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Potential of oxazolidines and zirconium oxalate crosslinkers on the hydrothermal and thermo-mechanical stability of collagen fiber

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Abstract

Collagen, a unique connective tissue protein finds extensive application as prosthesis, artificial tissue, drug carrier, cosmetics and leather industry. This work has been undertaken to stabilize collagen using oxazolidine and zirconium oxalate as crosslinking agents. It has been found that both oxazolidine and zirconium oxalate imparts thermal stability to collagen, and oxazolidine exhibits a marked rise in the peak temperature when compared to both native and zirconium oxalate tanned collagen. On the other hand, zirconium does not seem to alter the tensile strength of collagen fibers significantly in wet condition as well as oxazolidine. The study is expected to enhance the biomaterial applications of collagen tissues.

Keywords: Shrinkage temperature; Collagen Crosslinking; Mechanical; Oxazolidine; Zirconium

1. Introduction

Collagen, one of the most abundant proteins in mammals, accounts for about 20–30% of total proteins in human body and other animals. It is an important biomaterial finding several applications as prosthesis, artificial tissue, drug carrier, cosmetics and leather industry [1]. Modifications of Collagen film are the common methods to improve its integrated performance. Chemical crosslinking is an efficient approach for Collagen crosslinking in tanning processing compared to weak physical crosslinking. The widespread use of collagen highlights the need to understand the mechanism of stabilization of collagen against biodegradation and heat, as these studies will have far reaching implications in both industrial and biological applications of collagen. It is well known that collagen crosslinked with various crosslinking agents such as oxazolidines, zirconium, and glutaraldehyde which is made resistant against the degradation by collagenase and also that the thermal and mechanical stability of collagen is increased owing to the crosslink's formation [2– 5]. This crosslinking is the main objective of tanning, a process that converts raw animal hide/skin into leather. The role of metals as a protein stabilizer has gained an attention in the tanning industry [6,7]. Tanning, as a process introduces intermolecular crosslinks between collagen molecules, thereby improving the thermal, enzymatic and mechanical stability of the fibrous network [8,9]. In zirconium tanning, various theories have been proposed to explain the mechanism of binding of zirconium to collagen. It has been shown that zirconium Orth sulfate, which was used in tanning was predominantly anionic or nonionic in character and binds with amino groups of collagens [10]. On the other hand, oxazolidines are heterocyclic derivatives obtained by the reaction of amino-hydroxy compounds with aldehydes. It was observed that oxazolidines react with the hide protein and impart special characteristics to leather when tanned in combination with synthetic tanning agents (syntans), chrome and vegetable tanning materials. The bifunctional behavior of these oxazolidines is responsible for their reaction with proteins.

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In the current research work, an attempt has been made to understand the stabilization of collagen by oxazolidine and zirconium oxalate, to study the role and effect of coordinate and covalent crosslink on the hydrothermal and thermo mechanical stability of collagen.

2. Material and methods

2.1. Materials

Oxazolidine (4,4-dimethyl-1,3- oxazolidine) and Zirconium oxalate (C4O6Zr, molecular weight 267.2592) was supplied by Sigma Chemicals Co., USA , and where of analytical grade.

2.2. Preparation of collagen

Teased fiber collagen was prepared by washing with 1% sodium chloride and deionized water at 10 °C to gid rid of any trace materials attached. Two set of collage was tanned with 5&10% oxazolidine at pH 6 and 8 for 24 h. And another set was tanned with zirconium oxalate at pH 3 for 24 h.

2.3. Physical Analysis of collagen

2.3.1. Measurement of thermal shrinkage temperature

The thermal properties of native, oxazolidine, and zirconium oxalate tanned collagen fibers were investigated by using a differential scanning calorimeter (DSC 7, PerkinElmer). The samples were first air dried at room temperature and the weights recorded. 2–4 mg of collagen was treated in oxazolidine concentrated (3, 6, 9 and 15%) solution at pH 5 and 8 and zirconium oxalate (3, 6, 9 and 15%) solution at pH 3 for 24 h, then oxazolidine and zirconium tanned collagen fibers were saturated in water and 8 M urea solution for a period of 24 h [11]. Then the enthalpy changes ΔH (in Jg^{-1}) and peak temperature (Tp) and the onset temperature (Ts) (in °C), linked with the change of phase for the shrinkage process for native, oxazolidine, and zirconium oxalate tanned collagen fibers before and after urea treatment was determined [11].

2.3.2. Measurement of Tensile strength and percent of elongation

Measurements of tensile strength and percent of elongation were done using an Instron testing machine (model 1112 and 0–500 g load cell) designed for a liquid cell attachment. The sensitivity of the load cell was 1% at the maximum range. The specimen length was 1 cm and the elongation rate used was 0.5 cm min^{-1}. The tensile strength and percent elongation of native, oxazolidine and zirconium oxalate treated collagen in water medium at room temperature were calculated.

$$\text{Tensile strength} = \frac{\text{Maximum breaking load}}{\text{Cross sectional area}}$$

The Percent of Elongation at Break was measured according to the society of leather technologist and chemists [12].

- Calculation

$$\text{Elongation, \%} = \frac{\text{Final free length} - \text{Initial free length}}{\text{Initial free length}}$$

3. Results and discussion

3.1. Hydrothermal stability analysis

Differential scanning calorimeter is used for measurement of shrinkage temperatures and temperature at maximum tension of the collagen fibers tanned with oxazolidine and zirconium oxalate. Shrinkage temperature of the collagen fibers is a measure of the stability of the matrix as a whole, which arises due to the long-range ordering of the matrix. The shrinkage temperature for native collagen fiber was found to be 68 °C (Table 1). The collagen fibers treated with different oxazolidine (3, 6, 9, and 15%) concentration at pH 6 and 8 exhibited stability against wet heat even at low concentration (3%), whereas the collagen fibers treated with 3, 6, 9, and 15% zirconium oxalate produced shrinkage temperature of 74, 76, 76, and 77 °C, respectively (Table 1). It can be seen from Table 1 that oxazolidine exhibit an increase in the shrinkage temperature when compared to both native collagen and zirconium oxalate. This enhanced hydrothermal stability of oxazolidine tanned collagen is due to new crosslinks formed and consequent changes in the tertiary structure of collagen. In the case of oxazolidine tanning there is a general belief that the dimensional changes occurring beyond shrinkage temperature may be partially reversible [13].

In case of zirconium a rise in the shrinkage temperature were observed when compared to native collagen. This may be due to a net increase in the number of intermolecular crosslinks arising from electrostatic, co-ordinate, and covalent interactions between the Zr (IV) complexes and collagen.

Table 1 Shrinkage temperature and temperature at maximum tension on native, oxazolidine, and zirconium oxalate crosslinked collagen fibers

Parameters	**Shrinkage temperature (T_s), °C**	**Temperature at max. tension (T_t), °C**
Native collagen in water	68	70
Oxazolidine crosslinked collage at pH 6		
3% solution	85	>93
6% solution	82	>93
9% solution	81	>93
15% solution	87	>93
Oxazolidine crosslinked collage at pH 8		
3% solution	85	>95
6% solution	87	>95
9% solution	88	>95
15% solution	89	>95
Zirconium oxalate crosslinked collage at pH 3		
3% solution	74	>97
6% solution	76	>97
9% solution	76	>97
15% solution	77	>97

3.2. Role of secondary structure in the hydrothermal stability

Oxazolidine tanned collagen fibers are tried in urea solution. It is known that urea is capable of breaking down hydrogen bonds in collagen molecules [14]. Table 2 displayed shrinkage temperature of oxazolidine tanned collagen fibers in varying amounts of urea solutions. The reduction in shrinkage temperature of oxazolidine tanned collagen in urea solution is well known according to the previous studies [14]. In case of zirconium oxalate there is decline in shrinkage temperature of tanned collagen fibers when treated in diverse amounts of urea solutions as compared with that of zirconium oxalate tanned collagen in water. This reduction in the hydrothermal stability in urea solutions reveals modifications in the secondary structure of collagen are feasible even after tanning. The results clearly illustrate that zirconium impart thermal stability to collagen.

Table 2 Shrinkage temperature of oxazolidine and zirconium oxalate (5and 10%) solution crosslinked collagen fibers in water and different urea solutions

Parameters	**Shrinkage temperature (Ts ± 1) for crosslinked collagen fibers (^oC) at:**			
	pH 6		**pH 8**	
	5%	**10%**	**5%**	**10%**
Oxazolidine crosslinked collagen in				
Water	84	86	86	87
2 M urea	84	83	85	82
4M urea	81	83	84	86
6M urea	81	82	84	85
8M urea	81	82	84	85
	Shrinkage temperature (Ts ± 1) for crosslinked collagen fibers (^oC) at pH 3			
Zirconium oxalate crosslinked collage in				
	5%		10%	
Water	73		76	
2 M urea	71		73	
4M urea	68		71	
6M urea	67		68	
8M urea	66		64	

3.3. Tensile strength and elongation%

Table 3 Tensile strength and elongation, % of native, oxazolidine, and zirconium oxalate crosslinked collagen fibers at different pH levels and solution concentrations in water at 25 °C

Parameters	**Tensile strength (Mpa)**		**Elongation (%)**	
Native collagen	60±3		50±3	
Parameters	Tensile strength (Mpa)		Elongation (%)	
	pH 6	pH 8	pH 6	pH 8
Oxazolidine crosslinked collage with				
3% solution	150±8	155±9	65±5	68±4
6% solution	165±7	168±8	50±3	53±6
9% solution	175±8	170±7	35±3	30±6
15% solution	130±6	125±8	25±3	20±6
Parameters	Tensile strength (Mpa)		Elongation (%)	
pH 3				
Zirconium crosslinked collage with				
3% solution	60±6		68±7	
6% solution	61±4		53±5	
9% solution	64±6		38±5	
15% solution	44±4		22±5	

Table 3 shows the tensile strength and elongation %, of native, oxazolidine, and zirconium oxalate tanned collagen fibers (with 3, 6, 9, and 15% solutions at pH 6 and 8) in water at 25 °C. Oxazolidine increases tensile strength significantly till a critical concentration of oxazolidine is touched. Oxazolidine is capable of diffusing into the molecular pore dimensions. Rise in tensile strength can be understood in terms of the totality of covalent crosslinks formed during the tanning processes. On the other hand, a decline in tensile strength at higher concentration may be owing to the rise stiffness (shown by the decreasing elongation) results in a brittle fiber consequently it breaks more easily at reduced load. Oxazolidine tanned collagen measurement bound volume afford description of enlarged tensile strength of oxazolidine tanned collagen fiber. On the other hand, zirconium oxalate and native collagen fiber under the same conditions of temperature showed strength properties of 64±6, 44±4, and 60±3 MPa, respectively (Table 3). This value of native collagen fiber is nearly the same as those observed for zirconium tanned collagen fiber within experimental errors. In generals, zirconium tanning does not seem to change the tensile strength of collagen fibers significantly in wet condition as well as oxazolidine tanning, this due to the fact that deposition of material with a hardness higher than that of collagen fibers may well reduce thermos-mechanical properties [15].

4. Conclusion

The influence of oxazolidine and zirconium oxalate on the thermal and mechanical stability of collagen was investigated. Differential scanning calorimeter has been used for evaluating the thermal stability of collagen. Oxazolidine and zirconium oxalate has been found to increase the shrinkage temperature of the collagen fibers to about 18–24 °C more than that of the native collagen. On the basis of rise in thermal stabilization of tanning, a higher level of long-range order is involved in the case of oxazolidine tanned fibers in relation to zirconium oxalate treated analogue. This could be due to the differences in the type of interaction with collagen, which is also reflected in the differences in mechanical changes of collagen brought about by the two crosslinkers. In generals, zirconium does not seem to change the tensile strength of collagen fibers significantly as well as oxazolidine. Finally, it should be highlighted that there is a growing interest on the metal-free leather market, collaborating in this project in assessing the performance of oxazolidine-tanned leather.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no conflict of interests exists.

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