arose from a difference in lightness, which in fact indicated reduced dye uptake. It should be noted that for samples dyed in a depth of shade lower than 3% owf this colour difference was greater. Although the previous study demonstrated that the colour difference could be reduced and even compensated by varying the concentration of the applied chemicals [6], the reason for this shade change was still to be investigated. The impact of the various dyebath components on the colour change was therefore studied.

Interaction of dye and bleaching effluent components

Apart from the non-clearly identified composition of the substances extracted from the cellulose, the bleaching bath liquor contains typical bleaching chemicals, i.e. alkali, silicate and residual peroxide. According to our previous studies, to perform a dyeing procedure reusing the bleaching bath any residue of peroxide should be removed and the conventional dyebath components added [6]. Hence the resulting dyebath composition is rendered even more complex and contains in addition salt, enzyme and dye. Various interactions can be supposed from this dyeing liquor composition. The influence of the alkali and salt concentration on the dye absorbance as well as its temperature dependence has to be considered.

In the next stage of our study, the influence of each individual chemical on dye concentration depending on the temperature was investigated. For this purpose, solutions containing the various chemicals present in the standard dyebath and in the bleaching bath (in usual concentrations according to the recipe for dyeing) were subjected for 30 min to temperatures ranging from 30 to 100 °C.

Our results showed the dye absorbance in the dyebath prepared with catalase-treated bleaching effluent decreased compared to the absorbance in the conventional dyebath (Figure 3). Thus some interaction must be taking place in the dyeing solution prepared in such a manner. Additionally a temperature-dependent decrease of dye absorbance in the bleaching bath was observed. It is known that the extinction of anionic dyes decreases when aggregation occurs in the presence of salt and it may also change when the pH of the solution is varied [12]. However, no such influence of temperature on the dye absorbance when alkali and salt are present in the dye solution was detected in our study (Figure 3).

Some dyes give considerable changes in extinction with increasing temperature but this was not the case for CI Reactive Blue 198. Neither could a temperature-dependent influence of silicate on dye absorbance associated with possible dye/silicate interactions be concluded from the experiment (Figure 3). The absorbance of the dye solution containing silicate was 10% lower than the standard dyebath but this was fairly constant over the whole temperature range. Increasing the amount of enzyme added to the bleaching bath (bleaching bath volume 50 ml) from 0.20 to 0.60 ml did not substantially affect the dye absorbance. Higher amounts of enzyme have no practical significance considering that 0.20 ml of the product were sufficient to decompose the residual peroxide in 15 min.

Dye–enzyme temperature-dependent interaction

Based on the observation that dye absorbance decreased with increasing temperature in the dyebath prepared with catalase-treated liquor, a series of experiments were performed in order to investigate whether a temperature-dependent dye–enzyme interaction is taking place. Catalase solutions (concentration 0.5 g/l) were subjected for 30 min to temperatures ranging from 30 to 100 °C. The protein content was determined at 595 nm for both hot and cooled down samples to see whether the changes are reversible. The solutions were centrifuged if turbidity appeared. The well-known dye-binding method of Bradford, using an acid dye CI Acid Blue 90 (Brilliant Blue G, commercial grade dye obtained from Communise) for protein determination,

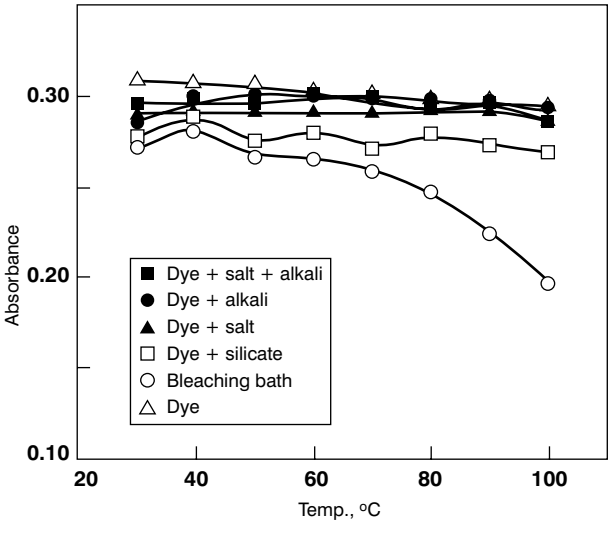

Figure 3 CI Reactive Blue 198 absorbance in different liquors, recorded at the wavelength of maximum dye absorbance (627 nm), depending on temperature after 30 min of incubation

was taken as a suitable model for studying the possible temperature caused dye–protein interaction [13].

The results in Figure 4 indicate there is a clear relationship between protein concentration and the temperature of the sample. The temperature-dependent decrease of catalase concentration suggested some dye–enzyme interaction, which remained irreversible after cooling indicating the enzyme has been denatured. Such a decrease in catalase concentration (and dye concentration, respectively) could be associated with the unfolding of the catalase structure above the corresponding temperature. The unfolded enzyme structure offers a greater opportunity for further interaction with the negatively charged acid dye molecules, possibly resulting in dye aggregation and precipitation.

The same experiment was then performed with CI Reactive Blue 198 being applied in the dyeing process and the profile of the temperature-dependent reactive dye concentration is shown in Figure 5. This profile is very similar to that of the temperature-dependent catalase concentration in Figure 4 although different dyes were used in each case (reactive and acid) and thus different dye–enzyme interactions would be possible. Compared to the acid dye concentration, the change in reactive dye concentration had a much lower magnitude, which would be expected since the acid dye should have higher affinity toward the denaturated protein than the reactive one under the corresponding conditions.

Thermal scanning fluorescence studies of enzyme unfolding

Changes in the fluorescence emission intensity have been shown to be a useful indicator of thermal unfolding of protein molecules [14]. This methodology detects changes in the environment of certain residues in the protein molecule. The wavelength of maximum fluorescence emission is a function of the polarity of the microenvironment surrounding a given residue – the more polar the environment, the longer the wavelength of maximum emission [15]. This principle is used to monitor the transfer of tryptophan side-chains from a relatively nonpolar (hydrophobic) environment in the interior of a globular protein to an essentially aqueous environment, as the protein is unfolded to an open structure.

The increase in wavelength of the maximum emission of the catalase that is seen with increasing temperature (Figure 6) indicates a more solvent-exposed environment for the tryptophan. The thermal inactivation behaviour of the catalase also appears to be related to the temperature-dependent thermal unfolding of the protein structure (Figure 7). It is apparent that over the temperature range from 50 to 80 °C the catalase undergoes significant structural changes. This observation was confirmed by the results from the thermal dependence of catalase concentration and the temperature profile of the enzyme activity.

The transition from globular to random coil conformation of the protein occurs during denaturation at elevated temperature. Amino acid residues, appearing from the interior of the globular structure, are exposed on the contact surface and are able to react with the dye. This thermally initiated process increased the hydrophobic dye–

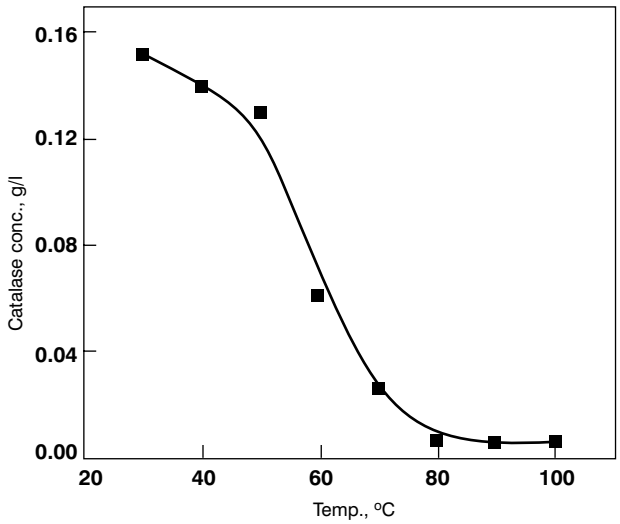

Figure 4 Temperature dependence of catalase concentration, converted from absorbance measurements at the wavelength of maximum absorbance of the dye/protein complex of the Bradford method (595 nm) after 30 min incubation in acidic medium

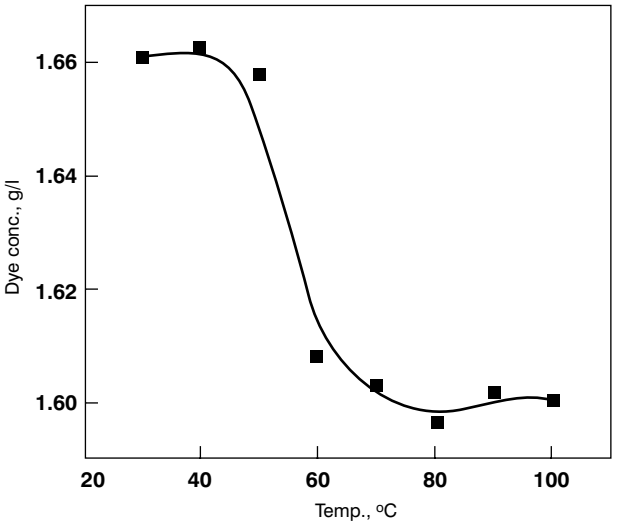

Figure 5 CI Reactive Blue 198 concentration converted from absorbance measurements at the wavelength of maximum absorbance of the dye (627 nm) in water solution, depending on the temperature in the presence of catalase

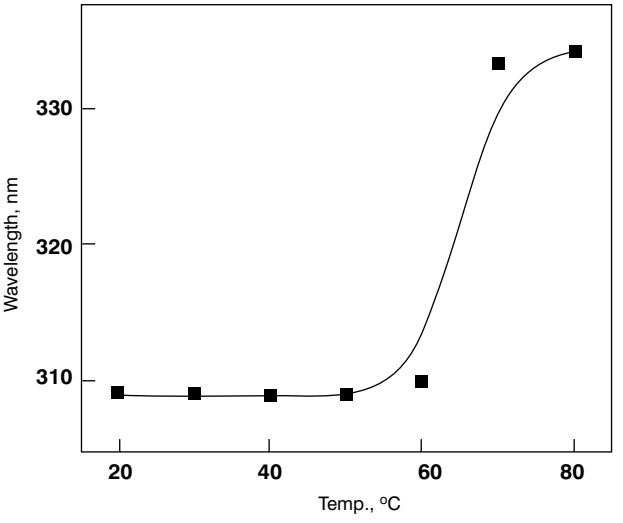

Figure 6 Temperature dependence of the fluorescence emission maximum of catalase in 50 mM phosphate buffer pH 7 (excitation monochromator set at 285 nm; scanning the emission monochromator from 300 to 450 nm)

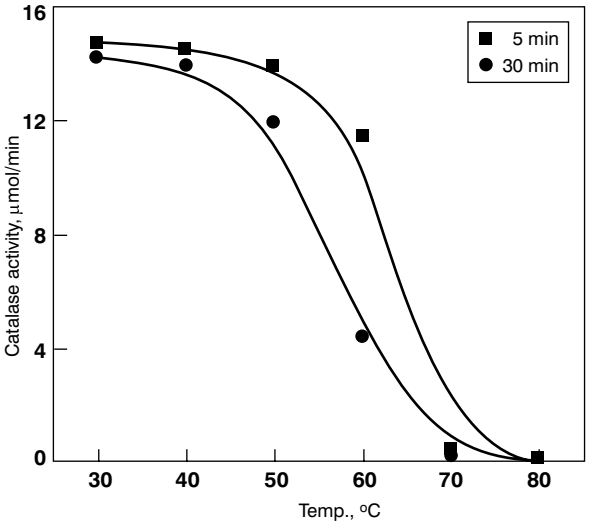

Figure 7 Temperature profile of catalase activity after 5 and 30 min of incubation at pH 7 (50 mM phosphate buffer)

enzyme or enzyme–fabric interactions, resulting in dye precipitation and poor dyeing results. The former constraint may be overcome by using immobilised enzymes. It should be noted that when a fabric is present in the dyebath the number of expected interactions increases, which is associated with the different substantivity of the chemicals toward the substrate and its own reactive chemical structure. The interaction between the fabric and the unfolded enzyme could be supposed also.

Conclusions

Dyeing in catalase-treated bleaching baths resulted in a shade change on the dyed fabrics. Following an increase of the dyeing process temperature, a corresponding decrease in the dye concentration of the bleaching liquor was detected. As a consequence the experimentally derived rates of exhaustion and fixation of the dye decreased. The experiments performed to study the possible temperature-dependent dye interactions in the standard dyebath, and in the dyebath prepared with bleaching effluent, showed that the dyeing chemicals and the typical bleaching additives did not provoke any significant alteration to the state of the dye in the dyeing solutions. However with the increase in temperature significant changes occurred in the structure of the catalase, related to the thermal unfolding and denaturing of the protein, as indicated by the shift in its fluorescence emission activity. Usually, the denatured protein precipitates taking with it the dye from the dyeing solution and thereby decreasing its concentration. Thus the colour difference on the dyed textiles could be attributed to the temperature-dependent dye–enzyme interaction and precipitation, as well as to the complexity of the bleaching bath composition. This includes a variety of substances extracted from the cotton, e.g. oils and waxes, pectins, proteins, organic acids, mineral matter, natural colouring-matter and size.

References

1. U Sewekow, *Melliand Textilber.*, **74** (1993), 153.
2. A Uygur, *J.S.D.C.*, **113** (1997) 211.
3. E Akertek and L Tarhan, *Appl. Biochem. Biotechnol.*, **50** (1995) 291.
4. L Goldstein and G Manecke, in *Applied Biochemistry and Bioengineering*, Vol. 1, Eds L B Wingard, Jr, E Katchalski-Katzir and L Goldstein (New York: Academic, 1976) 23.
5. K-H Weible, *US Patent*, 5 071 439 (1991).
6. T Tzanov, S Costa, G Guebitz and A Cavaco-Paulo, *Color. Technol.*, **117** (2001) 1.
7. H E Aebi, in *Catalase in Methods of Enzymatic Analysis*, Eds H U Bergmeyer and K Gawehn (Weinheim: Verlag Chemie GmbH, 1983) 273.
8. Y Qu and S Jamshidi-Barzi, *Text. Chem. Colorist*, **24** (1992) 13.
9. D Steen, *L'Industrie Textile* (1998) 55.
10. W Bauman, R Brossman, B T Grobel, N Kleinemeier, M Krayer, A T Leaver and H P Oesch, *Text. Chem. Colorist*, **19** (1987) 21.
11. R W Harold, *Text. Chem. Colorist*, **19** (1987) 23.
12. C E Garland, *Analytical Chemistry of Synthetic Dyes* (New York: Wiley Interscience, 1977) 149.
13. M M Bradford, *Anal. Biochem.*, **72** (1976) 248.
14. J Woodward, N E Lee, J S Carmichael, S L McNair and J M Wichert, *Biochim. Biophys. Acta.*, **1037** (1990) 81.
15. J R Lakowicz, *Principles of Fluorescence Spectroscopy* (New York: Plenum Press, 1983).