Dyes and Pigments 116 (2015) 65–73

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Study of the interaction between collagen and naturalized and commercial dyes by Fourier transform infrared spectroscopy and thermogravimetric analysis

Davide Pellegrini [a], Massimo Corsi [b], Marco Bonanni [b], Roberto Bianchini [b, **], Alessandro D'Ulivo [a], Emilia Bramanti [a, *]

[a] National Research Council of Italy, C.N.R., Istituto di Chimica dei Composti Organo Metallici-ICCOM-UOS Pisa, Area di Ricerca, Via G. Moruzzi 1, 56124 Pisa, Italy

[b] Department of Chemistry "Ugo Schiff", Via della Lastruccia 3-13, 50019 Sesto Fiorentino, Florence, Italy

ARTICLE INFO

Article history:
Received 25 September 2014
Received in revised form
10 January 2015
Accepted 12 January 2015
Available online 21 January 2015

Keywords:
Dyes
FT-IR
TGA
Cross-linking
Collagen
Secondary structure

ABSTRACT

The naturalized dyes (ND) and the traditional acid dyes (ADs) were compared by studying the different behavior during the leather dyeing process. NDs are glyconjugated compounds synthesized by the covalent union of a dye species with a natural sugar (*e.g.* lactose) able to confer water-soluble properties to the dye molecule as a whole. The interactions between the dyes and the leather proteins were studied by FT-IR spectroscopy and thermogravimetric (TG) analyses. The protein cross-linking of the dyed leather samples was investigated by studying the 1654/1690 cm^{-1} peak height ratio and a deconvolution procedure of the amide I peak. The helix secondary structure was the predominant component of the leather proteins of the samples dyed with low concentrations of NDs (2%), while the β-sheets prevailed when leather samples were dyed with the traditional ADs and high concentrations of NDs (>5%). The data were discussed with respect to TG results.

1. Introduction

The transformation of animal skin into leather is a complex process encompassing several steps, among which leather dyeing adds value to the material for market purposes [1]. Routinely, the dyeing is carried out with sulfonated colorants at acidic pH, in order to establish electrostatic interactions between the residual amino groups of collagen and the sulfonic groups of the dyes [2]. This process takes place in water and in the presence of a wide variety of auxiliary chemicals: from salts to adjust the pH of dyeing baths, to surfactants and mordants to aid dye penetration into leather and enhance the overall fastness properties. Therefore, effluents from a tannery include a broad spectrum of contaminants, impacting heavily on the environment [3].

Recently, we synthesized a new class of environmentally friendly naturalized dyes (NDs) [4]. Briefly, a naturalized dye is a glyconjugated chemical, which is the covalent union of a dye species (*e.g.*, azo, anthraquinone, aniline type chromophore) with a natural sugar, privileging lactose, able to impart remarkable water-soluble properties to the dye molecule as a whole. Moreover, dyeing processes involving NDs do not require the addition of chemical auxiliaries [5] and the resulting fastness properties of the dyed materials (*e.g.*, polyester and nylon) are competitive with those observed from commercial colorants [6]. On the basis of these findings we prepared a second generation of NDs, in view of replacing traditional acid dyes (ADs) to advance to a more eco-sustainable leather industrial process. In particular, we achieved a new chemical bridge linking the chromophore and lactose [5], introducing a more stable amide moiety in place of an ester (Fig. 1) [4].

This modification to the first reported structures generated NDs with an amphoteric character, which is essential for leather dyeing process [5]. Furthermore, we observed that chemically different glyconjugated dyes of this new class were able to dye leather when combined among them, giving a finished material with

* Corresponding author. Tel.: +39 050 315 2293; fax: +39 050 315 2555.
** Corresponding author. Tel.: +39 055 4573486; fax: +39 055 4574913.
E-mail addresses: roberto.bianchini@unifi.it (R. Bianchini), bramanti@pi.iccom.cnr.it (E. Bramanti).

http://dx.doi.org/10.1016/j.dyepig.2015.01.012
0143-7208/

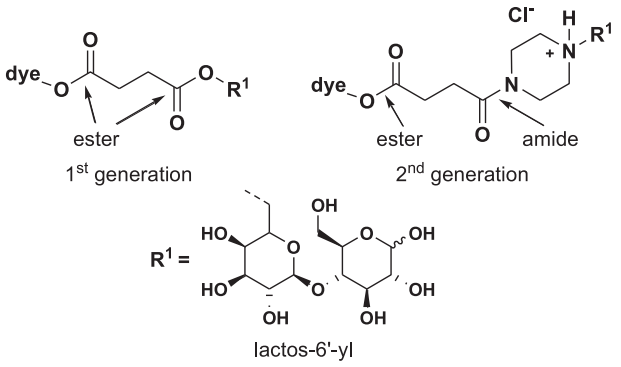


Fig. 1. Naturalized dyes.

homogeneous hue and fastness properties [6]. Elasticity, flexibility and longitudinal/tensile strength characterize collagen-based materials depending on the degree of intermolecular cross-links between the collagen triple helical units: lengthwise strength is enhanced by a parallel alignment of the fibrils (tendons), compliance is obtained through random layered arrangements (skin), flexibility is gained with laminated sheets, tensile strength is improved by means of concentric fibrillar layers [7]. These properties have been studied experimentally but also through successful computational materiomics studies [8–12]. While amino acid side chains play an important role in self-association of collagen helices and lateral packing of collagen helices, they are also crucial in the binding of a wide variety of molecular species which influence the microstructure and physical performance of the collagen matrix [13,14].

In this work, we investigated the interaction of second generation NDs **7**–**9** and **12** with leather at molecular level by Fourier transform infrared spectroscopy (FT-IR) and thermogravimetric analyses (TGA). IR spectroscopy is a well-established technique to analyze the secondary structure of polypeptides and proteins [15–19]. The IR spectral data of polypeptides and proteins are usually interpreted in terms of the vibrations of a structural repeat unit, which give rise to nine characteristic IR absorption bands, namely amide A, B and I-VII [17–19]. Among these, amide I and II bands are the two most prominent absorptions of the protein backbone, with amide I (1700-1600 cm^{-1}) being the most sensitive one of the spectral region. The amide I band is due almost entirely to the carbonyl stretch vibrations of the peptide linkages and the frequencies arising from each component of the complex absorption correlate closely to each secondary structural element of the proteins [20]. Thus, second derivative spectra allow the identification of the various secondary structures present in the protein [18] and the curve fitting can be applied to calculate the contribution of each component of the absorption band to the secondary structure [18,21–24].

The results were compared to those obtained from a group of commercial ADs. An interaction model between the dyes and the leather proteins was described, indicating that NDs interact better than ADs with the polypeptide matrix.

2. Experimental section

2.1. Materials and solutions

Chromium tanned leather samples and the commercial traditional ADs Acid Orange 37 (AO37), Acid Yellow 49 (AY49), Acid Red 249 (AR249) and Acid Blue 113 (AB113) were provided by BIOKIMICA. The NDs **7**-**9** and **12** were synthesized according to patented experimental procedures, elaborating commercial Disperse Orange 30 (DO30), Disperse Red 202 (DR202), Disperse Blue 27 (DB27) and Disperse Yellow 42 (DY42), respectively [5]. Formic acid was purchased from Sigma-Aldrich-Fluka (F0507, purity > 95%). All solutions were prepared with deionized water (18.2 MOhm cm) using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Synthesis of second generation ND: the (piperazin-6′-yl)lactose moiety

The selective protection of the hydroxyl groups of lactose **1** [25] generated the protected diol **2**, whose 6′ and 2′ positions were available for further elaboration. Regioselective tosylation of the primary alcohol [26] was followed by an S_N2 process using an excess of piperazine to obtain **4** in 62% overall yield for three steps (Scheme 1).

Next, the piperazine derivative **4** was coupled to the dye **5**, which had been prepared conveniently from chromophore DO30 [5]. The free carboxylic acid moiety was activated with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) in mild conditions [27], recovering compound **6** in good yield. Final deprotection of the acetonide moieties was carried out in acid conditions, to restore the original structure of the lactose portion within **7** (Scheme 2) [5].

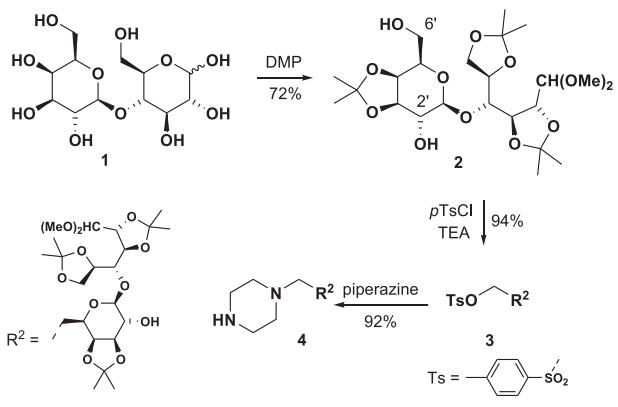
Adduct **7** showed a significant solubility in water, even though the piperazinil unit was present as a hydrochloride salt. A similar strategy was followed to synthesize compound **8** and **9** (Fig. 2) [5].

A slightly different approach was adopted for compound **12**. In this case, DY42 **10** was made to react with ethyl chloroacetate in the presence of a catalytic amount of potassium iodide [28]. The resulting ester derivative was hydrolyzed to acid **11**, which was converted to **12** following the route described previously (Scheme 3).

Thanks to this easy procedure to achieve the second generation NDs, it is possible to process chemically different chromophores in a reproducible manner, whenever a free carboxylic acid is available for amide coupling [5].

2.3. Leather dyeing

The operational procedure for leather dyeing was an adaptation to laboratory scale of the daily large scale dyeing of a tannery. Briefly, 0.2 g of chrome tanned leather specimen were put in a plastic tube with a 2 mL aqueous solution of dye at C1 concentration (4 mg, 2% w/w) and stirred (magnetically) for one hour at 20 °C. The dyeing bath was heated at 55 °C and 0.4 mL of water and



Scheme 1. Synthesis of protected (piperazin-6′-yl)lactose.