Ferrous-gluconic Acid Compound Tanning: A Cleaner Chrome-free Tanning System

by

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Abstract

In order to evaluate the reaction mechanism between Fe^{2+}-gluconic acid compound and collagen, the combination tanning of type-I collagen with Fe^{2+}-gluconic acid compound was studied by UV-Vis spectroscopy, infrared spectroscopy, circular dichroism, and differential scanning calorimetry. The molar ratio for the reaction between Fe^{2+} and gluconic acid was 1:3. The resulting increased thermal stability could be attributed to crosslinks formed between the compound and collagen. The tanning trials indicated favorable results. The shrinkage temperature observed was 96°C, with tensile strength of about 239 kg/cm , elongation at break of about 63% and tear strength of about 74 kg/cm. The leather tanned with the Fe^{2+}-gluconic acid compound was lighter in color compared to the single ferrous sulfate (Fe(II)) tanning system, and had neither a darkening of color nor a reduction in strength properties. It was also clear that COD and TS loads were decreased by 26.5% and 29.8%, respectively compared to Fe(II) system, 7.3% and 26.1%, respectively, compared to BCS system. Studies show that the disadvantages connected with Fe(II) tanning system including loss of strength and darkening of color have been solved successfully.

Resumen

En orden para poder esclarecer el mecanismo de la reacción de $Fe^{2}+$ y el compuesto ácido–glucónico y colágeno, el curtido combinado entre colágeno tipo I con el compuesto $Fe^{2}+$ –ácido glucónico se investigó por espectroscopia UV-Vis, espectroscopía infrarroja, dicroísmo circular, calorimetría por barrido diferencial, La proporción por mole entre la reacción entre $Fe^{2}+$ y ácido gluçónico fue de 1:3. La aumentada estabilidad térmica se puede atribuir a enlaces formados entre el compuesto y colágeno. Los resultados de los ensayos del curtido fueron favorables. La temperatura de contracción observada fue 96°C, con una resistencia aproximada de 239 kg/cm, la elongación al quiebre de 63% y resistencia al desgarre de aproximadamente 74 kg/cm. El cuero curtido por el compuesto $Fe^{2}+$-ácido glucónico resultó más claro en tono que el producido con el sistema basado en sulfato ferroso solamente ($Fe^{2}+$) y no demostró bajo condiciones de añejamiento ni gradual oscurecimiento de tono ni merma en valores de resistencias físicas. Fue también claro que las cargas de DQO y ST disminuyeron a 26,5% y 29,8% respectivamente en comparación por el sistema de curtición con solo sulfato ferroso (Fe(II)), 7,3% y 26.1%, respectivamente, comparado con el sistema basado en sulfato básico de cromo. Estudios entonces demuestran que las desventajas del curtido por el sistema Fe(II) que incluyen la pérdida de tenacidad así como oscurecimiento han sido resueltos a satisfacción.

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INTRODUCTION

Collagen is a unique connective tissue protein that is used in many applications.[1] Stabilization of type-I collagen using inorganic compounds, especially chromium sulfate, has been extensively studied. However, use of chromium sulfate is limited because of difficulties surrounding its safe disposal.[2] Iron has potential as a tanning agent and iron benefits include low toxicity, rapid breakdown, no bioaccumulation and reduced risk to both human health and environment.[3] But its commercial exploitation has been limited by loss of strength with tensile strength of 238.3 kg/cm to 188.58 kg/cm^2 and darkening of color with light brown to dark brown with time.[4] To overcome these problems, a ferrous (Fe^{2+})-gluconic acid tanning system has been investigated.

Gluconic acid is an abundant, non-toxic, inexpensive, renewable, and biodegradable natural material that is used as a raw material in various industries.[5] In the present study, stabilization of collagen by a Fe^{2+}-gluconic acid compound was investigated. The effect of this compound on the thermal, enzymatic, and conformational stability of type I collagen was evaluated. Formation of the compound was studied by UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FT-IR) and elemental analysis. Circular dichroism (CD) spectroscopy was used to study the overall helix to coil transition, and differential scanning calorimetry (DSC) was used to understand the thermal denaturation events that occurred when the collagen was heated.[6,7] Viscosity studies were used to determine if the heat transformation of collagen to gelatin was accompanied by a change in physical properties.

In this study, the application of Fe^{2+}-gluconic acid compound tanning system has been explored. The physical-chemical properties, the effect of aging and the environmental impact of Fe^{2+}-gluconic acid compound tanning system have been analyzed. The control tanning systems solved in single ferrous sulfate(Fe(II)) and basic chrome sulfate(BCS) tanning systems.

EXPERIMENTAL

Materials

All chemicals used for the experimental work were of commercial grade. Laboratory grade ferrous sulfate ($FeSO_4$) was obtained from Chengdu Shudu Ltd. Co. Chengdu, China Gluconic acid and acid soluble rat tail tendon (RTT) type I collagen were purchased from Sigma-Aldrich (St. Louis, MO). Pickled pigskins and all chemicals for tanning processes were supplied by Decision Chemical Ltd.

Preparation of the Fe^{2+}-gluconic acid compound

The Fe^{2+}-gluconic acid compound was prepared from $FeSO_4$ (1.0 wt.% in H_2O) and gluconic acid (1.0 wt.% in H_2O) (Figure 1) with shaking for 24 h at pH 3.0 and 60°C. The whole reaction process was conducted under nitrogen. Samples of the reagents and product (i.e. $FeSO_4$, gluconic acid, and the Fe^{2+}-gluconic acid compound) were dried at 100°C to constant weight. The prepared samples were analyzed as described in the following sections.[8,9]

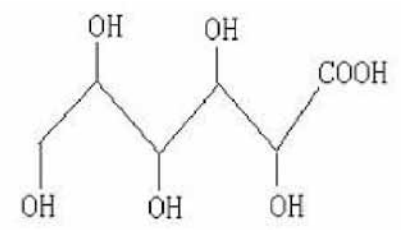


Figure 1. Structure of gluconic acid.

UV-Vis Spectral Studies

To study the compound formation between $FeSO_4$ and gluconic acid, solutions were analyzed in 10 mm quartz cells using a 752 UV spectrophotometer (Unic Shanghai Analysis Co.) between 200 nm and 600 nm. In this experiment, the solvent was distilled water.[10]

FT-IR Spectra

FT-IR(P.E., USA) spectra were obtained in the solid state using KBr pellets and were recorded from 4000 to 400 cm^{-1}.[11]

Elemental Analysis

Carbon (C) and hydrogen (H) contents were determined using a PE-2400 elemental analyzer, and metal ion contents were determined using ICP-AES.[10]

Viscosity Measurements

Viscosities were determined using a Hangping NDJ-8SN viscometer(Shanghai Jingke Analysis Co.) (Φ 0.6mm). The collagen concentration (10.0×10^{-6} mol L^{-1}) was fixed and flow time measurements were carried out in the presence of various concentrations of Fe^{2+}-gluconic acid (10.0×10^{-6} to 100×10^{-6} mol L^{-1}). The viscosity (η) contribution of collagen was measured. The viscosity was calculated according to the formula, $\eta=t/t_0$, where t_0 is the flow time of distilled water and t is the flow time for each sample. A plot of relative viscosity (η/η_0) against I (I=[compound]/[collagen]) was obtained.[9]

CD Spectroscopy

A Jasco 715 (Easton, MD) CD spectrometer with quartz and a light path of 1 mm was used. CD spectra of the collagen samples were recorded under nitrogen at 0.2 nm intervals. The measurements were performed at 25°C, and 10 scans were averaged for each sample in the far UV region (190–250 nm). The concentration of collagen was kept constant at 10.0×10^{-6} mol L^{-1}. Different concentration of Fe^{2+}-gluconic acid (0–90 μmol L^{-1}) and the reaction mixture was investigated for any conformational changes compared with the structure of native collagen.[12,13]

DSC Determination

Thermal denaturation of collagen was studied using a Perkin Elmer DSC calorimeter (Waltham, MA). The samples were sealed in a DSC cell and heated at a constant rate of 5°C/min .[14,15] RTT type I collagen fibers were treated with a 6% solution of the Fe^{2+}- gluconic acid at pH 3.8–4.2 which was adjusted with 10% formic acid (wt.% in H_2O) and 10% sodium bicarbonate solutions(wt.% in H_2O) and stirred overnight.

Native and Fe^{2+}-gluconic acid treated RTT fibers were blotted uniformly to remove excess water, and then hermetically sealed in aluminum pans. The peak temperature (*Tp*) for the collagen-to-gelatin transformation was recorded using standard methods. [16,17]

Tanning Trials

The pickled pigskins (at pH 2.8) were applied for shoe lining leather tanning processes. The control tanned leathers included ferric sulfate(Fe(II)) and basic chromium sulfate(BCS), and the trial tanned leather used Fe^{2+}-gluconic acid compound. The tanned leather was obtained by the following procedures.

Fe2+-gluconic acid compound Tanning Process

Pickled liquor	50%		Check pH 2.8~3.0
Fe^{2+}-gluconic acid compound	4%	60 min	Check penetration
sodium tartrate	2%	45 min	
Water	50%	10 min	
Sodium bicarbonate	1.5%		
Water	15%	3×10 min+2 hrs	Check pH 5.0-5.4

Control Fe(II) Tanning Process

Pickling liquor	50%		Check pH 2.8~3.0
Fe(II) (as FeO)	4%	60 min	Check penetration
Sodium tartrate	2%	45 min	
Water	50%	10 min	
Sodium bicarbonate	1.5%		
Water	15%	3×10 min+2 hrs	Check pH 3.8-4.2

Control BCS Tanning Process

Pickling liquor	50%		Check pH 2.8~3.0
BCS (as Cr_2O_3)	6%	60 min	Check penetration
Water	50%	10 min	
Sodium bicarbonate	1.5%		
Water	15%	3×10 min+2 hrs	Check pH 3.8-4.2

Post Tanning Regime for Both Experimental and Control Shoe Lining Leathers

Neutralization			
Water	100%	60 min	Check penetration
Sodium formate	1%		
Sodium bicarbonate	1%	3×10 min+10 min	Check pH 5.0-5.4
Washing			
Water	200%	10 min	
Retanning			
Water	100%		
Lanxess RE	5%	40 min	
Lanxess OS	5%	60 min	
Fatliquoring			
Decision QL	4%		Emulsified with hot water at 60°C (1:20 dilution)
Decision QW	4%	60 min	
Fixing			1:20 dilution;check exhaustion and drain
Formic acid	1%	3×10 min+10 min	
Washing			
Water	200%	10 min	
Drain, pile over night			

Analysis of Strength Characteristics

Samples of standard dimensions for various physical tests were obtained according to the IULTCS method.[18] Each value reported is an average of three experiments. Physical properties such as shrinkage temperature(Ts), tensile strength, tear strength and % elongation at break were tested as per the standard procedures.[19] In order to determine the impact of aging on physical properties, samples for different tanning systems were aged for three months and six months under the natural light and were then examined for varied physical characteristics by above standard method.

Spent Tan Liquor Analysis

The spent tan liquors for the Fe^{2+}-gluconic acid compound, Fe(II) and BCS were collected and determined for Chemical Oxygen Demand(COD) and total solids(TS) by conventional analytical methods.[20]

RESULTS AND DISCUSSION

UV-Vis Spectral Studies

In a UV-Vis spectrum of a physical mixture of Fe^{2+} and gluconic acid (Figure 2), the λmax of Fe^{2+} remained constant at 248 nm and 275 nm. By contrast, in the spectrum of the Fe^{2+}-gluconic acid compound, the λmax of Fe^{2+} had shifted from 248 nm and 275 nm to 239 nm and 269 nm. This clearly showed that Fe^{2+} and gluconic acid formed a compound.

FT-IR Studies

Figure 3 shows FT-IR spectra of gluconic acid and its compound with Fe^{2+}. The FT-IR spectrum of gluconic acid had an intense band at about 3215.47 cm^{-1} for the ν_{O-H} vibration of the carboxyl group. The band at 1763.25 cm^{-1} was assigned to the $\nu_{C=O}$ vibration, and the 919.38 cm^{-1} band to the δ_{CH} vibration. After Fe^{2+} was reacted with gluconic acid, the characteristic band for the carboxyl group disappeared, and

new bands appeared at 1415.12 cm^{-1} and 1838.21 cm^{-1}. These results indicate the carboxyl groups of gluconic acid coordinated with the metal ion.[21]

Elemental Analysis

The elemental analysis results for the Fe^{2+} -gluconic acid compound are given in TABLE I. The molar ratio of Fe^{2+} to gluconic acid was 1:3. Chelation via the carboxyl group was proposed, and the structure of Fe^{2+} -gluconic acid is shown in Figure 4.

Viscosity Analysis of the Collagen-Fe^{2+}-gluconic Acid System

The Fe^{2+}-gluconic acid–collagen interaction and the influence of Fe^{2+}-gluconic acid on the viscosity of collagen were investigated by relative viscosity measurements. A plot of relative viscosity (η/η_0) against I is given in Figure 5. Compared with the viscosity of native collagen, the relative viscosity decreased (from 0.976 to 0.824) as the Fe^{2+}-gluconic acid concentration increased. This indicates that the reaction of Fe^{2+}-gluconic acid with collagen did not result in protein aggregation.

CD Studies

To investigate if the Fe^{2+}-gluconic acid compound stabilized collagen by changing its conformation, CD spectra of native collagen and collagen treated with Fe^{2+} and Fe^{2+}-gluconic acid were obtained. The CD spectra are shown in Figure 6. The spectrum of collagen showed a minimum at 197 nm and a maximum at 220 nm, which is characteristic of the collagen triple helix.[22,23] As the concentration of Fe^{2+}-gluconic acid increased, the dichroic intensity at 220 nm remained constant while that at 197 nm decreased. This could occur because of cross-linking of native collagen molecules with the Fe^{2+}-gluconic acid compound. It also explained that the change CD spectrum of collagen in the presence of Fe^{2+}- gluconic acid which was not because of loss of the triple helix conformation, but collagen undergoes drastic denaturation when this occurs. The ratio of the positive peak intensity to the negative peak intensity (R_{pn}) is characteristic for the collagen triple helix.[24] The R_{pn} values for a native collagen solution and a solution of collagen treated with Fe^{2+}-gluconic acid are given in TABLE II. The R_{pn} ratio for the native collagen solution (0.1290) was less than that of the treated collagen solutions (0.1293–0.1340) but this difference was not significant. Therefore, no major conformational changes took place in collagen after treatment with Fe^{2+}-gluconic acid.

DSC Studies

DSC was used to analyze thermal denaturation of collagen in various aqueous environments, and to measure the corresponding temperature of the collagen gelatin transition.[25] Figure 7 shows the DSC results of native collagen fibers, and collagen fibers treated with Fe^{2+} or Fe^{2+}-gluconic acid. After

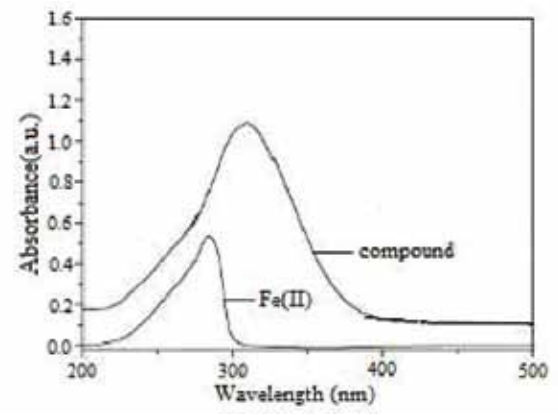


Figure 2. UV-Vis spectra of Fe and Fe^{2+} -gluconic acid.

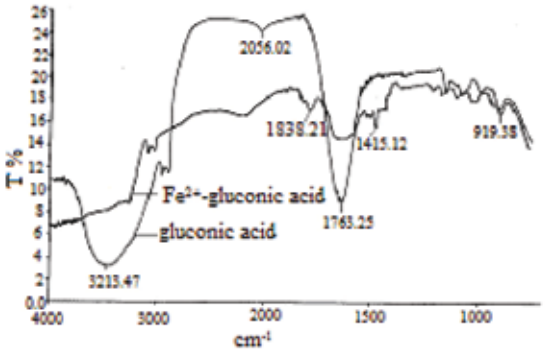


Figure 3. FT-IR spectra of gluconic acid and Fe^{2+}-gluconic acid.

TABLE I
Elemental analysis results for the Fe^{2+}-gluconic acid compound.

Parameters*	Experimental value
C(%)	30.94
H(%)	6.02
Fe(%)	8.02

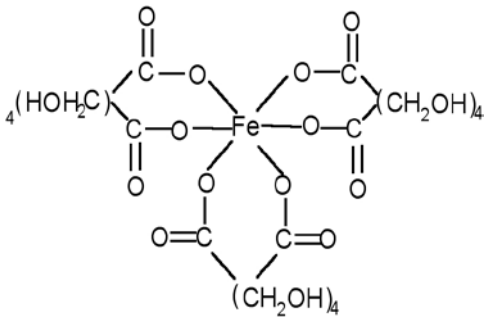


Figure 4. The structure of the compound between Fe2+ and gluconic acid.

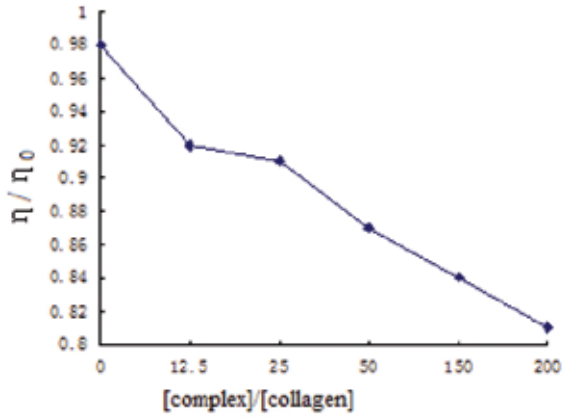


Figure 5. A plot of relative viscosity (η/η0) against I.

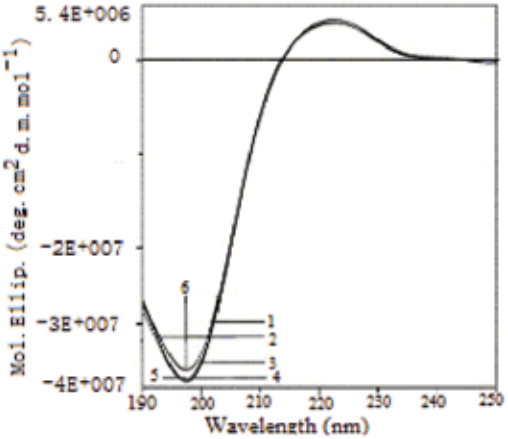


Figure 6. Circular dichroism spectra of a collagen solution treated with different concentrations of Fe^{2+}-gluconic acid.

TABLE II
The R_{pn} ratios of native collagen and collagen treated with Fe^{2+}-gluconic acid.

Concentration of Fe-gluconic acid compound /μM	R_{pn} (characteristic ratio)
0	0.1290
0.9	0.1293
9	0.1298
20	0.1301
50	0.1315
90	0.1340

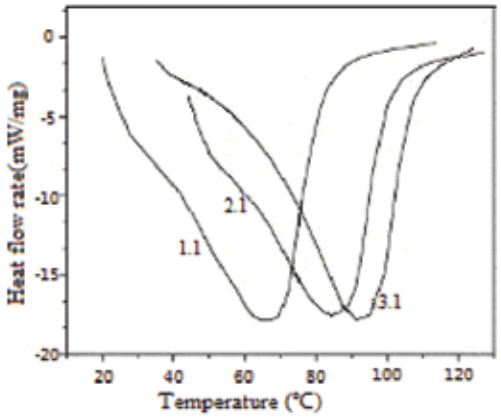
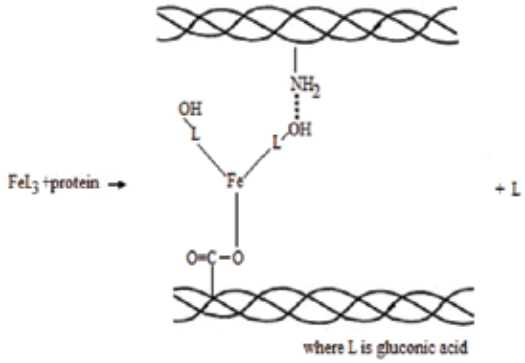


Figure 7. Differential scanning calorimetric thermograms of native collagen fibers, and collagen fibers treated with Fe^{2+} or Fe^{2+}-gluconic acid ([1.1 native collagen; [2.1] collagen treated with Fe; [3.1] collagen treated Fe- gluconic acid compound].



Scheme 1. The reaction process of Fe-gluconic acid compound with protein.

treatment with Fe^{2+}-gluconic acid, the thermal stability of collagen increased from 68°C to 92°C because this compound created crosslinks in the collagen. The thermal stability of collagen treated with Fe^{2+} (88°C) was lower than that of the Fe^{2+}-gluconic acid treated collagen. Iron is known to form a coordination complex with collagen. [26] It can be concluded that the increase in the average number of cross-links per molecule with Fe^{2+}-gluconic acid increases the denaturation temperature of collagen. The results also indicated that the long range ordering of collagen improved because of the interaction between the Fe^{2+}-gluconic acid compound and collagen. According to all above results, the interaction of the Fe^{2+}- gluconic acid compound with collagen molecules can be described in Scheme 1.

Physical Properties Analysis of Leathers

TABLE III and TABLE IV were the Ts and physical testing data for all samples. It indicated that the tanned leather owned a Ts of after fatliquoring with 6% Fe^{2+}-gluconic acid compound and 2% sodium tartrate. The Fe(II) tanned leather gave a Ts of 88°C. They are close to one another but are evident less than

for BCS(113°C). According to physical properties involving strength and organoleptic characteristics, it is clear that Fe^{2+}-gluconic acid compound tanned leather have better values for tear strength and % elongation at break than those of Fe^{2+}-gluconic acid compound, BCS systems. For tensile strength, the value for Fe^{2+}-gluconic acid compound is higher than that of Fe(II), but lower than that of BCS. The ageing results showed that there was no significant reduction in Ts and strength characteristics for Fe^{2+}-gluconic acid compound tanning regime and BCS. It was obvious that after aging all kinds of physical properties has change for Fe(II) tanning system. It owns an easy oxidation tendency of Fe(II) to Fe(III) under natural condition. After aging 3 months, the color of tanned leather with Fe(II) has varied from yellow brown into dark brown and relative strength properties has revealed significant reduction.

Spent Tanning Liquor Analysis

The spent tanning liquors were collected from control and experimental tanning systems in TABLE IV. COD and TS require the calculation of the emission load for environmental impact. The COD and TS values and calculated emission leads are given in TABLE IV. It is obvious that a reduction in COD and TS load has been achieved in Fe^{2+}-gluconic acid compound tanning system than that of single Fe(II) and BCS tanning regimes. It is clear that COD and TS loads have been decreased by 26.52% and 29.82%, respectively compared to Fe(II) system, 7.34% and 26.10% respectively compared to BCS system.

TABLE III
Comparison of physic-chemical characteristics for leather after fatliquoring.

Parameters		Tanning systems		
	Aging time	Fe^{2+}-gluconic acid compound	Fe(II)	BCS
Ts/°C	After fatliquoring	96±0.5	88±1.0	113±0.5
	Aging for 3 months	94±1.0	82±0.5	112±1.0
	Aging for 6 months	93±0.5	78±1.0	110±1.0
Tensile strength (kg/cm^2)	After fatliquoring	239±2.3	217±2.0	289±0.5
	Aging for 3 months	236±3.1	207±1.9	288±2.0
	Aging for 6 months	233±4.1	181±2.1	281±1.9
Tear strength (kg/cm)	After fatliquoring	74±2.9	57±3.7	65±3.4
	Aging for 3 months	72±3.4	50±2.7	63±4.1
	Aging for 6 months	70±3.3	45±2.3	62±3.9
% Elongation at break	After fatliquoring	63±2.9	62±2.5	63±2.0
	Aging for 3 months	58±31	54±3.1	61±1.8
	Aging for 6 months	58±4.5	47±2.0	61±2.7
Organoleptic properties	After fat liquoring	Yellow leather with soft, fine grain	Yellow brown leather with stiff grain	Blue leather with soft, fine grain
	Aging for 3 months	Yellow leather, with soft, fine grain	Dark brown leather with stiff grain	Blue leather with soft, fine garin
	Aging for 6 months	Yellow leather, with soft, fine grain	Dark brown leather with stiff grain	Blue leather with soft, fine grain

TABLE IV
Composite liquor analysis spent liquor.

Parameters	Fe^{2+}-gluconic acid compound	Fe(II)	BCS
COD (ppm)	2574	3503	2778
TS (ppm)	35,235	50,211	47,683
Volume of effluent (L/ton of shaved weight)	1367	1579	1480
Emission Load-COD (kg/ton of shaved weight)	28.1	40.3	32.7
Emission Load-TS (kg/ton of shaved weight)	37.7	50.9	42.1

CONCLUSION

Fe^{2+} and gluconic acid form a compound in a 1:3 molar ratio that improves the thermal stability of type-I collagen. The increased thermal stability can be attributed to cross links formed between this compound and collagen. And Fe^{2+}-gluconic acid compound tanning system can produce leathers with shrinkage temperature of 96°C. The tanned leathers were lighter in color and kept slight change for physical characteristics after aging when compared to the control Fe(II) tanning system. It also indicated COD and TS values have been reduced for Fe^{2+}-gluconic acid compound tanning system when compared to single Fe(II) and BCS tanning systems.

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