Studies on Phosphonium Based Combination Tanning: Less Chrome Approach

by

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Abstract

A new combination tanning system based on tetrakis (hydroxymethyl) phosphonium sulfate (THPS) and chromium as a chrome saver approach has been developed. The shrinkage temperature of the leathers obtained was 110°C. The tanning system is versatile in terms of processing both upper and garment leathers. The physical strength characteristics and organoleptic properties of the leathers obtained are comparable to that of chrome tanned leather. Environmental impact assessment shows that there is significant reduction in effluent (TS and sulfates) load when compared to conventional chrome tanning. The molecular level studies show that high level of enzymatic stability is imparted to collagen stabilized using Cr-THPS combination tanning. Mechanistic insights into the stability of collagen against collagenase have been addressed by studying the conformational changes occurring in collagen and collagenase as a result of interaction with Cr-THPS using circular dichroism (CD). The triple helical conformation of collagen is not altered due to interaction with Cr-THPS. Changes in the conformation of collagenase have been observed, which indicates that Cr-THPS stabilizes collagen by altering the conformation of the enzyme.

Resumen

Un nuevo sistema de curtido combinado sobre la base de Sulfato Tetrakis Hidroximetil Fosfonio (THPS) y Cromo, como un acercamiento al ahorro de cromo, se ha desarrollado. La temperatura de contracción de los cueros obtenidos fue de 110°C. El sistema de curtido es versátil en cuanto a procesos de cueros para calzado y vestimenta. Las características físicas de resistencia y las propiedades organolépticas de los cueros obtenidos son comparables a las del cuero curtido al cromo. Evaluación del impacto ambiental indica que hay una reducción significativa en la carga del efluente (TS y sulfatos) en comparación con curtido al cromo convencional. Los estudios de nivel molecular muestran que altos niveles de estabilidad enzimática es impartido al colágeno estabilizado empleando la combinación curtiente Cr-THPS. Puntos de vista mecanicistas en la estabilidad del colágeno frente a la colagenasa se han abordado mediante el estudio de los cambios conformacionales que ocurren en el colágeno y la colagenasa como resultado de la interacción con Cr-THPS usando dicroísmo circular (CD). La conformación de la triple hélice del colágeno no se altera debido a la interacción con Cr-THPS. Los cambios en la conformación de la colagenasa se han observado, lo que indica que el Cr-THPS estabiliza el colágeno mediante la alteración en la conformación de la enzima.

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INTRODUCTION

Leather making has evolved through ages. Earlier vegetable tanning was the predominant form of tanning that was practiced. Later, the advent of chrome tanning revolutionized the process of leather making. Nevertheless, it is till today the most widely used tanning system. The reason for such dominance is the unmatched properties, which chromium imparts to the leather. The stringent norms on disposal of chromium and the possibility of formation of Cr(VI) made researchers to explore the possibility of other materials capable of tanning.[1] One of the ways to alleviate chrome pollution is to follow less-chrome tanning technology, wherein partial substitution of chromium is done with another tanning agent. This tanning agent is expected to provide properties to leather, which are on par with that of chromium if not better. Selection of such a material is bound to be a challenging task in the wake of the excellent performance of chromium as a tanning agent. The partially substituted tanning agent is expected to add value to the final leather by working in tandem with chromium. Several tanning agents were screened for their tanning potentials both individually and in combination with chromium.[2-6] In recent years, Tetrakis (hydroxymethyl) phosphonium sulfate (THPS) as an alternative to chrome tanning has been explored.[7-15] This substance in combination with various metals has been shown to produce leathers of quality on par with that of chromium. It has been shown that THPS negates the disadvantages associated with iron tanning successfully by complexing with iron.[8,9] The advantages associated with silica tanning had been judiciously utilized in the combination tanning of Si-THPS. The combination of Si-Al-THPS resulted in making good quality wet white leathers.[10] A metal free organic tanning with tannic acid and THPS has also been established.[11] Wet pink leather as a substitute for wet blue leather has been made using the combination of zirconium with THPS.[12] Recently, it has been shown that a combination of aldehydes with THPS results in leather with good light fastness properties.[13]

In this study, a combination tanning of THPS with chromium to make leathers has been investigated. The objective behind the study is to develop a less-chrome tanning methodology. The physico–chemical properties of the leathers made, the impact of the system on the environment and the molecular level interaction of this combination tanning on collagen have been detailed in this report.

EXPERIMENTAL METHODS

Materials

Wet salted goatskins were used for tanning trials. All chemicals used for leather processing were of commercial grade. Tetrakis (hydroxymethyl) phosphonium sulfate (THPS) liquid formulation (Albrite^R AD75E, 75% w/w concentrated THPS) was procured from Rhodia Consumer Specialties Ltd., UK. The chemicals used for analysis of spent liquors were of analytical grade.

Determination of Shrinkage Temperature

The shrinkage temperature, which is a measure of hydrothermal stability of leather, was determined using a Theis shrinkage meter.[16] Each value reported is an average of three experiments with respective standard deviation.

Chromium-THPS Combination Tanning

Wet salted goatskins were processed to pickled stage by conventional method. The pickled skins at a pH of 2.8 were taken for both control and experimental tanning system. Eight pickled skins were taken. Two skins were used for each trial. The optimization of THPS was carried out as reported earlier.[8] Pickled skins were treated with 50% pickle liquor, optimized amount (1.5%) of THPS and drummed for 45 min.[7] Different percentages of basic chromium sulfate (BCS) (with 24% Cr_2O_3 content) viz., 0.5, 1, 1.5 and 2% was then offered and the drum was run for another 60 min. The drum was flooded with 50% water and run for 10 min. Basification was then carried out by the addition of 0.5% sodium formate, drummed for 15 min and 1.0-1.2% sodium bicarbonate (1:10 dilution and given in 3 feeds with 10 min interval). Finally, the drum was run for 2 hrs and the pH was checked to be 3.8-4.0. Then the leathers were piled over night. Next day, hydrothermal stability of wet tanned leathers was measured using a shrinkage tester.[16] The optimized experimental tanning and control chrome tanning process was followed using five goatskins for each to make upper leathers. Control chrome tanned (5 goatskins) leathers were obtained following the conventional procedures.[11] Then the leathers were piled overnight. Next day, the hydrothermal stability of the wet tanned leather was measured using a shrinkage tester.

Post Tanning Process

Tanned leathers were shaved to a uniform thickness of 1.1-1.2 mm and 0.7-0.8 mm and post tanned to obtain upper and garment crust leathers, respectively, using the recipes as described earlier[11] for both control and experimental leathers. After post tanning operations, the leathers were piled overnight. Next day, the leathers were set, hooked to dry, staked and buffed.

Physical Testing of Leathers

Samples for physical testing were obtained as per IULTCS method.[17] The samples were conditioned at 80 ± 4°F and 65 ± 2% R.H. for 48 hrs. Physical properties such as tensile strength, % elongation at break and tear strength were examined as per the standard procedures for upper leathers (both experimental and control).[18,19] Each value reported is an average of four (2 along the backbone, 2 across the backbone) experiments with standard deviation.

Objective Assessment of Softness through Compressibility Measurements

Softness of leathers can be numerically measured based on their compressibility.[11] Circular leather pieces (2 cm x 2 cm area) from experimental and control crust leathers were obtained as per IULTCS method[17] and conditioned at 80±4°F and 65±2% relative humidity over a period of 48 hrs. The samples were spread uniformly over the solid base of the C & R (compressibility and resilience) tester. The initial load acting on the grain surface was 100 g. The thickness at this load was measured 60 seconds after the load was applied. Subsequent loads were added (100, 200, 200, 300, 300, 100, 100, 400, 400, 200, 200 g) and the change in thickness was recorded one minute after the addition of each load. Logarithm of change in leather thickness (Y axis) was plotted against logarithm of load (X axis) and tan^{-1} of the slope is calculated as the negative slope angle.

Color Measurement

Reflectance measurements were made for Cr-THPS tanned leathers using Gretagmacbeth Spectrolino hand held spectrophotometer. The L, a, b and c values were calculated. 'L' indicates the lightness, 'a' represents red and green axis, 'b' represents yellow and blue axis and 'c' represents chromacity. The values reported are average of three values.

Scanning Electron Microscopic Analysis

Samples from experimental and control crust leathers were cut from the official sampling position.[17] A Quanta 200 series scanning electron microscope was used for the analysis. The micrographs for the grain surface and cross section were obtained by operating the SEM at low vacuum with an accelerating voltage of 30 KV in different lower and higher magnification levels.

Isolation and Characterization of Type I Collagen

Acid soluble rat tail tendon (RTT) collagen type I was isolated according to the method described by Chandrakasan et al.[20] The procedure included acetic acid extraction and salting out with NaCl. The purity of collagen preparation was confirmed by SDS- polyacrlyamide gel electrophoresis. The collagen concentration in the solutions was determined from the hydroxyproline content according to the method of Woessner.[21]

Circular Dichroic (CD) Measurements

Circular dichroic spectra were measured using Jasco 715 Circular Dichroism spectropolarimeter using a quartz cell with a light path of 1 mm at 0.2 nm intervals, at 25°C, with 3 scans averaged for each sample. CD spectra were recorded in the far UV region (190 - 250 nm), under nitrogen, to estimate the conformational changes of Cr-THPS treated collagen sample. Concentration of collagen was kept constant at 0.6×10^{-6} M throughout and the concentration of THPS was varied from 0.6 to 30×10^{-6} M.

RESULTS AND DISCUSSION

Chrome-THPS Combination Tanning: Optimization of BCS with 1.5% THPS

The amount of THPS was kept constant at 1.5% and the amount of BCS was varied from 0.5 to 2%. The shrinkage temperatures of the leathers made are presented in Table I. The shrinkage temperature of the leathers made using 1.5% THPS along with 0.5 and 2%, is 95 and 110°C, respectively. It is seen that an increase in the amount of chromium increases the shrinkage temperature.

TABLE I
Optimization of BCS

BCS (%)	T_S (°C)
0.5	95±2
1.0	98±1
1.5	102±2
2.0	110±3

Physical Strength Characteristics of Cr-THPS Tanned Leathers

Tensile, tear and grain crack strength tests were carried out along and across the backbone line for chrome tanned control and Cr-THPS tanned crust leathers (both upper and garment). The mean values corresponding to each experiment were averaged and values for upper and garment leathers are given in Table II. It is seen from table that both control and experimental upper leathers exhibit tensile, tear and grain crack values acceptable according to the UNIDO norms.[22] It is also seen from the table that the tensile strength of garment leather made from Cr-THPS combination is higher than that of the control leather. The other strength properties are acceptable according to UNIDO norms.

Color Measurements

The reflectance measurements were carried out for leather tanned using Cr-THPS combination system. The absorption maximum is around 592 nm. Absorption maximum is the wavelength at which the reflectance is minimum. The 'L', 'a', 'b' and 'c' values, the parameters used to assess color are given in Table III. As stated earlier in the experimental section, 'L' represents whiteness, which on a scale of 0-100, 100 means pure white, in this case the value of 'L' has been found to be 81.55. This indicates that the color obtained is lighter in shade. 'a' represents red and green axis, where 'a'>0 means red and 'a'<0 means green. The 'a' value for this combination-tanned leather is more than 0. 'b' represents yellow and blue axis, where 'b'>0 means yellow and 'b'<0 means blue. The 'b' value for the leather is greater than 0 indicating that the color

TABLE II
Physical Strength Characteristics of Upper and Garment Leathers (Control: Chrome Tanning and Experiment: Cr-THPS Tanning)

Tanning System	Tensile Strength (Kg/cm2)		% Extension at Break		Tear Strength (kg/cm)		Grain crack for upper leather	
	Upper	Garment	Upper	Garment	Upper	Garment	Load (Kg)	Distance moved (mm)
Experiment	225±8	196±9	48±5	52±4	48±3	50±3	26.5±1	7.0±0.4
Control	230±10	146±13	53±6	61±6	52±2	53±4	30.0±2	8.0±0.5
UNIDO norms22	200	120			40	20		

TABLE III
'L', 'a', ' b', 'c', values for Cr-THPS Tanned Leather

Parameters	Values
L	81.55
a	1.85
b	3.05
c	3.57
H	58.83

obtained has yellow shade. 'c' represents the chromacity of the color, which means the intensity of the color. The value indicates that the color obtained has less intensity or depth of shade. Thus the color measurement indicates that the leathers made using this new combination system are suitable for making pastel shades.

Softness Measurements

The versatility of this combination tanning system to make garment leathers as well was tested by quantitative assessment of softness through compressibility measurements. The softness measurement was carried out for both upper as well as garment leathers made using the preferred combination and the same is shown in Fig. 1. The logarithm of change in thickness was plotted against logarithm of load for the leathers, which exhibits a linear fit.[23] The corresponding equation of the line was obtained. The negative slope angle was calculated and the value is -9.14 for garment leather. Higher negative angles imply more softness in the leather. A slope angle value of above -6 is considered as sufficient for garment leathers. It is evident that the garment leather exhibits higher negative slope angle (compressibility index, CI). This shows the extent of softness achieved by the combination system. The negative slope angle for Cr-THPS upper leather is -5.5 and it is the same as that of control chrome tanned leathers.

Scanning Electron Microscopic Studies and Hand Evaluation of Chrome-THPS Tanned Leathers

Scanning electron microscopic analysis of the control and experimental tanned crust leathers was carried out to study the influence of the combination tannage on the fiber structure. The micrographs showing the grain of control and experimental tanned leathers are given in Fig. 2. The grain surface of the experimental tanned leather at a magnification of x500, as seen in Fig. 2b shows no surface deposition of tanning agents. Scanning electron micrographs of crust leather samples from control and experimental processes showing the cross section at a magnification of x250 are given in Figures 2c and 2d, respectively. It is seen from the figures that the fiber structures are comparable. The control and experimental upper and garment leathers were assessed for organoleptic properties by three experienced tanners and it was found that all the properties of experimental leather are comparable with that of conventionally processed chrome tanned leather for both upper and garment (Figure not shown).

Environmental Impact of Chrome-THPS Combination Tanning System

The chlorides, TS, COD and sulfates values for both upper and garment leathers tanned using Cr-THPS tanning system are shown in Table IV. It is seen from the table that there is significant reduction in TS and sulfates when compares to control chrome tanning system. COD values are comparable to that of conventional Cr tanning.

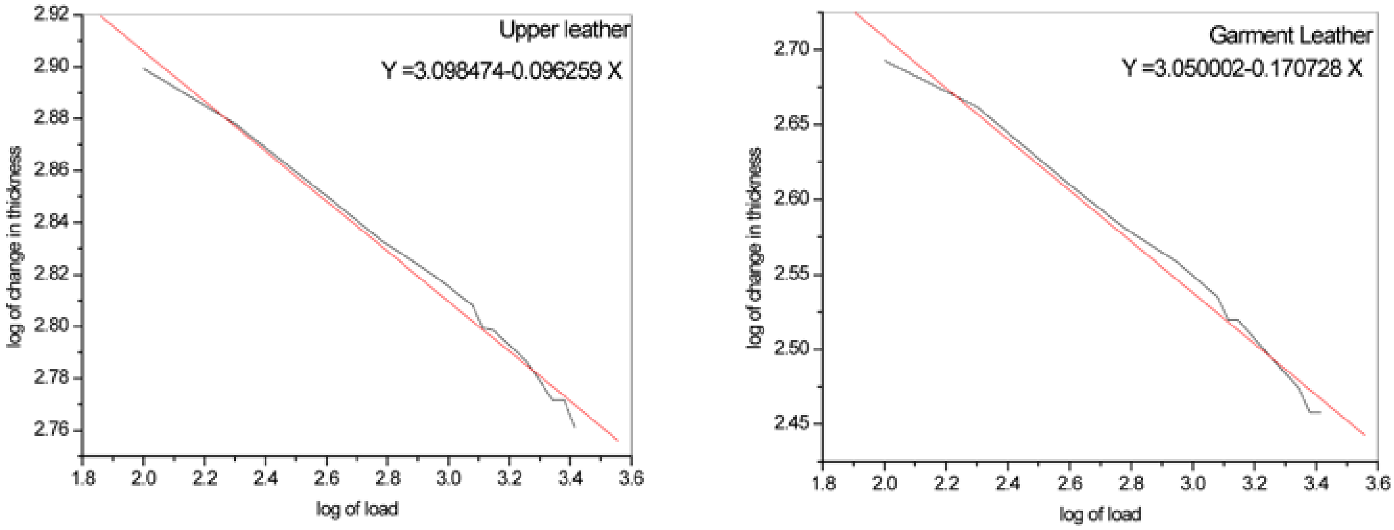

Figure 1. Plot of log of load *Vs* log of change in thickness for Cr-THPS tanned a) upper leather, b) garment leather (The negative slope angle □ = Tan^{-1} (m) (here m=0.0962 for upper; m=0.1707 for garment) obtained from compressibility measurements).

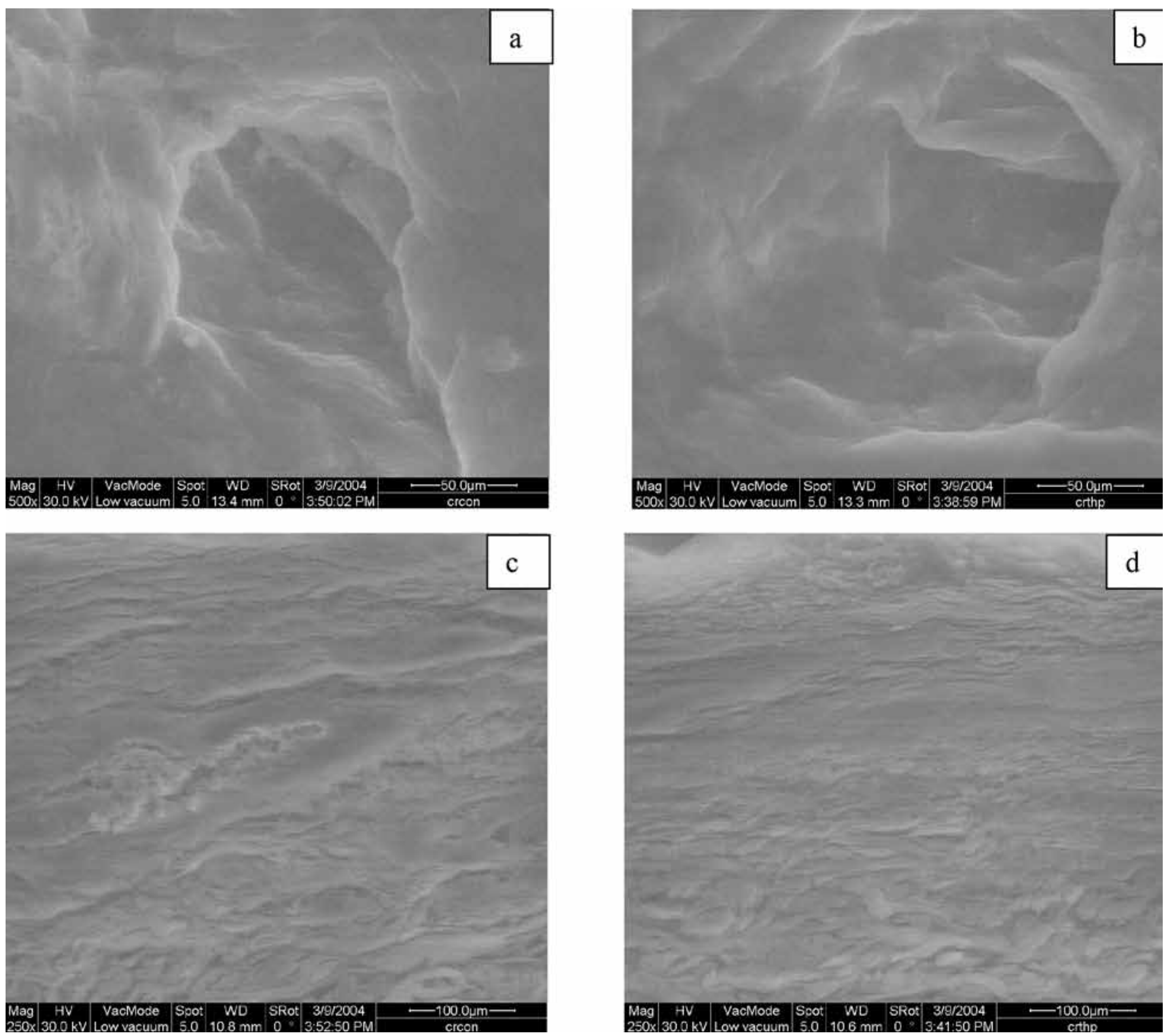

Figure 2. Scanning electron micrograph showing the grain surface (X500) of (a) chrome tanned garment (control) and (b) Cr-THPS tanned garment leather and showing the cross section (X250) of (c) chrome tanned garment (control) and (d) Cr-THPS tanned garment leather.

TABLE IV
Spent Tan Liquor Analysis of Upper and Garment Leathers Made from Chrome-THPS Tanning System

Tanning	Chlorides (ppm)		COD (ppm)		TS (ppm)		Sulfates (ppm)	
	Upper	Garment	Upper	Garment	Upper	Garment	Upper	Garment
Chrome-THPS	17514±23	17101±34	2081±47	2670±39	26270±49	25940±20	4897±37	4600±45
Control (Chrome)	17375±42	18308±89	2168±14	2700±10	48090±13	48260±12	14400±23	13600±26

TABLE V
The % Degradation of Native and Cr-THPS Tanned Collagen Fibres on Treatment with Collagenase at Various Intervals of Time

Time of incubation (hrs)	Native (%)	Cr-THPS treated (%)
12	10	No degradation
24	48	No degradation
48	77	No degradation
72	90	No degradation
96	96	No degradation

Studies on Molecular Level Understanding of Cr-THPS-Collagen Interaction

(1) Enzymatic stability of Cr-THPS Tanned Collagen

Cr-THPS interaction with collagen was studied at molecular level in order to gain insight into the mechanism of stabilization of collagen. The stability of the tanned collagen fibers against enzyme degradation was studied by estimating the hydroxyproline content released after treatment of Cr-THPS tanned collagen with collagenase. Mammalian collagenase is highly specific in its activity against collagen where it specifically cleaves collagen at 772-773 amino acid residue of Gly-Ile or Gly-Leu bond in the polypeptide chain of collagen.[24,25] In the present investigation, bacterial collagenase has been used for understanding the enzymatic stability of collagen and it cleaves the peptide bond between Y and Gly (Y is most frequently a neutral amino acid) at several regions in the collagen triple helix. The native RTT and RTT treated with Cr-THPS tanning system was incubated with collagenase. The % degradation of the tendons has been calculated based on the release of hydroxy proline into the solution and the same for native and treated RTT are given in Table V. From the table, it is seen that native completely degrades within 96 hrs of the treatment with collagenase whereas RTT treated

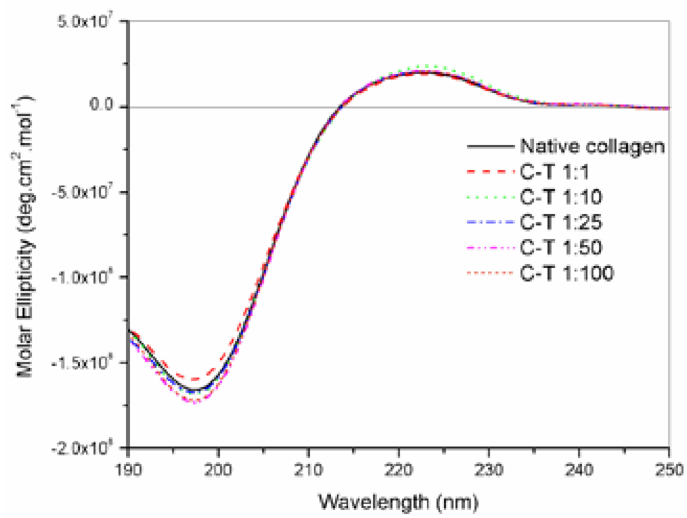

Figure 3a. Far-UV CD spectra of collagen in the presence of Cr-THPS (C-T). 1.Collagen (0.6μM), 2.C-T(0.6μM), 3.C-T(6μM), 4.C-T(15μM), 5.C-T(30μM), 6.C-T(60μM).

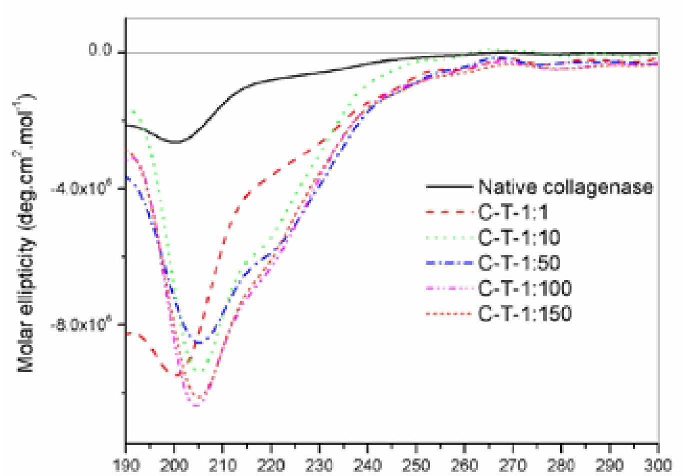

Figure 3b. Far-UV CD Spectra of collagenase in the presence of Cr-THPS (C-T). 1.Collagenase (1.0μM), 2.C-T (1.0μM), 3. C-T (10μM), 4.C-T (50μM), 5.C-T (100μM), 6.C-T (150μM)

with Cr-THPS is found to exhibit high stability against the enzymatic hydrolysis and no degradation even after 96 is observed. Hence, this table clearly displays the stabilization effect of Cr-THPS on the collagen fibers.

(2) Conformational stability of Cr-THPS Tanned Collagen

CD spectral studies were carried out to find out the effect of Cr-THPS tanning on conformation of collagen. The CD spectra of collagen in the presence of increasing concentrations of tanning agent are shown in Figure 3a. In the far UV region, collagen exhibits a minimum at 197 nm and a maximum at 220 nm.[26] The maximum at 220 nm in CD spectrum of native collagen solution is characteristic of triple folded helix. It could be seen from the figure that there are no major alterations in the conformation of collagen. Increase in Cr-THPS concentration has resulted in only minor changes in the amplitude of the spectra, a slight unwinding of triple helices and no further modification are observed. This decrease in dichroic intensity at 197 and 220 nm in a concentration dependent manner could be due to the crosslinking of native collagen molecules with the Cr-THPS complex.

In order to investigate whether inhibition of collagenase by Cr-THPS is due to alterations in the secondary structure of collagenase, CD spectral studies on the Cr-THPS–collagenase systems were carried out. In the far UV region, collagenase exhibits a minimum at about 210 nm and a maximum at 195 nm with a crossover point at about 200 nm. The CD spectra of collagenase in the presence of increasing concentrations of Cr-THPS are shown in Figure 3b. It could be observed from the figure that conformational changes in collagenase are being brought about by Cr-THPS treatment.

The nature of inhibition of Cr-THPS tanned collagen fibers against collagenase could be due to:

1. Blocking of the specific sites in the collagen
2. Conformational changes in collagen which would have rendered collagen as an unknown substrate for collagenase to recognize
3. Interaction of Cr-THPS with collagenase so that the activity of collagenase is lost.

As is evidenced from the CD studies, the stability of collagen brought about by Cr-THPS tanning could not be due to change in conformation of collagen. Hence, it could be due to the blocking of the specific sites in collagen and change in conformation of collagenase if free Cr-THPS is available.

Conclusion

The present study explores the possibility of developing a less chrome tanning system, based on chrome and THPS. The amount of BCS and THPS has been chosen as 2 and 1.5%, respectively, for this combination tanning system, to get better leather characteristics. The shrinkage temperature of the leathers tanned using this new combination tanning system is about 110±0.2°C. The physical strength characteristics of the leathers meet the standard norms. There is considerable reduction in sulfates and TS loads using Cr-THPS tanning system.

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