# A New Depigmentation and Fiber Opening Method for the Conversion of Stingray Skins into Leathers

*by*

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## Abstract

A study has been conducted to produce leather from stingray skins without using the conventional lime and sulphide combination, which results in harder and rigid skin making further processing difficult. Sodium lauryl sulphate (SLS) was employed in the present investigation to remove the pigments present in the dorsal portion of the skin as well as to open up the hard fiber structure. The green fleshed skins were treated with SLS, subsequently bated and pickled using hydrochloric acid. Histological and scanning electron microscope studies were carried out on SLS treated stingray skins to test the efficacy of SLS on fiber opening. The spent liquor was analyzed for the presence of glycoprotein using FT-IR. Histological studies with alcian blue, scanning electron microscope analysis and FT-IR analysis confirms the opening up of fiber bundles as well as removal of proteoglycans by the treatment of SLS.

## Resumen

Un estudio ha sido llevado a cabo para producir cuero de piel de raya sin necesidad de utilizar la combinación convencional de cal y sulfuro, que se traduce en pieles más duras y rígidas, haciendo más difícil su procesamiento. Lauril Sulfato de Sodio (LSS) fue empleado en la investigación actual para eliminar los pigmentos presentes en la parte dorsal de la piel, así como para abrir la dura estructura de la fibra. Las pieles frescas descarnadas fueron tratadas con LSS, posteriormente purgadas y piqueladas con ácido clorhídrico. Estudios histológicos y de microscopía electrónica de barrido se llevaron a cabo en pieles de rayas tratadas con LSS en la apertura fibrilar. El licor empleado fue analizado para determinar la presencia de la glicoproteína con FT-IR. Los estudios histológicos con Azul Alciano, análisis de microscopio electrónico de barrido y análisis FT-IR confirma la apertura de los haces de fibras, así como la eliminación de los proteoglicanos en el tratamiento con LSS.

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## Introduction

The important byproducts of meat industry viz. hides and skins are commonly converted into leather. Similarly, fish skins are a by-product of fish processing industry that can also be successfully processed into leather.[1] India is the second largest producer of fish in the world, produces annually about 6.9 million metric tons of fish[2] and hence there is a scope to use the fish skins as a potential raw material for the leather industry. According to Food and Agriculture Organization of the United Nations (FAO), about 15% of the animal protein consumed by the entire human population comes from seafood.[3] Processing of fish leads to enormous amounts of waste. It is estimated that fish processing waste after filleting accounts for approximately 75% of the total fish weight[4] and 30% of the waste is in the form of bones and skins.[5] But the only problem with fish skin is its size. In contrast with cow or buffalo skin, the length and width of the fish skin is smaller in size and not uniform. However the skins of chondrichthyans are very hard and tough, possess very high tensile strength. Chondrichthyan fishes include the sharks, skates, and rays. Stingrays are existing in enormous numbers in the Indian seas and India is one of the world's leading stingrays fishing nation. This stingray belongs to the family Dasyatidae of the class Elasmobranchi.[6] This cartilaginous fish lives both in salt and fresh coastal waters including some rivers around the world.[7] The dorsal portions of the stingrays have denticles instead of scales.[8] Since the stingray skin is calcified during the mature stage[9], becomes very hard and tough and hence the skins are not generally used by the consumers. The photographic picture of stingray skin with denticles is presented in Figure 1. The stingray reaches a maximum size of just over 6 feet wide, 4 feet long, and about 100 pounds in weight, with the females being larger than the males.[10]

The United States of America (USA) was known to be the globally dominant importer of fish leather. The United States Fish and Wildlife Service (USFWS) records showed that fish leather was imported from a total of 57 countries, over 90% of all fish leather products reportedly originated in the Republic of Korea (71%), mainland China (16%) and Thailand (4%), while other imports came from East and South-east Asian nations and from Mexico.[11] Eels and hagfishes, stingrays and sharks are the important types of fish which dominates the fish leather trade. Stingray leather was put forward as one of the world's most prized and priciest decorative materials.

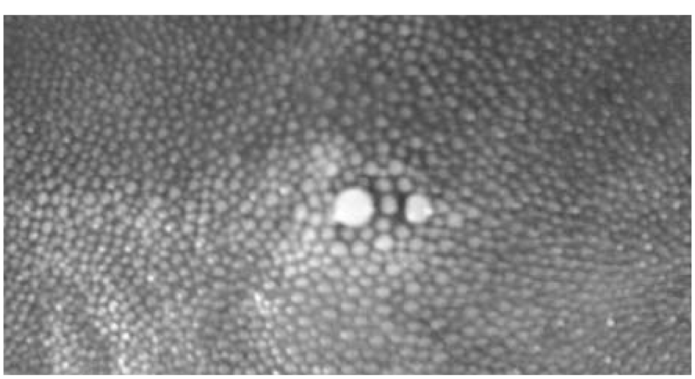


Figure 1. Stingray skin with denticles

Thus unlike other fishes it is possible to get skins of uniform size and thickness from stingrays and converting this byproduct into value added leather would help to strengthen the economy of country. But in India, conversion of stingray fish skin into leather is unfamiliar and most of the stingray skins are exported to foreign countries in the form of dried fish and are also wasted by the local vendors. A limited quantity of fish skins are converted into leather thus showing little or no interest in the tanning of fish skins. To create awareness and to highlight stingray skin processing, studies have been conducted to develop technologies for the preparation of leathers from stingray skins. In our earlier studies on stingray skins, a modified chrome tanning system has been developed for the preparation of soft leathers.[12] Further, a special footwear termed as massage footwear has been developed from stingray leathers where the stingray leather was used as insole/insock material to stimulate the reflex points present in the foot.[13] Apart from producing massage footwear from stingray leather, efforts have been made at CLRI to utilize the leather with unique grain structure for the preparation of outer covers for steering wheel, motor bike handle, tennis racket and cricket bat handles where the stingray leather imparts more grip to the user and these products could also be used to stimulate the reflex points present in our palms.[14] In the present investigation, a lime and sulphide free de-pigmentation and fiber opening method for the preparation of leathers from stingray skins has been developed. Sodium Lauryl Sulphate was used to remove the pigments adhered with the stingray skin, as well as to open up the hard fiber structure.

## Experimental

### Materials and Methods

Stingray fish skins were collected from local fish markets in Chennai. Hydrochloric acid was purchased from SD Fine chemicals, Chennai. Microbate was purchased from Tex Biosciences (P) Ltd, (earlier called Textan Chemicals (P) Ltd), Chennai. Sodium lauryl sulphate, Alcian blue were purchased from SRL chemicals, Chennai. The chrome tanning agent used in this study was prepared in Bioproducts pilot plant, CLRI, by a patented procedure.[15]

### Lime and Sulphide Free Tannage

The process followed for the conversion of stingray skins into leather without using lime and sulphide is given below.

*Raw Material*: Wet salted stingray skins. Soaking and Fleshing: The skins were soaked for 4 h with 300% water and then washed with 2 changes of fresh water. The skins were fleshed and soaked weight was noted.

*De-pigmentation and Fiber Opening*: Sodium Lauryl Sulphate 5% (percentage based on soaked weight); Water 50% (pH adjusted to 11.0); Run the drum for overnight (4-5 rpm). Next day, the skins were rubbed with gunny bag or brush to remove the pigments and fleshed using fleshing machine.

*Bating:* Water 100%; Microbate 2% 60 min; Fleshed and washed thoroughly.

*Pickling:* Water 80%; Salt 8% 10 min; Hydrochloric acid 1%(in 10% water) 3×10 min + 60 min. Leave over night. Next day add Hydrochloric acid 0.5% (in 5% water) 2×10'+30' pH 2.6-2.8.

*Chrome Tanning:* 50% pickle water; Chrome tanning agent 4%; Cationic fatliquor 1% 40 min; Chrome tanning agent 4%; Cationic fatliquor 1% 40 min; Water 50% 30 min; Sodium formate 1% (in 10% water) 30 min; Sodium bicarbonate 1% 3×10min + 60min (in 10% water); pH 3.8. Drain, rinsed and piled overnight. Next day, the tanned leathers were shaved and taken for further processing.

**FT-IR Analysis of SLS Treated Spent Liquor**

The SLS treated spent liquor was collected, filtered, and dried in a rotary evaporator. The dried material was subjected to FT-IR analysis using a Thermo Nicolet avatar 320 FTIR spectrometer (Nicolet Instrument Co., USA). The powder sample was mixed with KBr of spectroscopic grade and made in the form of pellets at pressure of about 1 MPa. The pellets were about 10 mm in diameter and 1 mm in thickness. The measurements were carried out in the mid-infrared range from 4000 to 400 $cm^{-1}$ after baseline correction and analyzed by OMNIC (Version 6.0) software supplied by Thermo Nicolet.

**Differential Scanning Calorimetric Studies (DSC)**

The thermal denaturation of tanned and raw stingray skin samples (wet samples) were studied using a DSC Q 200 differential scanning calorimeter (TA Instruments). The DSC method offers a much more objective and comprehensive way of evaluating the thermal shrinkage process of collagen.[16,17] 5–10 mg of samples collected from dorsal portion of the skin were sealed in aluminum pan and an empty pan was used as a reference. The heating rate 5°C per minute and temperature range between 0°C and 200°C in an $N_2$ atmosphere were maintained.[18]

**Histological Studies with von Kossa's Stain**

The von Kossa staining method was used to represent the amount of calcification.[19] This stain does not react with calcium but reacts with phosphate in the presence of acidic material.[20] The staining procedure originally derived by von Kossa[21] actually detects phosphate, it is being used routinely for the determination of calcification. Initially the raw stingray skin sample was cut and preserved in 10% formalin for 48 h. The fixed samples were dehydrated in a series of solutions of alcohol of different concentrations (50 to 100%) and then cleared in xylene. They were finally embedded in paraffin wax into moulds. The moulds were labeled and stored until use. Thin sections (10$\mu$m thick) were cut on a microtome, mounted on glass slides. Then the sections were treated with 5% silver nitrate solution in the dark at 37°C for 30 min and exposed to sunlight for 20 min and then washed well in distilled water. Sodium thiosulphate (2%) was added for 3 min and the sections were counterstained with Hematoxylin.

**Histological Studies with Van Gieson Stain**

The histological studies for the soaked, SLS treated and tanned stingray skin samples were carried out. After the completion of the above mentioned processes, the samples were dehydrated and the procedure adopted was same as outlined in the above experiment. The thin sections (10$\mu$m thick) mounted on glass slides were stained with Van Gieson.[22] Van Gieson's stain is a mixture of Picric Acid and Acid Fuchsin. It is the simplest method of differential staining of collagen and other connective tissue. It was introduced in 1889 by American Neuropsychiatrist and Pathologist Ira Van Gieson. To prepare this stain 100 mL of saturated aqueous solution of picric acid was added to 5 mL of 1% aqueous solution of acid fuchsin.

**Histological Studies with Alcian Blue**

To demonstrate the effect of SLS on glycosaminoglycans histological examination was carried out on the soaked and SLS treated stingray skins. Alcian blue has been recommended as a useful dye for the histochemical staining of acid mucopolysaccharides. The thin sections were stained with alcian blue at pH 1.0. The staining solution was prepared by dissolving 1gm of alcian blue 8GX in 90 ml of distilled water and 10 ml of 1N hydrochloric acid.[23]

**Post Tanning**

The shaved stingray leathers were neutralized to pH 5.5–6.0 before dyeing with 2% acid dye followed by fatliquor, retan, dry and buff. The process details are given in Table 1.

**Scanning Electron Microscope (SEM) Analysis of Stingray Leather**

To demonstrate the degree of fiber opening using SLS, scanning electron microscope analysis has been carried out on the soaked, SLS treated and tanned stingray skin samples.

**Physical Testing of Stingray Leather**

The samples for physical testing were cut from the tanned stingray crust leathers. The samples were conditioned at 20±2° C and 65±4% relative humidity for 48 h. The tensile strength, and tear strength were determined as per the IUP 6 and 8 methods respectively.[24,25] Lastometer test was carried out as per the method SLP 9.[26]

**TABLE I**
**Post tanning procedure for stingray leathers**
**Raw material: Shaved stingray leather**

| Process | Chemicals | % | Time | Remarks |
|---|---|---|---|---|
| **Neutralization** | Water | 100 | | |
| | Vernatan AKM (Neutralizing syntan) | 1.0 | 20 min | Check pH -5.5 -6.0 |
| | Sodium formate | 0.75 | 3×10+30min | Wash/drain |
| | Sodium bicarbanate | 0.75 | | |
| **Retanning Dyeing and Fatliquoring** | Water | 100 | | |
| | Sellasol PR (Protein retanning) | 4 | 30 min | |
| | Acid Dye 2% | 2 | 30 min | Check penetration |
| | Vernaminol ASN (synthetic fatliquor) | 3 | 15 min | |
| | Basyntan DI (Phenolic syntan) | 4 | | |
| | Basyntan FB 6 (Resin Syntan) | 4 | 40 min | |
| | Balmol SXE (synthetic fatliquor) | 3 | | |
| | Balmol BL II (semi synthetic) | 3 | | |
| | Lipsol LQ (synthetic fatliquor) | 3 | | |
| | Lipsol BSFR (synthetic fatliquor) | 3 | 60 min | |
| **Fixing** | Formic acid | 1.5 | 3×10+30min | |

Drain, wash and pile over night. Next day, hook to dry, stake, buff, and trim

## RESULTS AND DISCUSSION

### Effect of SLS on Stingray Skin

Sodium lauryl sulphate (SLS)—an anionic surfactant effectively removes the pigments adhered with stingray skin. SLS also plays an important role in the disruption of fiber bundles of stingray skin thereby extracting the proteoglycans as well as opening up the rigid fiber structure. This result is confirmed by FT-IR, histological analysis and scanning electron microscope studies. Removal of glycosaminoglycans and particularly proteoglycans is necessary to allow the fiber structure to split apart. Otherwise, the fiber structure may be cemented together during drying. SLS is safe and easy to use, non-toxic material and it is used in toothpastes, shampoos, and shaving foams. SLS has the ability to bind with glycoproteins and proteins[27] and is generally considered a potent denaturant.[28] The optimum amount of SLS employed in this process has precise action on stingray skin and it is obvious that it would not contribute much pollution load to the effluent. The photographic picture of soaked and SLS treated stingray skin is presented in Figure 2. After treatment with SLS, the pigmentation is removed completely and it is clearly presented in the picture.

### FT-IR Spectra of SLS Treated Spent Liquor

The FT-IR spectra of SLS treated spent liquor sample is presented in Figure 3 and it is the sum of the contributions mainly from the proteins and carbohydrates i.e proteoglycans. The absorption near at 1638.72 is the characteristic absorption band of amide I and it occurs in the range of 1700-1600 $cm^{-1}$. The peak at 1395.23 is the characteristic absorption band of cis-peptide bond. The absorption near at 1226.62 corresponds to amide III band occurs in the range of 1220-1300 $cm^{-1}$. The peak at 1094.94 corresponds to the absorptions of carbohydrate moieties occur in the range of 1100-1005 $cm^{-1}$. Thus from the FT-IR analysis it is confirmed that proteoglycans are extracted from stingray skins by the treatment of SLS.

### DSC Studies of Stingray Skin

The temperature of denaturation of raw stingray skin (dorsal portion) determined by DSC shows a value of 78°C and it is higher compared to other conventional animal skins used for leather making. This is because the dorsal portion of the stingray is made up of cartilaginous skeleton and it is composed of calcified cartilage. Cartilage tissue is a three dimensional network of collagen II fibers connected by a network of proteoglycan fibers composed mainly of

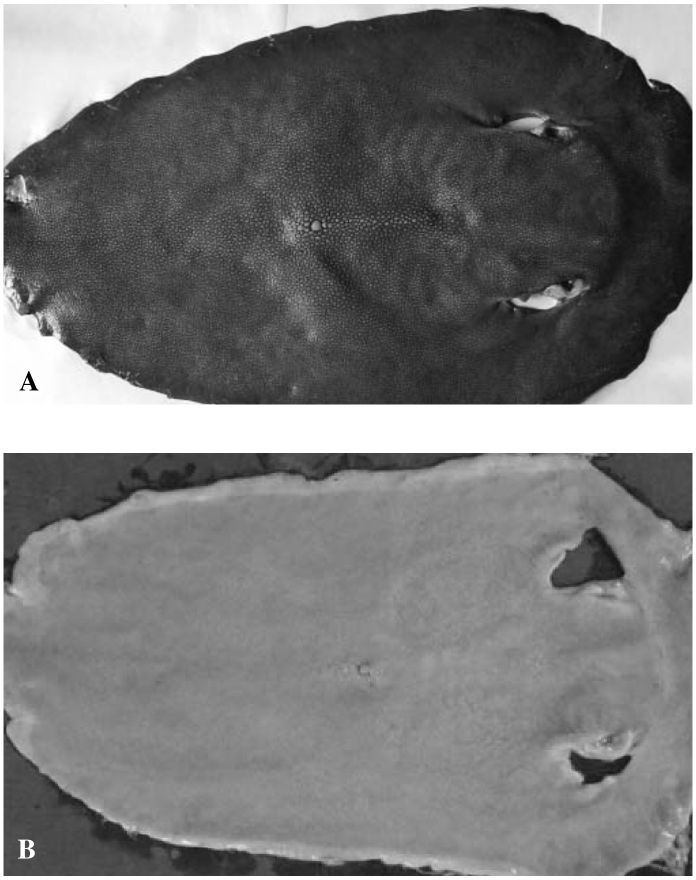



Figure 2. Photographic picture of Soaked (A) and SLS treated (B) stingray skin

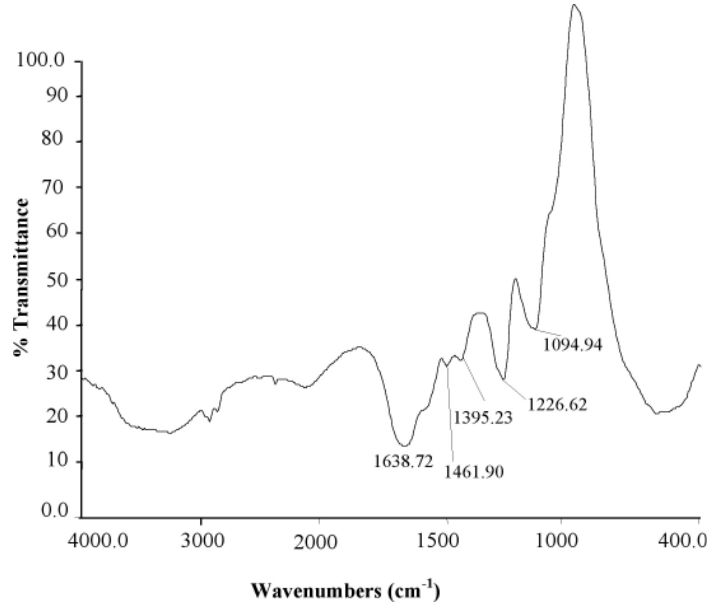



Figure 3. FT-IR spectra of SLS treated spent liquor

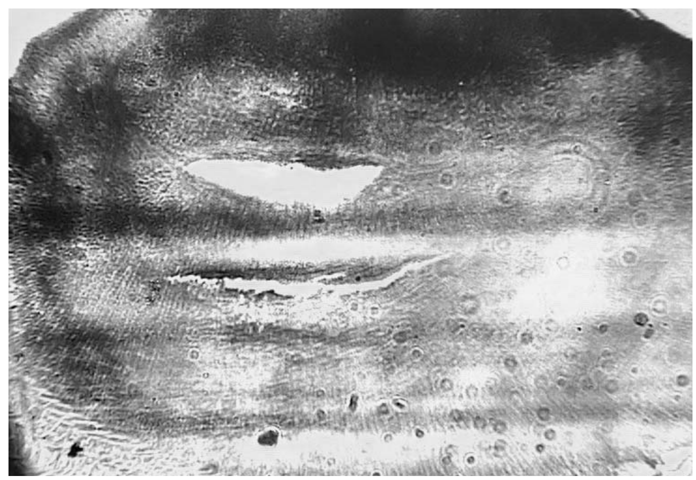


Figure 4. Optical microphotograph of raw stingray skin (dark precipitate indicates the presence of calcium phosphate

glycosaminoglycans.[29] During processing into leather, substantial amount of proteoglycans and calcium are removed from the skin but due to tanning the denaturation temperature of tanned leather goes up to 109°C. The amount of calcification and proteoglycan–collagen interactions determine the thermal stability of stingray skin.

**Demonstration of Calcification with von Kossa Stain**

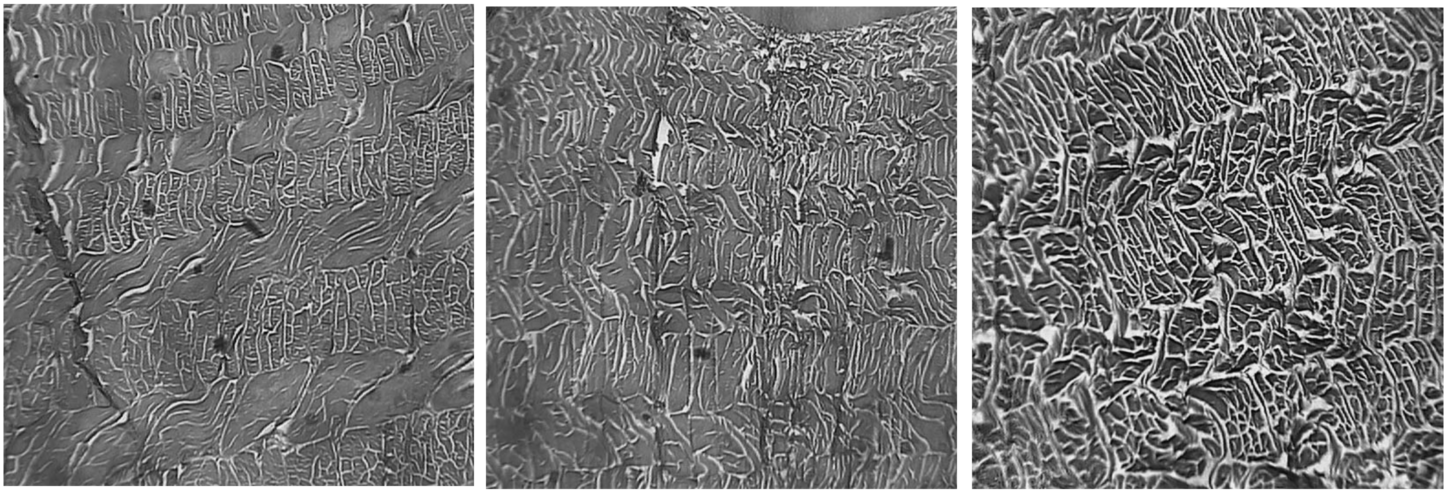
The optical microphotograph taken for the raw stingray skin stained with von Kossa is set out in Figure 4. The presence of dark precipitate in the figure confirms the calcification of raw stingray skin. This is due to specific reaction of von Kossa with phosphate ion. Although von Kossa stain is a measure of phosphate ion precipitation, this method has been used to represent the amount of calcification in samples.[30]

**Effect of SLS on Fiber Opening: Histological Studies with Vangieson**

The optical microphotographs taken for the soaked, SLS treated and tanned stingray skin are set out in Figure 5. The microphotographs reveal that SLS treatment opens up the rigid parallel fiber bundles of stingray skin. SLS has the ability to rupture the hard fiber structure by breaking the non covalent bonds. The optimum amount of SLS added during fiber opening splits the rigid fiber structure and makes the skin loosen. This opening up allows penetration of tanning and lubricating agents to produce leather with range of organoleptic properties required by the fish leather manufacturers and consumers. The opening up of fiber structure is sustained even after tanning, which is shown in the microphotograph tanned leather.

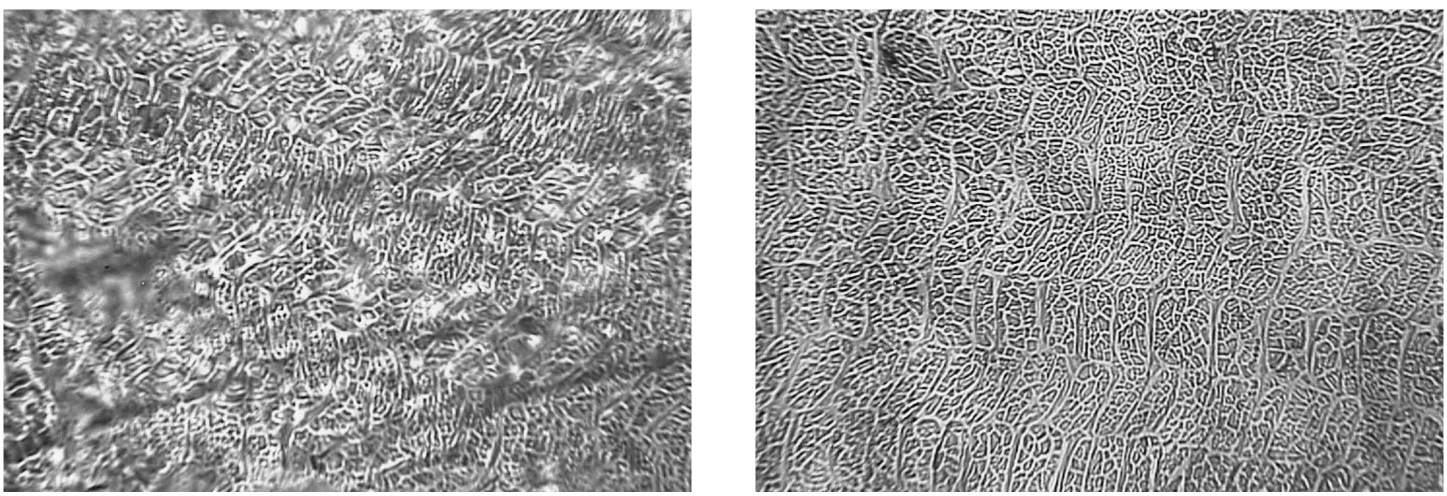
**Effect of SLS on Glycosaminoglycans: Histological Studies Stained with Alcian Blue**

The optical microphotographs taken for the soaked and SLS treated stingray skin stained with alcian blue are set out in Figure 6. Glycosaminoglycans are strongly anionic, unbranched long-chain polysaccharides containing aminated monosaccharide. The complex molecules formed by the attachment of glycosoaminoglycan chains to a protein core constitute the proteoglycans. Some of the significant constituents of connective tissue matrices of stingray skin are proteoglycans. Glycosaminoglycans and acidic glycoproteins are strongly anionic because of their high content of carboxyl and sulfate groups and they react strongly with alcian blue dye[31] and forms bluish green complex which is presented in the microphotograph of soaked stingray skin. After treatment with SLS, significant amount of proteoglycans are extracted from the skin and the alcian blue dye could not able to form



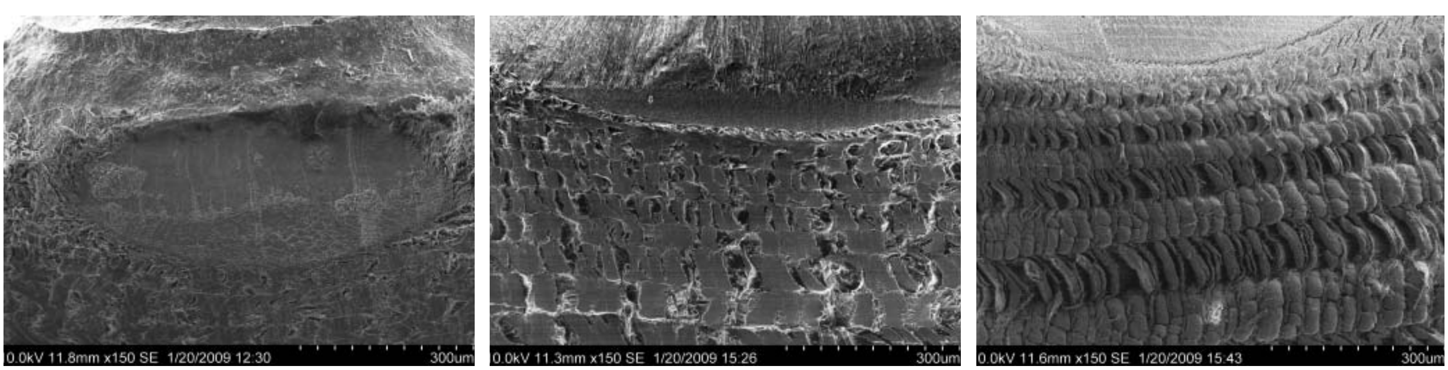
**A** **B** **C**

Figure 5. Optical microphotograph of soaked (A), SLS treated (B), and tanned (C) stingray skin



**A** **B**

Figure 6. Optical microphotograph of soaked (A) and SLS treated (B) stingray skin stained with alcian blue




**A** **B** **C**

Figure 7. Scanning electron microphotograph of soaked (A), SLS treated (B), and tanned (C) stingray skin at 150x magnification

**TABLE II**

**Strength properties of stingray leathers**

| Strength Properties | Value Obtained |
|---|---|
| Tensile Strength Kg/cm$^2$ | 282±8 |
| Elongation at break % | 34±2 |
| Tear Strength Kg/cm | 94± 6 |
| Grain crack load, Kg | 33± 2 |
| Distension at grain crack in mm | 5.1±0.2 |

complex with SLS treated skin. Removal of the non-collagenous proteins is necessary to produce soft leather.

### Effect of SLS on the Fiber Structure of Stingray Skin: SEM Studies

Scanning electron microphotographs of soaked, SLS treated and tanned stingray skin showing their cross-section at magnification of 150× are presented in Figure 7. The microphotographs confirm that SLS has the ability to open up the skin structure by disrupting the fiber bundles. From the SEM cross section of tanned leather it is observed that the fiber structure remains separated even after tanning result in soft leather.

### Physical Properties of Stingray Leather

The strength properties of SLS treated stingray crust leathers are given in Table 2. The stingray leathers possess high tensile and tear strength properties and this is due to the association of parallel collagen fiber bundles with calcified denticles.

## Conclusion

This study highlights the preparation of leathers from stingray skins and offers a novel route to the tanners to manufacture soft and pliable leathers. SLS could be effectively used for the removal of pigments adhered with stingray skin. FT-IR analysis of SLS treated spent liquor reveals that proteoglycans are extracted from stingray skin by SLS treatment. SEM analysis and histological analysis confirm that SLS could be effectively used for the fiber opening of stingray skins.

## Acknowledgment

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# PEABODY LEATHERWORKERS MUSEUM—CAN YOU HELP???

The Peabody Leatherworkers Museum opened in July 2009 and is located on Washington Street in Peabody, Massachusetts.

The museum is designed to tell the story concerning the 300 year history of tanning that at one time made Peabody the leather capital of the USA. Over 80 tanneries operated in the city at its peak but this has now been reduced to where only one tannery is left.

Several items and photographs have already been donated to the museum. However, it is hoped that more artifacts could be found and donated that would help to make the museum of even greater interest especially to young people who may have no idea about this unique history.

If you have any photos, documents or artifacts that you could donate and think would be appropriate, please contact the museum curator Merritt Kirkpatrick at MerrittKirkpatrick@gmail.com.