Stabilization of collagen using dialdehyde alginic acid - an ecofriendly tanning system

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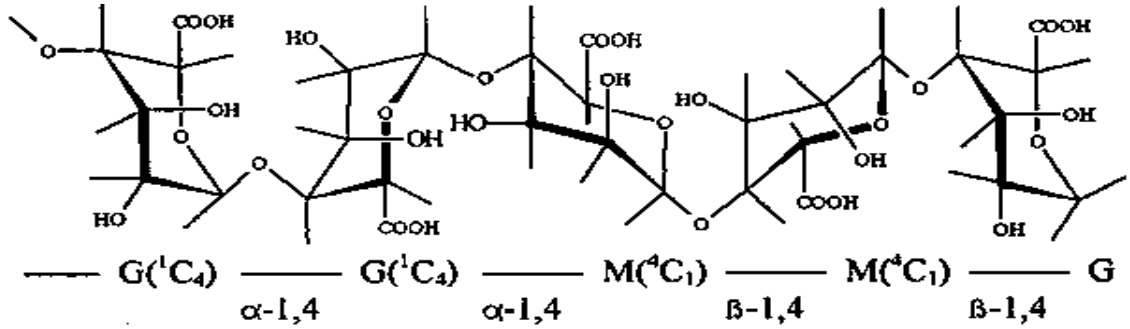
ABSTRACT

Alginic acid is renewable inexpensive non-toxic polysaccharide found abundant in nature, possessing high degree of functionalization. Alginic acid is modified and widely used as raw materials in various industries. Alginic acid on selective oxidation gives dialdehyde alginic acid (DAA), a biopolymeric dialdehyde. DAA could be explored as an alternative tanning system. The characteristic effect of this biopolymeric polyaldehyde as a tanning agent has been investigated. Thermal and enzymatic stability of collagen on treatment with the DAA at varied concentration, pH, time and temperature has been studied. DAA treatment of goatskins resulted in a maximum hydrothermal stability of 82^{o}C at pH 10. The DAA treated goatskins have shown resistance to collagenase. The formation of the crosslinked network between DAA and collagen has brought about significant stability to collagen against thermal and enzymatic degradation by collagenase. Scanning electron microscopic analysis showed a well-coated fibre matrix. The characteristic leather properties of DAA tanned leathers have been discussed and the conditions of tanning have been optimized. The optimized tanned leathers have been compared with chrome tanned leathers for organoleptic properties.

INTRODUCTION

Polysaccharides, inexpensive natural biopolymers are widely used as raw materials in several industries.[1-4] Being a natural compound, most polysaccharides are easily biodegradable.[5] Alginic acid is a naturally occurring hydrophilic colloidal polysaccharide obtained from the various species of brown seaweed (*Phaeophyceae*) but is also present in some species of bacteria.[6,7] Alginic acid is one such material, which has already established its applications in food, pharmaceutical, and medical industries[8-11]. To fulfill the demand for tailored end use applications, the native biopolymers often need to be modified. It is a linear copolymer consisting mainly of residues of 1,4-linked α-L-guluronic acid and β-D-mannuronic acid[12]. These monomers are often arranged in homopolymeric blocks separated by regions approximating an alternating sequence of the two acid monomers. Along the polymer chain the two residues are arranged in an irregular blockwise pattern[13-15]. There are three types of blocks: homopolymeric sequence of mannurronate (MM blocks) and guluronate residues (GG blocks) and a region where the two residues alternate (MG blocks). The relative proportions of these block types are affected by several factors[16-18] such as the botanical source, plant maturity, collection site and seasonal variations. Alginic acid is the only polysaccharide, which naturally contains carboxyl groups in each constituent residue, and possesses various abilities for functional materials. The formula weight of the each structural unit is 176.13 (theoretical) and 200 (actual average). The molecular weight of the macromolecule varies between 10,000 - 600,000 Daltons (typical average) [19]. Alginic acid occurs as white to yellowish brown filamentous, grainy, granular or powdered form insoluble in water and organic solvents. It dissolves slowly in solutions of sodium carbonate, sodium hydroxide and trisodium phosphate. The pH of alginic acid (0.3 in 10 suspension) is 2.0-

3.5. In solution, alginates behave like flexible coils[20]. However on interaction with metal ions, they form ordered structure as evidenced by their X-ray diffraction patterns[21]. Attempts have been made to covalently crosslink sodium alginate with gelatin and ethylenediamine using water-soluble carbodiimide[22]. To fulfill the demand for tailored end use applications the native biopolymers often need to be modified.



Scheme 1: Structure of alginic acid

There are two hydroxyls attached to carbons at 2,3 position and carboxyl attached to C_6 in each dehydrated guluronic unit. There are many reactive groups available in the alginic acid molecule that can react with different chemical reagents As seen in Scheme 1, there is a carboxyl group in C_6 position and two hydroxyls in C_2 and C_3. Periodate oxidation of polysaccharides like alginic acid selectively cleaves the C_2-C_3 bond between the two adjacent hydroxyl groups[23-26] and the 1,2--diol group is converted into a dialdehyde. The advantage of periodic acid lies in the specificity of its oxidation. It forms aldehydes within the polysaccharide molecule. It is established that the oxidation of the alginate polymers results in low molecular weight water-soluble forms.[27] The extent of oxidation can be readily controlled, and a complete range of aldehyde derivatives of alginic acid is made available as the oxidation level varies between 0 and 100%. At 100% oxidation hydroxyl groups of each guluronic residue in the alginic acid is

converted to its corresponding dialdehyde structure; at 50% oxidation half of the guluronic residues of the alginic acid are converted. Dialdehyde alginic acid is thus an open chain biopolymer containing large number of aldehyde groups.

Chrome tanning is a primary tanning system in most commercial tanning practices, as they result in leathers for different end uses with good physical and organoleptic properties. In recent times because of the ecological concerns, the leather industry is looking for an alternative tanning system. It has been shown that there is a possibility for the formation of chromium(VI) during the tanning conditions using chromium.[28] It is well established that hexavalent chromium is carcinogenic and can cause damage to skin, mucous membrane, respiratory tract, kidney etc.[29] Recent reports suggest that at higher levels and under certain ligand environments even chromium(III) is toxic.[30] Use of formaldehyde has been restricted in some countries as it is established to be carcinogenic.[31] Dialdehyde alginic acid has been proved to be biodegradable and toxologically acceptable chemical.[32] In the search of an affective tanning alternative from a sustainable resource, dialdehyde alginic acid, a modified biopolymer of alginic acid appeared to be an attractive option for leather processing as it is abundant and one of the cheapest and eco-acceptable raw material. A cleaner and sustainable tanning agent/system that can result in leathers with good functional properties is the current need of the tanning industry. In this context, the dialdehyde alginic acid has immense potential to be used as a tanning agent in leather processing as it is abundant and one of the cheapest raw material with large number of aldehyde groups in a single molecule. Tanning with aldehydes has been known for a very long time and aldehydes such as formaldehyde, glyoxal and glutaraldehyde and biopolymeric tanning agents like dialdehyde starch have been used for tanning.[33] Aldehydes are excellent cross-linking

agents for the electron microscopy study of proteins, cells, etc.[34] Galactose dialdehyde has been used as protein cross-linker and was very reactive at basic pH leading to complex reaction mixtures.[35] Dextran dialdehyde based crosslinking hydrogel films and in vitro characteristics of bioactive molecules were studied.[36] There are large numbers (more than 1000) of glucose residues present in each alginic acid molecule, which on 100% oxidation will result in large number of dialdehyde groups that can be used to stabilize the collagen matrix. Studies on the use of dialdehyde starch biopolymers as a tanning material have been carried out earlier.[37-42] They have studied the tanning conditions at different oxidation levels of DAA.

DAA has been proved to be biodegradable and toxologically acceptable. The growing demand for the application of biodegradable materials calls for the development of ecologically acceptable leathers. It is well known that 18 billion square feet of leathers is being manufactured[43], used and finally disposed. The ecological imbalance due to this solid waste is being addressed. To attain a safe disposal of this solid waste, it is essential to develop leathers, which on disposal after usage could be easily acceptable to the environment. The sustenance of the industry needs to look at complete life cycle analysis of leather industry. In the present scenario, leather research is being focused in the development of cutting edge technologies to produce leathers that are eco-acceptable. The use of DAA for tanning seems to have an immense potential for the reason that this polyaldehyde polymer is eco-aceptable.[34] Though its application in leather making is not reported, there seems to be an immense potential for the reason that polyaldehyde alginate biopolymer is a biodegradable polymer. This paper describes the results of experimental work carried out to examine the feasibility of using this polyfunctional aldehyde as a tanning agent.

EXPERIMENTAL

Reagents and Chemicals

DAA (33%, 66% and 99% oxidized) was prepared using a modified oxidation process using different amount of sodium metaperiodate to obtain different degrees of oxidation.[44] Type IA collagenases have been obtained from SIGMA ALDRICH, U.S. The chemicals used for collagenase treatment and determination of hydroxyproline were of analytical grade and have been obtained from S.D fine chemicals, India. Basic chromium sulfate (BCS) and other post tanning auxiliaries used for post tanning are of commercial grade. The raw material used for all trials was pickled goatskins (at pH 2.8 – 3.0).

Tanning Trials using DAA - Effect of Various Conditions for Tanning

Various experimental trials (trial 1 to trial 4, mentioned below) were carried out at different conditions. Two skins were taken for each experimental trial.

Effect of pH (Trial 1): Tanning trials at different pH values viz., 4, 5, 6, 7, 8, 9 and 10 were carried out as described in the process mentioned in Table 1. The leathers were aged for 24 hours at room temperature (~30^{o}C) and taken for shrinkage temperature measurement, % aldehyde fixed and enzymatic degradation

Effect of Concentration (Trial 2):. DAA at four concentrations viz., 5, 10, 15, 20% were used for tanning the pelts as described in the process mentioned in Table 1. The leathers were aged for

24 hours at room temperature and taken for shrinkage temperature measurement, % aldehyde fixed and enzymatic degradation

Effect of Time (Trial 3): Tanning trials of pickled goatskins were carried out using as described in the process mentioned in Table 1 and the shrinkage temperature measurement, % aldehyde fixed and collagenase hydrolysis was determined at different time intervals.

Effect of Temperature (Trial 4): Tanning trials at varied temperature conditions viz., 30, 40 and 50^{o}C were carried out as described in the process mentioned in Table 1. The leathers were aged for 24 hours at room temperature and taken for shrinkage temperature measurement, % aldehyde fixed and enzymatic degradation

Comparison of Dialdehyde Alginic Acid Tanned Leathers with Control Leathers

Matched pair comparison of experimental and control processing were carried out using eight pickled goatskins. Right half of eight pickled goatskins were tanned using DAA (10% DAA; pH 8; temperature 30^{o}C; tanning duration 24 hrs) following the process mentioned in Table 1. Four leathers from experimental tanned leathers at pH 8 and 10 (DAA tanned using 10% DAA; pH 8 and 10; temperature 30^{o}C; tanning duration 24 hrs) were oiled with 4% neats foot oil based fatliquor in a fresh float of 100% water and finally fixed with formic acid (10% dilution in three feeds) after tanning in the same drum. Corresponding left halves of the eight pickled goat skins were processed using control chrome tanning process as mentioned in Table 3. All the experimental leathers and control tanned leathers were post tanned using the process mentioned in Table 2. The resultant crust leathers were analyzed for SEM and organoleptic properties.

Estimation of Aldehyde Fixed to Collagen

All the leathers from the experimental process after ageing for 24 hours were taken for the determination of moisture, nitrogen and ash content as per standard procedure.[45,46] The amount of aldehyde fixed to collagen during tanning, calculated on protein weight was determined.[41]

Collagenase Hydrolysis of the Dialdehyde Alginic Acid Tanned Leather

The enzymatic degradation of delimed skin and dialdehyde alginic acid treated leather by collagenase was analyzed by estimating the amount of hydroxyproline released in the solution after hydrolysis. The native and stabilized collagen was treated with bacterial collagenase (Type IA) from *Clostridium histolyticum*. Collagenase treatment was carried out in 0.04 M $CaCl_2$ solution buffered at pH 7.2 using 0.05 M tris HCl. The collagen: enzyme ratio was maintained at 40:1. The samples were incubated at a temperature of 37^{o}C for a period of 72 hrs. The cleavage of native and stabilized collagen was monitored by the release of soluble form of hydroxyproline from insoluble collagen.[47] Aliquots of 750 µl of supernatant were withdrawn after centrifuging at 10,000 rpm for 10 min. The collagenase hydrolysate was hydrolyzed in sealed tubes with 6N HCl for 16 hrs at 110^{o} C. The hydrolysates were evaporated to dryness in a porcelain dish over a water bath to remove excess acid. The residue, free of acid was made up to a known volume and the percentage (%) of hydroxyproline was determined using the method of Woessner.[48] Woessner method of determining hydroxyproline involves the oxidation of hydroxyproline to pyrrole-2-carboxylic acid, which complexes with p-dimethylaminobenzaldehyde exhibiting maximum absorbance at 557 nm.

The enzymatic degradation of autoclaved dialdehyde alginic acid treated leathers by collagenase was analyzed by estimating the amount of hydroxyproline released after hydrolysis (degradation) of the tanned leathers. Hydroxyproline is a unique amino acid for collagen and it offers itself as a useful marker for identifying collagen in the presence of non-collagenous proteins.

Shrinkage Temperature Measurements

The shrinkage temperature of tanned leathers, which is a measure of hydrothermal stability of leather, was measured and determined using a Theis shrinkage meter.[49] The values reported are an average of three measurements for each experiment

Scanning Electron Microscopic (SEM) Measurements

Samples from experimental crust leathers made from DAA tanning (10% DAA; pH 8; temperature 30^{o}C; tanning duration 24 hrs) followed by post tanning (Table 2), were cut into specimens from official sampling position.[50] 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 20 KV at different magnifications.

Organoleptic Properties of Tanned Leathers

The matched pair control and experimental crust leathers were assessed for fullness, grain smoothness, softness and general appearance by hand evaluation technique. The functional properties of the leathers in a scale of 0 -10 points has been rated by three experienced tanners and the average values are reported. Higher values indicate better property.

RESULTS AND DISCUSSION

Tanning trials with DAA

Shrinkage temperature and Fixation of DAA at varying tanning conditions

Effect of pH

The results obtained with respect to the effect of pH on the fixation of dialdehyde alginic acid to the collagen matrix are shown in Figure1. The shrinkage temperature of the leathers is given in Table 4. From the figure it can be seen that the fixation of dialdehyde alginic acid increases gradually with increases in pH and a similar trend is also observed in the case of shrinkage temperature of the leathers (Table 4). Maximum fixation and shrinkage temperature of 7.5% and 82^{o}C respectively is observed at pH 10. The higher fixation of polyaldehyde alginic acid in the alkaline pH can be explained on the basis of the high sensitivity of dialdehyde alginic acid towards alkali. Periodate oxystarch, like periodate oxycellulose, has been shown to be extremely sensitive to degradation by alkali, by virtue of the presence of dialdehyde groups[51]. Hydrolysis may take place either at the 1,4-bonds or at the C_2-O bonds[52-53], giving depolymerized and decomposition products, which may take part in the reaction in the alkaline range. Since these cleavage products will have a lower molecular weight than the original dialdehyde alginic acid, the intermediate dialdehyde products will be able to diffuse much better and have access to more functional sites. This results in more fixation and higher shrinkage temperature at high pH values. There is also proportionally an increase in uncharged amino groups (NH_2) of the side chain functional groups of amino acids like lysine and arginine with increasing pH, which could also have favored improved fixation as it is known that aldehydic groups covalently crosslink with amino functional groups of the protein.[54]

Effect of Concentration

The shrinkage temperature and fixation of dialdehyde alginic acid at different concentrations of DAA treatment are given in Table 5 and Figure 2. From the figure it is seen that the uptake of polyaldehyde alginic acid increases with increasing offer of DAA. Above 10% offer of DAA there no significant increase in the fixation of the dialdehyde. As seen from Table 5, the shrinkage temperature also does not vary substantially after an offer of 10% DAA. Hence this percentage offer appears to be sufficient for stabilization of collagen matrix.

Effect of time

The fixation of DAA at different time intervals is shown in Figure 3 and the shrinkage temperature of the same are given in Table 6. From the figure it is seen that the uptake of polyaldehyde alginic acid increases gradually with time. It requires minimum of 12 hours to bring about significant stabilization of collagen resulting in shrinkage temperature of 76.5^{o}C. At 24 hours of treatment time the shrinkage temperature was found to be 80^{o}C. At higher time intervals, the shrinkage temperature and fixation of dialdehyde alginic acid with collagen did not increase much.

Effect of temperature

Penetration of DAA into collagen is slower than that of the simpler aldehydes because of its higher molecular weight and size. Hence thermal agitation may favor the diffusion of DAA into the collagen matrix. The results obtained for the fixation of polyaldehyde alginic acid at different temperatures are given in Figure 4. From the figure it is seen that there is only a marginal

increase in the fixation and shrinkage temperature of DAA with increase in temperature. The increase in shrinkage temperature could have been augmented with the distribution of DAA aiding access in finding more sites for interaction with collagen with increase in temperature. Hydrolysis takes place in the alkaline pH at higher temperature. Diffusion of cleaved products that have lower molecular weight intermediate aldehyde chemical constituents could have favored improved fixation and enhanced tanning resulting in higher and maximum fixation.

Characteristics of Leathers Tanned with DAA

The leather tanned using DAA at pH 8 results in a shrinkage temperature of 80^{o}C. Maximum polyaldehyde fixation of 7.2% and shrinkage temperature of 80^{o}C has been observed at a pH of 8 for 99% oxidized DAA. It is seen that the fixation of DAA increases with increases in degree of oxidation and a similar trend is also observed in the case of shrinkage temperature of the leathers. It is essential to study the characteristics of leathers tanned with DAA at different oxidation levels to optimize the conditions for tanning and producing different kinds of leathers. At 33% oxidation levels, the quality of DAA tanned leathers can be graded as untanned. And drying was hard and stiff. It almost looked like parchment leather on drying. The same was also observed for 66% oxidation levels. The leather was thin and flattest and was of poorest quality. At 99% oxidation level the leathers were tanned (at pH 8), looked off white in colour and slightly darker at pH 10.The leather like drying of these leathers (tanned at pH 8 & 10) was however not as expected. The leathers dried out little harder. Hence the leathers made from optimized tanning conditions (matched pair comparison at pH 8) were fatliquored with 4% neats foot oil in the drum for 1 hour after tanning. The fatliquored DAA tanned leathers exhibited better

characteristics and on drying resembled slightly light colored vegetable tanned leathers. Best of the leather characteristics were obtained by tanning goatskins at pH 10 and fatliquoring.

Collagenase Hydrolysis of the Collagen Stabilized by Dialdehyde Alginic Acid Treatment at Varying Tanning Conditions

The % degradation of collagen for the delimed collagen and collagen stabilized using DAA at varying conditions viz., % offer, pH and temperatures are given in Table 8. It can be clearly seen from the Table that complete degradation of collagen has occurred in the case of delimed pelt treated with collagenase. There has been tremendous increase in the stabilization and resistance to degradation of collagen by crosslinking with polyaldehyde (DAA). The resistance to degradation to collagenase is high for pelts treated with DAA at higher pH compared to DAA treatment at lower pH ranges. There is considerable increase in the stability against collagenase attack when collagen has been treated with DAA at increasing pH conditions. Only 6% collagen degradation has been observed when the tanning has been maintained at pH 8 and above. The trend as observed in thermal stability has been observed in enzymatic stability of DAA treated collagen with varying pH. The enzymatic stability of the leathers have increased with increase in the offer of DAA, when the DAA offer has increased from 5 to 10%, the resistance to collagenase has increased by 50%. However, there was no significant increase in collagenase resistance with further increase in DAA offer. Hence 10% offer of DAA appears to be optimum for tanning. While monitoring the DAA treated collagen against collagenase at different time interval, it has been observed that treatment for 24 hours offered 95% inhibition to degradation compared to untreated collagen. The degradation levels have been found to be further reduced when the DAA treatment was carried out at elevated temperatures. This observation on the

enzymatic stability is been almost similar in trend with the values obtained for shrinkage temperatures. The stability against collagenase hydrolysis is again an adequate proof that DAA can be used effectively as an alternative tanning agent for ecobenign leather making.

Scanning Electron Microscopic (SEM) Analysis of Polyaldehyde Tanned Leathers

It is also essential to study the fibre structure orientation on the structural changes in tanned leathers with DAA. The Scanning Electron photomicrographs of the cross section of leathers tanned using DAA from matched pair DAA post tanned leathers (99%) are shown in Figure 5. The cross sectional view of the DAA tanned leather at a magnification of 300X is shown in Figure 5. The cross sectional SEM micrograph of dialdehyde alginic acid treated collagen fibres as seen from figure appears to be coated. The coating could be due to the presence of DAA around the collagenous fibres.

Organoleptic Properties of Crust Leathers from DAA Tanned Leathers

Tanning trials at 99% oxidation levels for the matched pair and chrome tanning process has been carried out. Post tanning process as given in Table 2 for both control and DAA tanned leathers experimental leathers were assessed for bulk properties. The organoleptic properties of 99% oxidation DAA tanned crust leathers; tanned-fatliquored and post tanned crusts were compared with control chrome tanned crust leathers are shown in Figure 4. The softness and the grain smoothness of DAA tanned crust leathers appear to be comparable to chrome tanned crust leathers. The leathers showed firm grain but lower in rating than chrome tanned crusts. Fullness of these leathers appears to be higher than chrome tanned leathers. The colour of the DAA tanned leather is light brown. The general appearance of the DAA tanned leathers at the moment

is rated lower compared to chrome tanned leather. Although DAA exhibits tanning property as seen from the shrinkage temperature measurements, most of the chemical, physical and functional properties need to be addressed to achieve good quality leather for proper end use. The processing condition for the DAA treatment has to be further fine tuned to make leathers with good tactile properties. Studies on tanning/post tanning processing conditions are in progress to achieve good saleable leathers for it to be comparable to that of chrome tanned crust leathers.

CONCLUSIONS

The present study explores the possibility of making leathers using a modified product obtained from a natural renewable source i.e. a polyaldehyde prepared by oxidation of alginic acid. Approaches have been made to identify suitable processing conditions at which the tanning agent can be best suited to provide better stability to the collagen matrix. Evidences have been gained from the study that DAA can be good tanning agent in stabilizing the collagen matrix. The DAA tanned leathers have been found to have shrinkage temperatures greater than $80^{o}C$ and the leathers exhibited stability against collagenase, the main enzyme which is involved in the degradation of collagen. Offer of 10% DAA at pH 8.0, temperature ~$30^{0}C$ and tanning time of 24 hours have been considered as optimum conditions for tanning. Hence, DAA can be an effective tanning agent, which has the potential to dominate the tanning industry. Best tanning conditions from the analysis made in this investigation need not be necessarily the true optimized conditions and other leather properties like strength properties like tensile, tear and burst strength, flexibility, grain crack resistance etc. need to be evaluated so as to produce leathers of

different end uses. Efforts are continuing in improving the processing conditions of the DAA tanning/post tanning to obtain leathers with comparable organoleptic properties as those of chrome tanned leathers.

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Table 1 Tanning process using DAA from pickled goat skins

Process	Chemicals	%	Duration	Remarks
pH adjustment	Pickle liquor	50		
	Sodium formate	1	15 min.	
	Sodium bicarbonate (1:10 dilution with water)	1-3.0	5x15 min.	Adjust pH to each experimental trial;
Tanning[#]	Water	50		Maintain the required
	DAA	10	24 hrs.	pH
Fatliquoring*	Water	50	1 hr.	Drain tan liquor,
	Neats foot oil based fatliquor	4		Check exhaustion of fatliquor
	Formic acid	0-2		
	Water	10	3x10+30 min	pH checked/adjusted to 4; Drain; Aged for 24 hrs; Sammed; Shaved to thickness 1.0-1.1 mm

[#] - Trial 1 – Tanning process at varied pH viz., 4, 5, 6, 7, 8, 9 and 10

Trial 2 – Tanning process at varied amounts viz., 5, 10, 15 and 20 % (pH 8)

Trial 3 – Tanning process at varied time viz., 4, 12, 24, 48 and 72 hrs (10% offer, pH 8)

Trial 4 – Tanning process at varied temperature viz., 30, 40 and 50^{o}C (10% offer, pH 8)

* - Fatliquoring done for trials of experimental matched pair leathers at pH 8 only

Table 2 Post tanning process for experimental (optimized) and control leathers

Process	Chemicals	%	Duration (min.)	Remarks
Wetting	Water Wetting	250	2 hrs	(% chemical addition for subsequent operation is based on set weight)
Neutralisation	Water	150		
	Sodium bicarbonate	0.5%	30	pH adjusted to 5 Drain
Washing	Water	200	15	Drain
Retanning, Dyeing and Fatliquoring	Water	150		
	Relugan RE	2	30	
	Basyntan DI	4		
	Basyntan FB6	4	45	
	Lipoderm Liquor SO (Cationic Fatliquor)	3	30	
	Acid dye	2	30	Check penetration
	Lipoderm Liquor SLW (synthetic fatliquor)	3	60	
	Basic dye	1	45	Check exhaustion
	Lipoderm Liquor SO (Cationic Fatliquor)	2		
	Basyntan FB6	2	60	Check exhaustion
Fixing	Formic acid	1		
	Water	10	3x10+30	Drain
Washing	Water	100	10	Drain, Piled O/N, Set, Dry, Stake, Trim & Buff

Table 3 Tanning process for control leathers

Process	Chemicals	%	Duration (min.)	Remarks
Tanning	Pickle Liquor	50		(% chemical addition is based on pelt weight)
	BCS	8	120	
	Water	50	30’	
	Sodium formate	1	30’	
	Sodium bicarbonate (1:10 dilution with water)	1.2	3X15 + 60	pH adjusted to 4.0; Drain
Washing	Water	100	10	Drain, Piled and aged for 2days; sammed, shaved to thickness 1.0-1.1 mm

Table 4: Shrinkage temperature of DAA tanned leathers* at different pH

pH	Shrinkage Temperature (^{o}C)
4.0	66±1
5.0	69±0.5
6.0	72±0.5
7.0	76±1
8.0	80±1
9.0	81±1
10.0	82±1

* - DAA offered at 10%, at 30^{o}C for 24 hours

(Shrinkage temperature of pickled pelt - 56^{o}C**)**

Table 5: Shrinkage temperature of DAA tanned leathers* at varying concentrations

DAA offer	Shrinkage Temperature (^{o}C)
5%	70±1
10%	80±1
15%	81±1
20%	82.5±1.5

* - Treated at pH 8.0, 30^{o}C for 24 hrs

Table 6: Shrinkage temperature of DAA tanned leathers* at varying time

Treatment time (hrs)	Shrinkage Temperature (^oC)
2	66±1
4	69±1
8	74±1
12	76.5±0.5
16	79.5±0.5
20	80±1
24	80±1
48	81±1
72	82±1

* - Treated at pH 8.0, 10% offer and 30^oC

Table 7: Shrinkage temperature of DAA tanned leathers* at varying temperatures

Temperature of the bath (^oC)	Shrinkage Temperature (^oC)
30	80±1
40	82±1
50	83±1

* - Treated with 10% DAA at pH 8.0 for 24hrs

Table 8: Collagenase hydrolysis (72 hrs) of native and collagen treated with polyaldehyde starch at varying crosslinked conditions

S.No	Process	Value	% Collagen degradation
	Native (Delimed)	-	99.3
	Chrome tanned leather	-	0.23
1.	pH of tanning	4.0	39.9
	(10% offer of DAA at	5.0	28.97
	30^{o}C)	6.0	17.39
		7.0	11.58
		8.0	6.13
		9.0	5.08
		10.0	5.01
2.	Amount of dialdehyde	5%	13.79
	alginic acid offered	10%	6.13
	(DAA used at pH 8.0 and	15%	6.02
	30^{o}C)	20%	5.97
3.	Time of tanning	4 hrs	19.36
	(10% offer of DAA at pH	12 hrs	7.81
	8.0 and 30^{o}C)	24 hrs	6.13
		48 hrs	6.12
		72 hrs	6.1
4.	Temperature of tanning	30^{o}C	6.13
	(10% offer of DAA at pH	40^{o}C	6.04
	8.0)	50^{o}C	5.98

LEGEND TO FIGURES

Figure 1: Effect of pH on the amount of DAA fixed to the skin during tanning at room temperature for 24 hours (offer 10% DAA)

Figure 2: Effect of % offer of DAA during tanning at pH 8, room temperature for 24 hours on % DAA fixed to the skin

Figure 3: Effect of time on 10 % offer of DAA during at a pH of 8 at room temperature on % DAA fixed to the skin

Figure 4: Effect of temperature on 10 % offer of DAA during tanning at a pH of 8 for 24 hours on % DAA fixed to the skin

Figure 5: Scanning Electron Micrograph of DAA tanned leather a) Grain structure (X 100) and b) Cross-section (X 300)

Figure 6: Graphical representation of organoleptic properties of control and DAA tanned leather

Figure 1

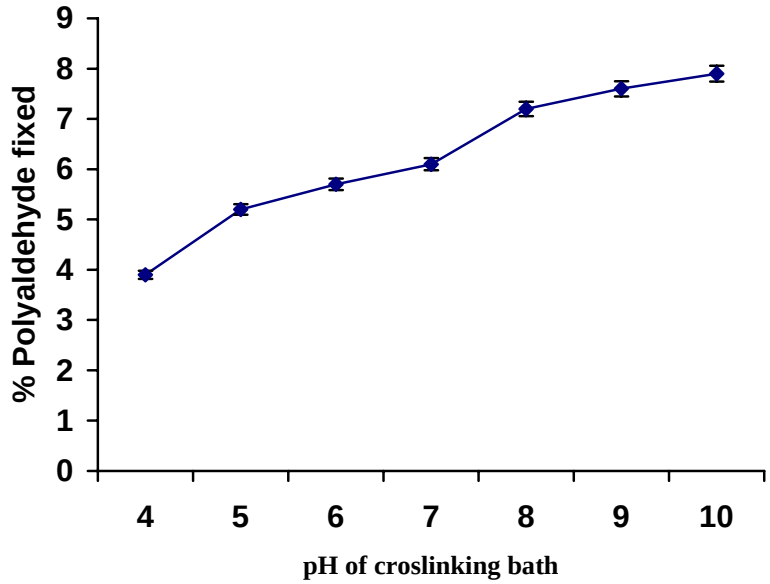


Figure 2

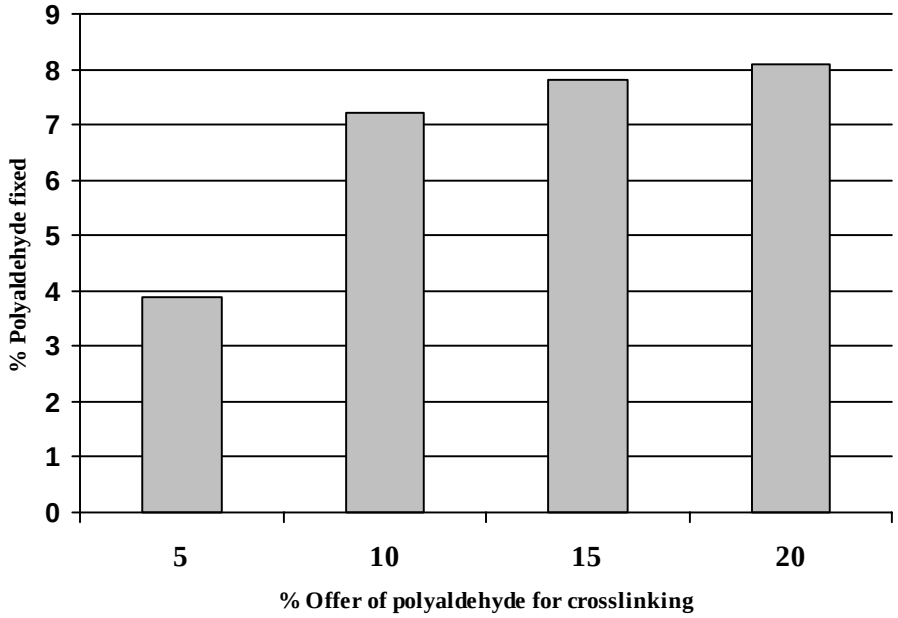


Figure 3

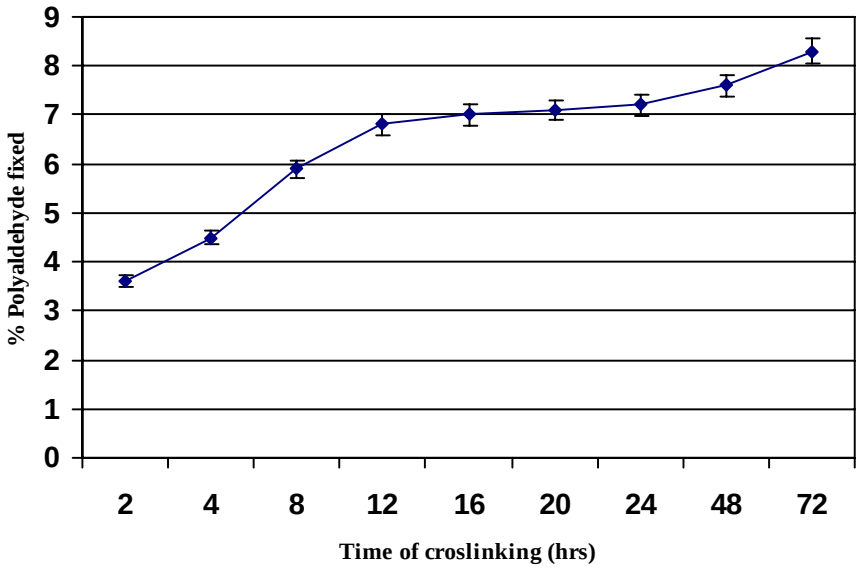

% Polyaldehyde fixed
0
1
2
3
4
5
6
7
8
9
2
4
8
12
16
20
24
48
72
Time of croslinking (hrs)

Figure 4

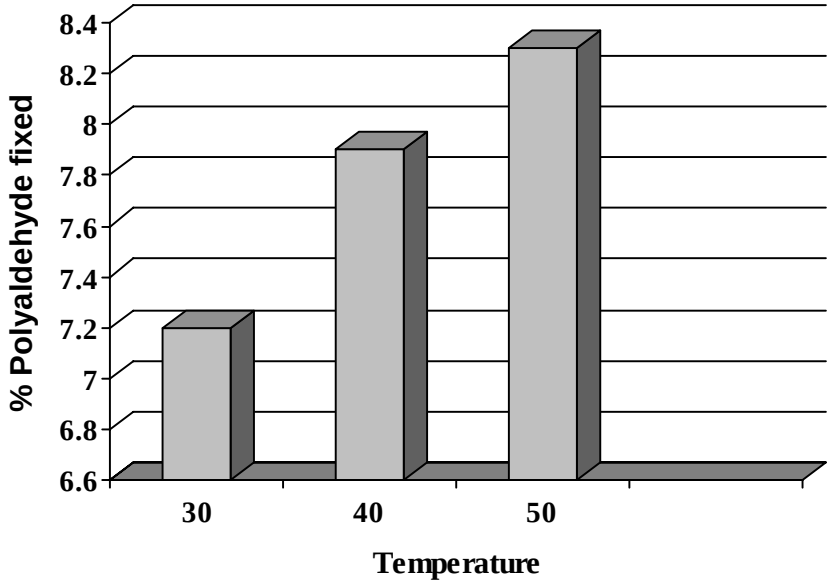


Figure 5

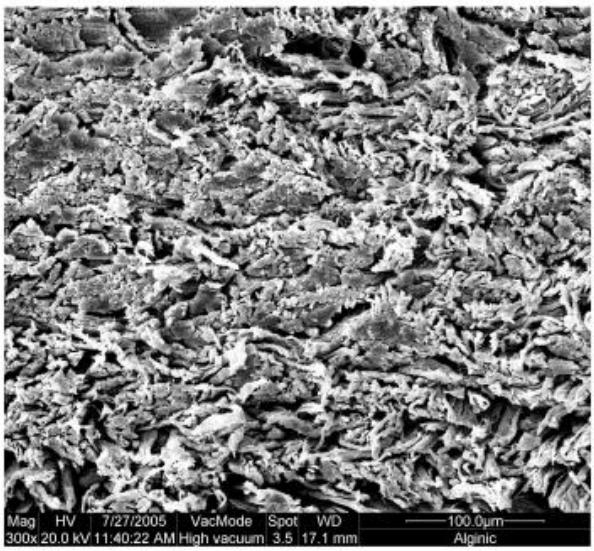
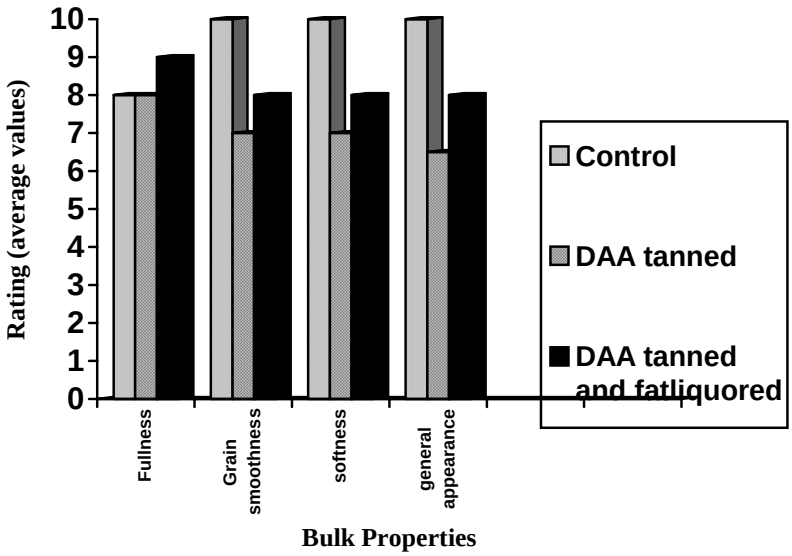


Figure 6


Rating (average values)
10
9
8
7
6
5
4
3
2
1
0
Fullness
Grain smoothness
softness
general appearance
Bulk Properties
Control
DAA tanned
DAA tanned and fatliquored

b