Click chemistry approach to tanning processes: Accelerated vegetable tanning process with improved properties

Ganesan Krishnamoorthy, Sayeed Sadulla*, T.P. Sastry and Asit Baran Mandal*

CSIR - Central Leather Research Institute,

Adyar, Chennai - 600 020, Tamil Nadu, India

*Corresponding authors email ID: sadullams@hotmail.com; abmandal@hotmail.com

Abstract

Click chemistry approaches are tailored to generate molecular building blocks quickly and reliably by joining small units together selectively and covalently, stably and irreversibly. The vegetable tannins such as hydrolysable and condensed tannins are capable to produce rather stable radicals or inhibit the progress of radicals and are prone to oxidations such as photo and auto-oxidation and their anti-oxidant nature is well known. A lot remains to be done to understand the extent of the variation of leather stability, colour variation (lightening and darkening reaction of leather) and poor resistance to water uptake for prolonged periods. In the present study, we have reported click chemistry approaches to accelerated vegetable tanning processes based on periodates catalyzed formation of oxidized hydrolysable and condensed tannins for high exhaustion with improved properties. The distribution of oxidized vegetable tannin, the thermal stability such as shrinkage temperature (Ts) and denaturation temperature (T_d), and resistance to collagenolytic activities, and organoleptic properties of tanned leather as well as the evaluations of eco-friendly characteristics were investigated. Scanning electron microscopic (SEM) analysis indicates the surface morphology and roughness of the leather. Differential scanning calorimetric (DSC) analysis shows that the Td of leather is more than that of vegetable tanned or equal to aldehyde tanned one. The leathers exhibited fullness, softness, good colour and general appearance when compared normal vegetable tannin. The developed process benefits from significant reduction in total solids and better biodegradability in the effluent, compared to non-oxidized vegetable tanning.

Key words: Click chemistry approach; Collagen; Vegetable tannins; Wattle; Myrobalan.

1. Introduction

Vegetable tannins have been considered as a suitable eco-friendly option to replace chromium and other inorganic tanning agents. Vegetable tanning is most ancient one and being employed for making some kinds of leathers. Although the technology of tanning process has evolved over centuries, even in recent years, the basic principles for the production of vegetable tanned leather have remained the same. The skin/the proteins, mainly triple helical collagen, are rendered insoluble and dimensionally more stable by treatment with vegetable tannins able to fix on

collagen fibrils and render them both more resistant to mechanical wear and less biodegradation and other types of attack. These tannins are used mainly for the manufacture of heavy and rigid leathers for shoe soles, saddles, belts, and other implements subject to high wear. These tannins have a strong astringent effect and give considerable body, hardness and toughness to the leathers produced with them.

However, this tanning leads to excessive loading in the leathers, which reduce its versatility to make different kinds of end products and also has low resource availability. These tannins are supple, yellow and brown in color, with the exact shade depending on the mix of chemicals and the color of the skin. These are the only form of leather suitable for use in leather carving or stamping. This tanned leather is not stable in water; it tends to discolor, and if left to soak and then dry it will shrink and become less supple and harder. In hot water, it will shrink drastically and partly gelatinize, becoming rigid and eventually brittle. They present, however, among others, the considerable disadvantage to have marked darkening (browning) problems when exposed to light. In this case, their capacity of photo-oxidation limits their use to applications where such a characteristic is of no consequence. The phenolic structure of the tannin renders photo-oxidation possible. Photo-oxidation and radical capture and retention by polyphenolic tannin structures have been shown to be closely related. Still remains unknown the extent of the variation of tanned leather color and its stability to radiation and radicals for prolonged periods and what type of law it follows. This leather is also known to be poorly biodegradable, which results in high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD).

Vegetable tannins are classified into two categories: (1) Pyrogallol tannins or hydrolysable tans and (2) Catechol tannins or condensable tans. In mechanism, acidic groups of tannins may combine with the basic groups of the hide collagen by poly-functional cross-linking via so-called H-bridges [Chen et al., 2004; Covington 1997, 2005a and b]. The hydrolysable tannin is on heating with hydrochloric or sulfuric acids, yields gallic or ellagic acids. At the center of a hydrolysable tannin molecule, there is a carbohydrate (usually D-glucose but also cyclitols like quinic or shikimic acids). The -OH groups of the carbohydrate are partially or totally esterified with phenolic groups such as gallic acid in gallotannins or ellagic acid in ellagitannins, These tannins are mixtures of polygalloyl glucoses/polygalloyl quinic acid derivatives containing in between 3 up to 12 gallic acid residues per molecule [Yoshida et al., 2000]. Condensed tannins are dimers or oligomers of catechin, epicatechin, or similar units. These units are polymers of 2 to 50 or more flavonoid units that are joined by C-C bonds between flavanol subunits which are not susceptible to being cleaved to hydrolysis. Mixtures of this oligomeric tannin are powerful antioxidants known as oligomeric proanthocyanidins [Schofield et al., 2001].

The variation of leather color as a function of aging time on prolonged irradiation with UV light of the leather produced with condensed and hydrolysable tannins is reported [Pizzi et al., 2004]. Under non-oxidizing conditions, tannins form non-covalent complexes with collagen through H-bonding and hydrophobic interactions and, may reduce the biodegradation and may contribute to chemical and biological defenses. Under oxidative conditions, tannins form covalent complexes. However, oxidation intermediates (semiquinone) or oxidation products (quinone) of vegetable tannins, including hydrolysable and condenzed, are electrophilic. In this condition, tannins form covalent complexes with nucleophiles, such as $–NH_2$ or thiol groups. Oxidative

activities of these tannins have been studied [Hartzfeld et al., 2002; Chen et al., 2004]. Given that interaction with collagen is characteristic of tannins and that collagen has nucleophilic functionality, collagens are likely to be modified by oxidized tannins. The covalent modification was observed in reaction between collagen and oxidized hydrolysable and condensed tannins [Chen et al., 2004].

Click chemistry approaches are tailored to generate molecular building blocks quickly and reliably by joining small units together selectively and covalently, stably and irreversibly [Moses et al., 2007]. These approaches have widespread applications such as preparative organic synthesis, modification of natural products and synthetic polymer, dendrimer design, drug discovery and design, modification of peptides and protein and widely used in polymer science, material science and nanotechnology. The execution of these approaches into tanning process could improve the organoleptic properties and thereby expected to yield a variety of leathers.

In this work, the development of click chemistry approach to tanning processes, periodate oxidized hydrolysable and condensed tannins has been attempted. The tanning conditions, physico-chemical characteristics and organoleptic properties of leathers and the environmental impact of this tanning have been compared with conventional tanning process.

2. Experimental procedures

2.1. Process chemistry of vegetable tanning and shrinkage temperature determination

Raw material used for leather processing was pickled goatskins processed from wet salted goatskins. The chemicals employed in subsequent operations were those normally used in leather industry. All the other chemicals were commercial grade products.

The tanning experiments were carried using pickled goatskins. Six pickled goatskins were tanned by (based on pickled weight) 3% syntan and 8% vegetable tannins Viz., Myrobalan and Wattle with 0.5 Sodium periodate ($NaIO_4$) at pH 3.5 for 3-4 h and then the basification was carried out by the addition of 1% formic acid and 1% sodium bicarbonate in four instalments at an interval of 15 min. Finally, the tanning drum was run for 1 h and shrinkage temperature (Ts) of each tanned leathers was determined.

2.2. Characterization

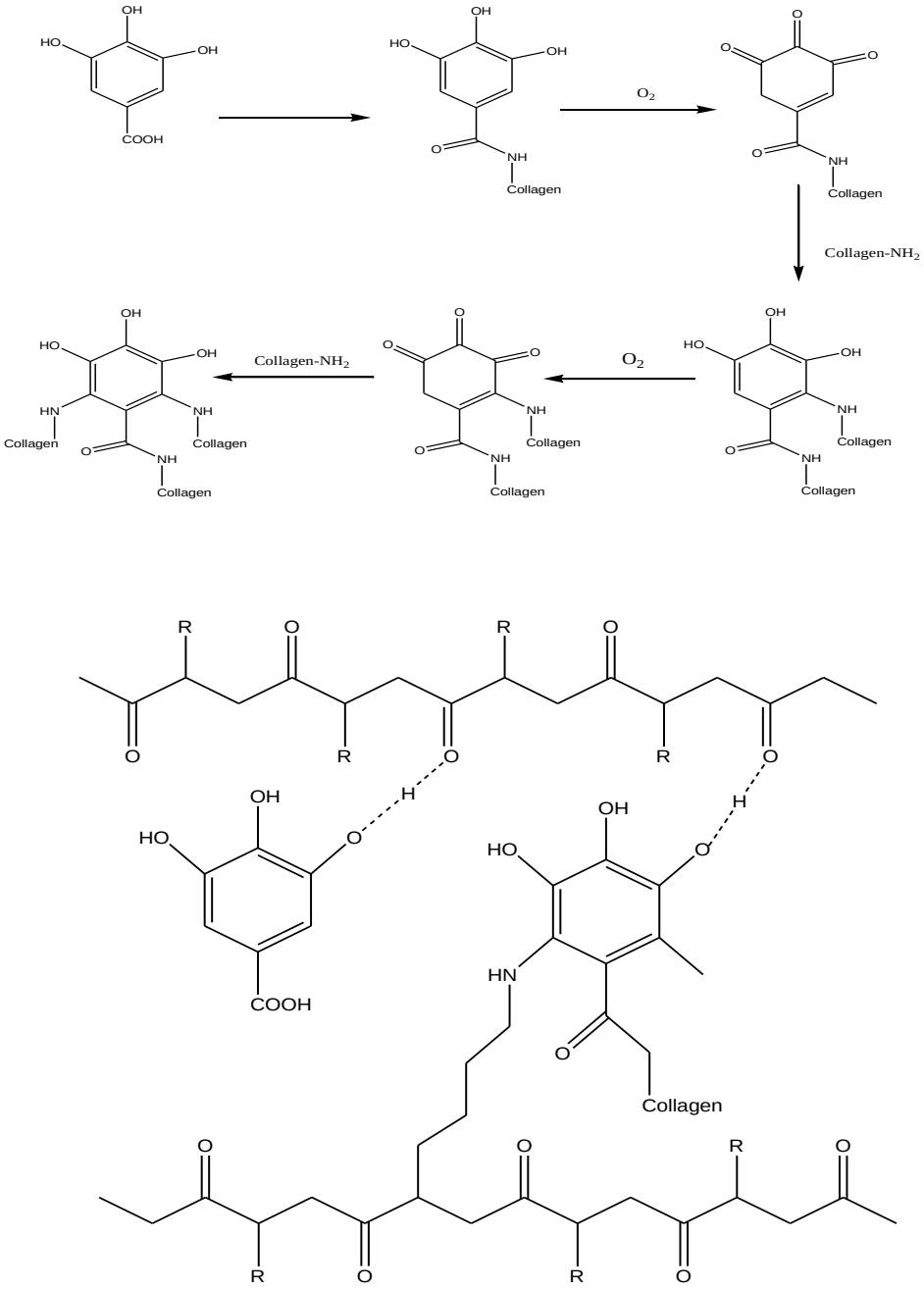
The thermal properties of tanned leathers were analyzed using Differential scanning calorimetric (DSC, TA-DSC Q 200). The organoleptic properties of tanned leathers were analyzed by visual hand evaluation. The % of degradation of tanned leather against collagenase activity was calculated by our earlier method [Krishnamoorthy et al., 2011]. The % of tannin exhaustion was calculated from amount of spent liquor collected. The tannin content in the spent liquor obtained in each of the trials was estimated by the perchloric acid digestion method. The tanned liquor from experimental processing was collected and analyzed for BOD and COD. Total solid (TS) content was calculated according to the standard procedure.

3. Results and discussion

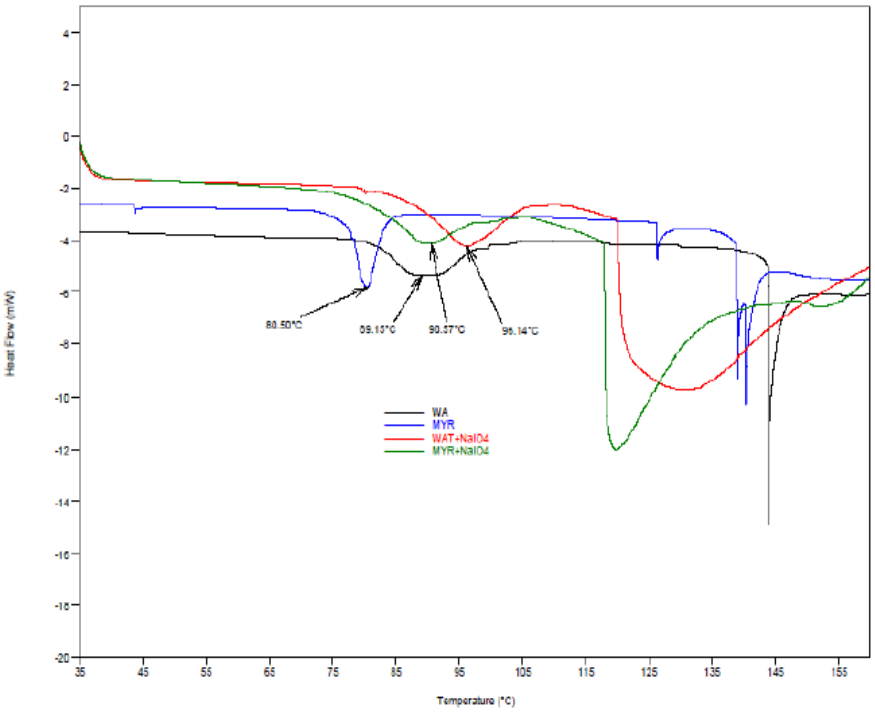
The functional groups of hydrolysable and condensed tannin are shown in scheme 1. These have been used as crosslinking agents and have -COOH groups that can react with active groups located in collagen, such as $-NH_2$ or -OH groups. These phenolic groups are able to form ionic bonds with positively charged amino acids and it can also form ion dipole interactions with water [Covington 1997]. These interactions are very important to the solubility properties of proteins. The effect of the oxidized tannins is used as high exhaustion on enhancing the ability of uptake tanning agent and reduction of tanning in waste. In the study delimed goatskin in partially anionic in charge is treated with sulfuric acid in order to convert the cationic charge, prior to tanning. Salt is used suppress the swelling of goatskin by maintaining the ionic balance. During tanning process, oxidized tannins are irreversibly bound with the skin matrix by cross linking with collagen $–NH_2$ groups through coordinate covalent linkage. The oxidized tannins tanned leather is negatively charged. The tanned leathers are neutralized in order to avoid the surface fixation of negatively charged post-tanning chemicals on the charged oxidized tanning cross linked matrix. This is achieved by using mild alkalis such as sodium bicarbonate. During neutralization, the pH is raised to 5.0-5.2 for further post tanning operation. Schematic representations of proposed structure of collagen side chain cross linking with oxidized tannins are shown (Scheme 1).

3.1. Physico-chemical properties

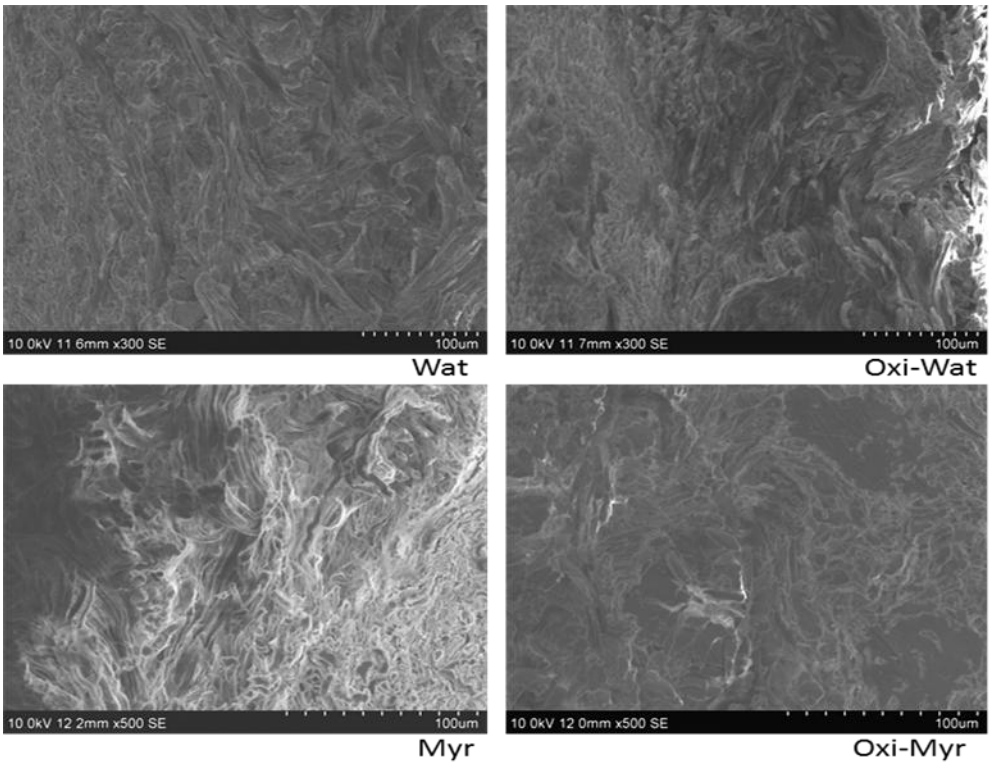
The denaturation temperatures (Td) of leather tanned with oxidized hydrolysable and condensed tannins were studied using DSC measurement. The T_d of leather is normally related to the onset, temperature of its peak in a DSC pattern and can be used as a measure of the hydrothermal stability of tanned leather subjected to oxidized tannins treatment. An increase in the TS is usually an indication of an increase in the stability of wet tanned leather. Fortunately, hide treated with oxidized tannins, the endothermic peaks are similar to vegetable tanned leather and become narrow and sharp, moreover, the T_d of Wattle, Wat+NaIO4, Myrobalan, Myr+NaIO4 final leather reach 80±3, 89±4, 90±2 and 92±3, respectively (Fig. 1A). Obviously, the oxidized tannins can efficiently improve the hydrothermal stability and the tanning effects are superior to non-oxidized tannin. The tanned leathers were found to be soft, full and possessed tight grain. The oxidized tannins show a strong tendency to form coordination compounds with molecules containing $–NH_2$ groups. There are a number of ways in which the tanning action could take place, and although an ionic compound may be formed initially, it seems certain that the main chemical action is by means of coordination complexes or covalent bonds. It also seems certain that the most important interactions are the free $–NH_2$ groups of the collagen structure [Covington 1997]. There is no significant change observed in the cross section of oxidized tannin tanned leather when compared with normal tanned leather (Fig. 1B).



Scheme 1. Schematic presentation of oxidized tannins complexation with collagen



A)



B)

Fig. 1. A) DSC analysis and B) SEM image of oxidized tannin tanned leather

3.2. Biodegradation

% leather biodegradation (based on hydroxyproline released) for oxidized hydrolysable and condensed tannins tanned leather by collagenase at various time periods have been determined. Significant reduction in the degradation of leather is observed for oxidized tannins as against 99% degradation in the case of pickled skin at 96 h period of incubation when compared to non-oxidized tannin tanned leathers. Previous report has also indicated the interaction of collagen with green tea polyphenols and vegetable tannin such as hydrolysable and condensed tannin and inhibition of collagenase activity [Krishnamoorthy et al., 2008; Madhan et al., 2007]. More recently, tannic acid molecules which also contain digalloyl unit have been shown to stabilize collagen not only through H-bonding but also by hydrophobic interactions [Krishnamoorthy et al., 2012].

3.3. Environmental impact assessments

The impact of oxidized tannins on the environment was assessed by the spent tan liquor analysis for TS, BOD_5 and COD. The COD value in tanning is higher than in non-oxidized tannins due to oxidized tannins. In comparison with non-oxidized tanning, the BOD_5/COD value is greater for oxidized tannins which demonstrate that the effluent from tanning process using oxidized tannins is easily biodegradable than that of oxidized tannins. The rate of fixation of oxidized tannins may be increased using these oxidized tannins. High exhaustion tanning may allow the reduction of 90 to 98 % of vegetable tannin from the liquor, reducing the concentration of vegetable tan in the effluent. The comparative statistical analysis of chrome and vegetable tanning effluents and their effects on related soil have been studied [Tariq et al., 2009]. The solid waste of this vegetable tanning process aggravates the water pollution by means of leaching of tannin and other associated pollutants [Mazumder et al., 2006].

3.4. Organoleptic properties

The organoleptic properties of oxidized tannins tanned leathers are given in the Table I. From the Table I, it is observed that oxidized tannins tanned leathers exhibited good fullness, softness, smoothness, colour and general appearance as compared to non-oxidized tannin. This is because of the improved uptake of oxidized tannins; generally, the appearance and overall performance of the tanned leather is good comparable to the normal tanned leather. It was found that the enhancement of fullness and tightness was directly proportional to oxidized tannin.

Table I. Rating of organoleptic properties

Processes	Fullness	Grain tightness	Grain smoothness	Softness	Colour of tanned leather	General Appearance
Wat	8.5	8	8	8	8	8
Wat+$NaIO_4$	9	8.5	9.2	9	9	8.5
Myr	9	8	9.2	8.5	8.5	8
Myr+$NaIO_4$	10	8.5	9	9	9	9

3.5. Leather-oxidized hydrolysable and condensed tannin complexes

3.5.1. Oxidation changed leather-tannin complexes

Under non-oxidizing conditions, non-covalent interactions including H-bonds and hydrophobic forces stabilize tannin-collagen complexes. Spectroscopic evaluation of soluble complexes indicates that the –OH groups of the tannin are H-bond donors, and the –COOH groups in the protein are H-bond acceptors [Simon et al., 2003]. Determination of the tanning degree of vegetable tanned leather by IR spectroscopy has been studied [Canals et al., 2011]. NMR studies suggest hydrophobic interactions involving the hydrophobic forces of aromatic rings of the tannin and the hydrophobic side chains of AAs in the collagen. Balancing of properties of vegetable tanned leathers and light stability/tannins antioxidant characteristics for leather made with vegetable tannins have been studied [Simon et al., 2003, George et al., 2004; Ozgunay 2008; Simon et al., 2003]. Analyses of vegetable tanning absorption during the tannage of hide/hide powder have been studied. Equilibrium and kinetics model can be used to predict the adsorption capacity of vegetable tannins in the vegetable tanning process [Liao et al., 2004]. These precipitates are typically completely solubilized by solvent with release of unmodified tannin and protein, suggesting that they are extended networks of tannin and protein that are insoluble only because of their size and polarity, not because of any irreversible covalent bond formation.

Oxidation alters the bonding in tannin-collagen complexes. For example, free radical oxidation of collagen-tannin yields insoluble aggregates that are not solubilized by collagen denaturants suggesting the formation of stable covalent linkages. Recent studies prove tannin oxidation: Intra-versus intermolecular reactions [Poncet-Legrand et al., 2010]. In this condition, oxidations created new inter and intra molecular bonds that were resistant to thiolysis. However, oxidation at high tannin concentration caused the weight average degree of multi polymerization to increase, indicating that intermolecular reactions took place as well, creating larger macromolecules. Green synthesis of soluble polyphenol: oxidative polymerization of phenol in water has been studied [Saito et al., 2007]. We found that oxidizing mixtures of tannin and collagen with $NaIO_4$ converted about 25% of the collagen-tannin complexes to stable form. The condensed tannin precipitated more collagen under oxidizing conditions than in the absence of oxidant, but hydrolysable tannin precipitated similar amounts of collagen under oxidizing and

non-oxidizing conditions. When quinones meet amino acids; chemical, physical and biological consequences and the formation and activity of this conjugates influences the colour, taste, and aroma have been studied [Bittner et al., 2006]. $NaIO_4$ is a two-electron oxidant. It is a good model oxidant for studying the mechanism of tannin oxidation, because it has no absorbance above 260 nm, which allows direct spectrophotometric monitoring of polyphenol oxidation. Within our experimental pH range (pH 2.1-7.4), the oxidizing species is IO_4, which is reduced to IO_3 by phenols. Periodate, not iodate or other more reduced forms of iodine, is the reactive species as demonstrated by the lack of reaction between tannin and IO_3 under experimental conditions that yielded instant oxidation of tannin by IO_4. In control experiments, when collagen or tannin was treated individually with $NaIO_4$ at pH 4.9, no precipitate was formed. The stabilization of fibrillar collagen matrices with 3, 4-dihydroxyphenylalanine have been studied [Gade et al., 1991].

3.6. Click chemistry approaches: An alternative option to high exhaust tanning

Using this click chemistry approach tools for high exhaust tanning process is an effective method that can be used to improve the organoleptic properties of leather, providing both economic and environmental benefits. The organoleptic properties of the leather are improved for example with regard to texture, thermal and mechanical strength. In general, click chemistry approaches to tanning involves implementation of the following strategies:

1) Be stereospecific and improve the structural integrity,
2) High exhaust process where the uptake of the tanning agent is greater,
3) Improve the organoleptic properties such as fullness, softness and general appearance etc.
4) Improve the conventional tanning processes which help reduce the TS.
5) Exhibit a large thermodynamic driving force to favor a reaction with a single reaction product.

In this scenario, the development of Click chemistry approaches to tanning process by rationally improving the conventional process provides a technically as well as economically viable alternative. These oxidized tannins based high exhaust tanning technologies are regarded as eco-friendly and hence contribute to the development of cleaner process.

3.7. What about the future? Remaining challenges…

The tanning in future direction will use click chemistry approaches, modification of collagen by cycloaddition, nucleophilic substitution, addition reactions and biocatalytic selective modification. The potential for use of this tanning processing lies mainly in areas in which pollution-causing chemicals are at present being used. Future may witness emerging of eco-labelled tannin as well as eco-labelled tanning process. Click chemistry approaches, and the experience gained by the leather sector in this area might greatly help to emerge as a global leader in leather industry. This approach in combination with combinatorial chemistry, high-throughput screening and building chemical libraries speeds up in emergence of new reactions by making each reaction in a multistep process speedy, efficient and predictable.

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