Can sodium silicates affect collagen structure during tanning? Insights from small angle X-ray scattering (SAXS) studies

Supplementary information

Yi Zhang,[a] Bridget Ingham,[b] Jérôme Leveneur,[c,†] Soshan Cheong,[d] Yin Yao,[d] David J. Clarke,[b] Geoff Holmes,[a] John Kennedy[c,†] and Sujay Prabakar*[a]

[a]Leather and Shoe Research Association of New Zealand, P.O. Box 8094, Palmerston North 4472, New Zealand.

[b]Callaghan Innovation, P.O. Box 31310, Lower Hutt 5040, New Zealand.

[c]National Isotope Centre, GNS Sciences, P.O. Box 31312, Lower Hutt 5040, New Zealand.

[d]Electron Microscope Unit, Mark Wainwright Analytical Centre, University of New South Wales, Sydney, NSW 2052, Australia.

[†]MacDiarmid Institute of Advanced Materials and Nanotechnology, Victoria University of Wellington, P.O. Box 600, Wellington 6140, New Zealand.

E-mail: sujay.prabakar@lasra.co.nz

Experimental details

Materials

Pickled lamb pelts (third grade) were obtained from Tomoana Pelt Processors Ltd in Whakatu, Hawkes Bay and transported to the New Zealand Leather & Shoe Research Association (LASRA), Palmerston North for processing in the pilot-tannery. This processing involved a sequence of mechanical and chemical operations using commercial equipment and reagents including sodium chloride, sodium bicarbonate, sodium formate, formic acid, sulphuric acid and sodium silicate (waterglass), which were used without further modification. Leather-specific reagents were obtained from their manufacturers, including Intrapol LTN (nonyl phenol ethoxylate) from the Shamrock Group Ltd; Chromosal® B (25% Cr_2O_3, 33% basicity) and Tanigan® PAK-N from Lanxess, Germany; Zoldine® ZE (oxazolidine E) from Angus Chemical Company, USA; Feliderm® DP liquid (disodium phthalate) and Tanicor® PW synthetic replacement re-tanning agent from Clariant, Germany; Clarotan Mimosa vegetable extract from Tanac, Brazil; Polyol AK sulphated natural oil from Smit & Zoon, Netherlands and Chromopol SG partially bisulfited natural oil from TFL, Germany.

Leather Processing

The skins were received at LASRA unpressed, and fleshed directly on the Rizzi SCA fleshing machine in the pilot tannery. A pre-tanning step followed, using a combination of Zoldine ZE and Intrapol LTN. The pH was raised to 7.5-8.0 to fix the oxazolidine and increase the hydrothermal shrinkage temperature to above 70°C. The increased thermal stability allowed the pelts to be subsequently washed at 42°C to increase the efficiency of degreasing. A final cold wash was used to reduce temperature prior to the main tanning step. The main tannage was carried out with either basic chromium sulphate (BCS), sodium silicate (So-Si) or a combination of the two (So-Si+BCS). Briefly, for chrome tanning the pelts were processed with BCS following the LASRA standard ThruBlu procedure[1] at an elevated pH and rising temperature, which allows the acidity of BCS to reduce the pH from 7.5-8.0 to around 4.0±0.2 at the end of an 12-hour run, without the need for additional basifying agent and with near complete exhaustion of the chrome bath. For the sodium silicate tanning process, the pelts were treated with sodium silicate for 1 hour at pH=12.0, after which gradual acidification over a period of 2 hours was used to reduce pH to 7.0, and promote fixation. The So-Si+BCS treated pelts were first processed using sodium silicate, and after acidification to pH=7.0 further processed with BCS and run over 12 hours to exhaustion of the bath. This step was followed by washing to remove excess salts and to adjust the temperature for the subsequent

neutralisation. After the main tanning steps, the pH of BCS and So-Si+BCS treated samples were brought up by mild basifying, followed by re-tanning, fatliquoring, fixing and the final drying.

Table S1 BCS processing steps

Step name	Chemicals/operations	Amount×	Temperature	Time
Pre-tanning	Water	50%	35°C	10min
	Sodium chloride	5%		
	Intrapol LTN	4%	35°C	20min
	Zoldine® ZE	2%		
	Sodium formate	1%	35°C	20min
	Sodium bicarbonate	1% × 2	35°C	20min×2
	Water	100%	42°C	60min
	Drain			
	Water	150%×4	42°C	20min×4
	Drain			
	Water	150%	20°C	20min
	Drain			
Main tanning	Water	100%	25°C	10min
	Feliderm® DP liquid	1%		
	Chromosal® B	4.50%	25°C	30min
	Increase temperature		40°C	12hr
	Drain			
	Water	100%	25°C	20min
	Drain			
Re-tanning	Water	100%		
	Tanigan® PAK-N	1%		
	Sodium formate	1%	35°C	60min
	Sodium bicarbonate	0.15%		
	Drain			
	Water	200%	35°C	15min
	Drain			
	Water	100%		
	Tanicor® PW	2%	35°C	45min
	Mimosa	3%		
Fatliquoring	Water	100%		
	Chromopol SG	1.50%	50°C	90min
	Polyol AK	1.50%		
Fixing	Water	10%		
	Formic acid 85%	0.50%	50°C	30min
	Drain			
	Water	200%	20°C	15min
	Drain			
	Naturally drying		20°C	3d
	Air drying		45°C	15min

×Amount is calculated as the percentages to the skin weight, same as followed.

Table S2 So-Si processing steps

Step name	Chemicals/operations	Amount	Temperature	Time
Pre-tanning	Water	50%	35°C	10min
	Sodium chloride	5%		
	Intrapol LTN	4%	35°C	20min
	Zoldine® ZE	2%		
	Sodium formate	1%	35°C	20min
	Sodium bicarbonate	1%×2	35°C	20min×2
	Water	100%	42°C	60min
	Drain			
	Water	150%×4	42°C	20min×4
	Drain			
	Water	150%	20°C	20min
	Drain			
Main tanning	Water	200%	20°C	60min
	Sodium silicate solution (Waterglass)	4%		
	Sulphuric acid 13%wt	1%×4	20°C	20min×4
	Keep running		20°C	60min
	Drain			
	Water	100%	25°C	20min
	Drain			
Re-tanning	Water	100%	35°C	45min
	Tanicor® PW	2%		
	Mimosa	3%		
Fatliquoring	Water	100%	50°C	90min
	Chromopol SG	1.50%		
	Polyol AK	1.50%		
Fixing	Water	10%	50°C	30min
	Formic acid 85%wt	0.50%		
	Drain			
	Water	200%	20°C	15min
	Drain			
	Naturally drying		20°C	3d
	Air drying		45°C	15min

Table S3 So-Si+BCS processing steps

Step name	Chemicals/operations	Amount	Temperature	Time
Pre-tanning	Water	50%	35°C	10min
	Sodium chloride	5%		
	Intrapol LTN	4%	35°C	20min
	Zoldine® ZE	2%		
	Sodium formate	1%	35°C	20min
	Sodium bicarbonate	1%×2	35°C	20min×2
	Water	100%	42°C	60min
	Drain			
	Water	150%×4	42°C	20min×4
	Drain			
	Water	150%	20°C	20min
	Drain			
Main tanning	Water	200%	20°C	60min
	Sodium silicate solution (Waterglass)	4%		
	Sulphuric acid 13%wt	1%×4	20°C	20min×4
	Keep running		20°C	60min
	Chromosal® B	4.50%	25°C	30min
	Increase temperature		40°C	12hr
	Drain			
	Water	100%	25°C	20min
	Drain			
Re-tanning	Water	100%	35°C	60min
	Tanigan® PAK-N	1%		
	Sodium formate	1%		
	Sodium bicarbonate	0.15%		
	Drain			
	Water	200%	35°C	15min
	Drain			
	Water	100%	35°C	45min
	Tanicor® PW	2%		
	Mimosa	3%		
Fatliquoring	Water	100%	50°C	90min
	Chromopol SG	1.50%		
	Polyol AK	1.50%		
Fixing	Water	10%	50°C	30min
	Formic acid 85%wt	0.50%		
	Drain			
	Water	200%	20°C	15min
	Drain			
	Naturally drying		20°C	3d
	Air drying		45°C	15min

Characterization Techniques:

Small-angle X-ray scattering (SAXS) experiments were performed at the SAXS/WAXS beamline at Australian Synchrotron. An X-ray energy of 12 keV and camera length of 3345.83cm was used. Data were collected using a Pilatus 1M detector with 1s exposures. Each sample was sandwiched between two pieces of Kapton™ tape to prevent drying, and mounted on an aluminium plate in a transmission geometry with the skin surface normal to the incident X-ray beam. Scans were recorded in 'gapless' mode by translating the detector. 10 such scans were performed for each sample, averaged, and radially integrated using the beamline software Scatterbrain.

SAXS data were fitted to a combined population and fibre d-spacing model implemented in a Java program developed in-house. The overall equation being fitted comprised three terms: $I(q) = I_{pop}(q) + I_{peaks}(q) + I_{bkg}(q)$ where each term is defined as follows:

The population scattering $I_{pop}(q) = c\int n(r)[f(qr)V(r)]^2 dr$

where $n(r)$ is a log-normal size distribution, $f(qr) = 3\frac{\sin(qr) - qr\cos(qr)}{(qr)^3}$ is the form factor for a sphere, and $V(r) = \frac{4}{3}\pi r^3$ is the volume of a sphere.

The diffraction peaks are modelled as Gaussians, $I_{peaks}(q) = \sum_i \frac{A_i}{w(q)\sqrt{\pi/2}} \exp\left(\frac{-2\left(q - \frac{2\pi i}{d}\right)^2}{w(q)^2}\right)$

where A_i is the area of peak i, d is the d-spacing (in Å), and $w(q)$ is the width, expressed as $w(q) = a + bq$ where a and b are fitted parameters.

Finally, the background term $I_{bkg}(q) = mq^{-p} + n$ is an empirical power-law plus constant function.

Differential scanning calorimetry (DSC) measurements for wet samples were carried out on DSC Q2000 (TA Instruments). Lyophilized samples were rehydrated with DI water in aluminium pans overnight, followed by running at 5°C/min from 20°C to 120°C under a N_2 purge. The measurements for dry samples were conducted on Q600 SDT (TA Instruments) using a N_2 atmosphere (flow rate of 100 mL/min), by heating samples from 20°C to 700°C at a rate of 10°C/min. Denaturation temperature in this study referred to the onset temperature of the endothermal peak analysed by TA universal analysis software (TA Instruments).

Atomic force microscopy (AFM) measurements were carried out on a Nanosurf FlexAFM system mounted on a Nanosurf Isostage active vibration cancellation stand. Samples of 10 µm thick were obtained using Leica CM1860 UV Cryostat and transferred onto glass slides. The skin sections were frozen, rinsed and naturally dried several days prior to the measurements. Prepared this way, the samples adhered to the glass without need for adhesive or mechanical support. However, due to the rinsing step during sample preparation it is likely that larger precipitates are removed in this step. The samples were imaged in tapping mode and phase contrast with PointProbePlus silicon cantilevers (PPP-NCH with 7 nm nominal tip radius, 132 µm cantilever length, ~30 N/m elastic constant, and resonance frequency ~250 kHz). Images were taken with using the Nanosurf C3000 acquisition software (v 3.7) with a scan rate of 1.5 s/line, 1024 pixels resolution and 5 µm scan size. Forward (left to right) and backward (right to left) topography, deflection amplitude and phase images were recorded. The images were then analysed using SPIP™ 6.6.2 (Image Metrology) and ImageJ [2] in order to retrieve the periodicity related to the collagen structure.[3] Three Fast-Fourier-Transform (FFT) analyses were performed. FFT of 20 cross sections of 20 singles fibrils was obtained for each image. 2D FFT was performed full picture as well as selected fibrils and compared to 2D-FFT of individual fibrils. Observed variations with the different FFT techniques were consistent with what has been presented in the literature.[3] However, with the number of fibrils and areas analysed, the variations observed with the three techniques were not significant against the distribution of the periodicity.

FTIR measurements were performed using iD5 ATR on Nicolet™ iS5 Spectrometer (ThermoFisher Scientific) in an attenuated total reflectance (ATR) mode. Dry leather samples were directly loaded and measured. The number

of scanning and resolution were set at 16 and 4, respectively. The data were analysed at a frequency interval of 500 to 4000 cm^{-1} using a crystal zinc selenide.

For scanning electron microscopy (SEM) studies, cross-sections from rectangular strips of leather crust were made by cutting from the grain surface to the flesh using a stainless steel blade and mounted on 45˚/90˚ SEM pin stubs such that the cross sections are facing the electron beam. The specimens were sputter-coated with ~40 nm platinum and imaged using an FEI Nova NanoSEM 450 FE-SEM operating at 5 kV with a spot size of 3 and a working distance of 5 mm.

Additional Figures:

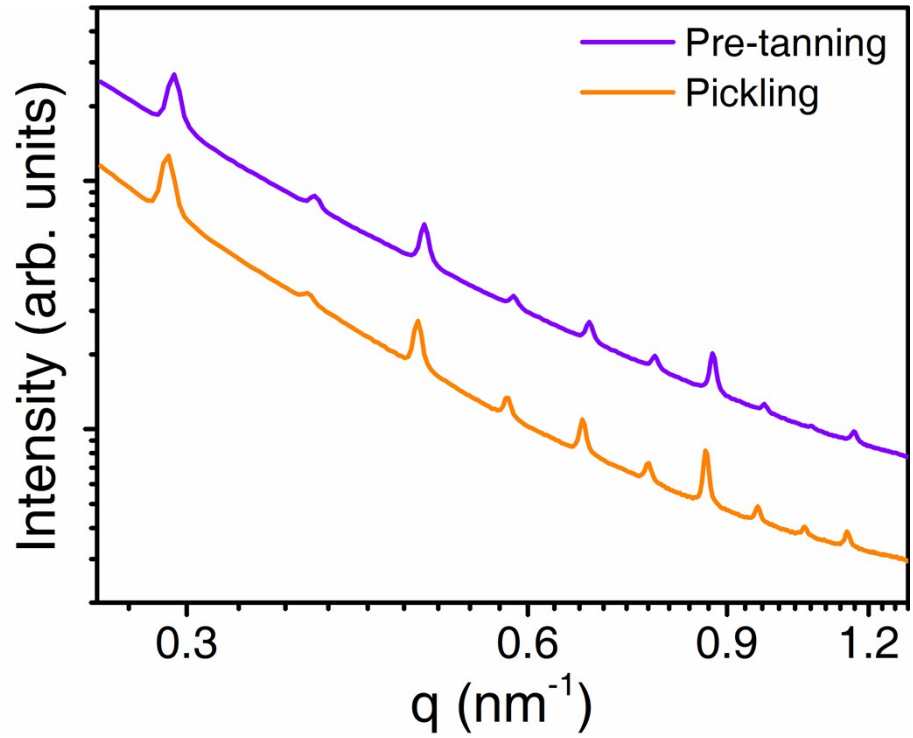

Figure S1: SAXS patterns for pickling stage and pre-tanning stage

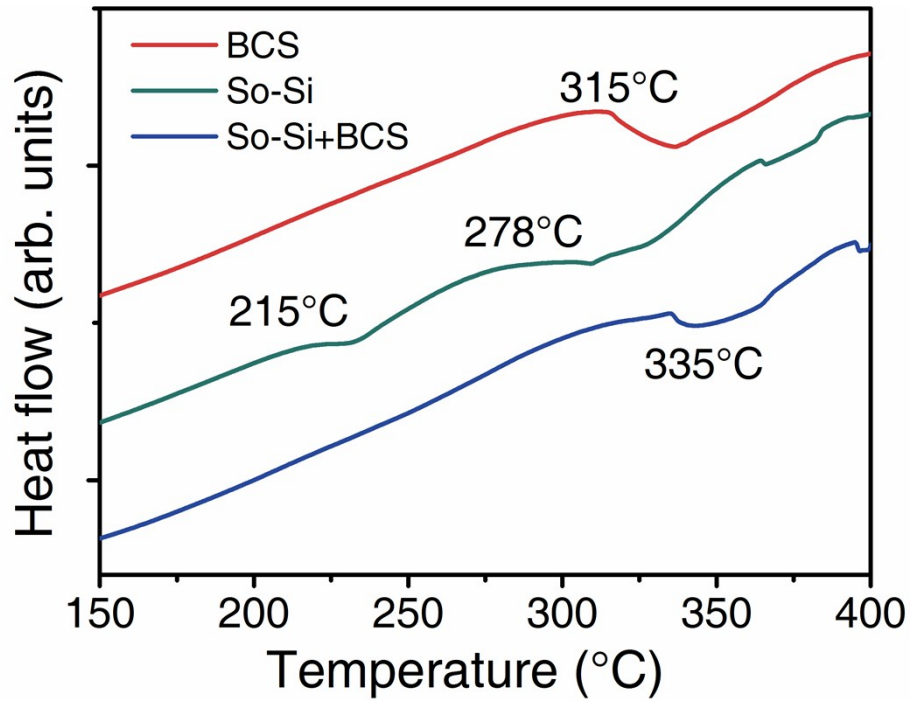

Figure S2: DSC plot of dry leather samples. Temperatures indicate the of denaturation of the samples in the dry state.

REFERENCES:

1. S. DasGupta, *Journal of the Society of Leather Technologists and Chemists*, 1997, **82**, 15-21.
2. C. A. Schneider, W. S. Rasband and K. W. Eliceiri, *Nat methods*, 2012, **9**, 671-675.
3. B. Erickson, M. Fang, J. M. Wallace, B. G. Orr, C. M. Les and M. M. Banaszak Holl, *Biotechnology journal*, 2013, **8**, 117-126.