



The influence of syntans on the assembly of collagen

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Since the introduction of the first syntan one hundred years ago by BASF, the role of syntans in the tanning process has changed significantly. While syntans were initially used as dispersers and accelerators for vegetable tannins, they were applied as sole tanning agents later, and with the wide acceptance of chromium or glutaraldehyde tanning, they are now mainly used in the re-tanning process.

However, increasing technical requirements of leather quality could be achieved through modification of their chemistry. In order to quantify the advantages of new syntans, the search for new methods for their evaluation continues.

To meet these needs a method to evaluate tanning agents is required to measure their influence on the kinetics within the assembly of collagen. In addition to that, to gain insight into the change of the fibrils via atomic force microscopy, after they have assembled in the presence of a tanning agent.

The investigation

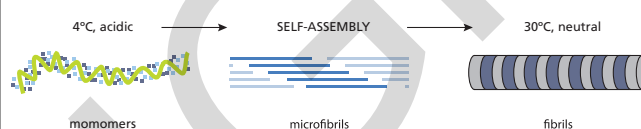
The *in vitro* self-assembly of calfskin collagen from monomers to fibrils was studied using UV/Vis spectroscopy. Fibrillation in an acidic monomer solution is initiated by increasing the temperature to 30°C and the pH to neutral conditions. The schematic build-up of fibrils from monomers via microfibrils is shown in *Panel 1*.

The self-assembly process can be monitored via kinetic spectra taken by UV/Vis spectroscopy. This unspecific size effect is measurable at wavelengths between 200nm and 400nm. Monomers do not scatter light at 340nm whereas the fibrils do, and the delayed slope or lag time proves that there is a transition state between monomers and fibrils, as shown in *Panel 2*.

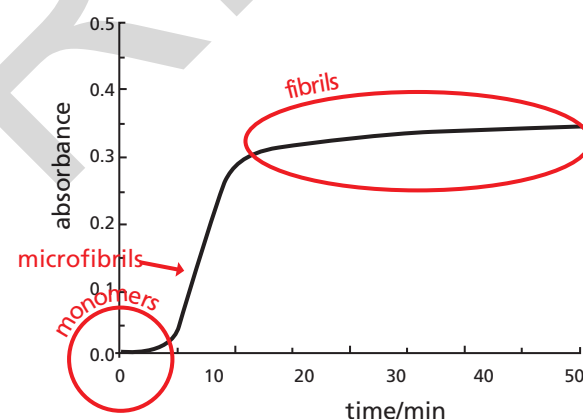
It can be concluded that changes to the spectroscopic measurements of turbidity may provide insight to the interaction between an additive, like a tanning agent, and collagen at different hierarchical levels. Accordingly, changes in the lag times and in the height of the turbidity plateaus are detected as a function of the amount and type of additive. These findings have been further investigated by means of a simple model that assumes microfibrils are an essential intermediate.

Changes in the morphology of the assembled fibrils were studied by atomic force microscopy (AFM) as complementary information. The AFM pictures were acquired with a Nanoscope IIIa from Digital Instruments in the tapping mode with a cantilever tip (Binnig 1985). The resolution chosen for the pictures was between 256 x 256 and 512 x 512 pixels (Westra 1994).

Panel 1: Representation of the self-assembly of collagen



Panel 2: Time dependence of the assembly of collagen from monomers to fibrils



Polycondensates based on formaldehyde and phenol (phenolic syntans) as well as polycondensates based on formaldehyde and dihydroxy diphenyl sulfone (sulfone syntans) are widely used in the tanning processes (Reich 1956). In order to compare these two generations of syntans, polycondensates of a comparable molecular size had to be synthesized, since different molecular weights – even within the same chemistry – can lead to different shrinkage temperatures and leather properties (Ammenn 2015). In order to measure the molecular weight of the different syntans, gel permeation chromatography (GPC) was applied, which is a kind of size exclusion chromatography (SEC) that separates analytes on the basis of size. For characterisation, the average molecular weight was used (Lathe 1956). Calibration was achieved with polyacrylates of defined molecular sizes.

The sole tanning was carried out with bovine pelts in a procedure published by Lollar and Tu (Lollar 1950). A two hour fixation in a separate float generally increased the shrinkage temperature by 1° to 2°C as recommended in the stated article.



Panel 3. Structure of syntans, results of GPC analysis, and results of sole tanning

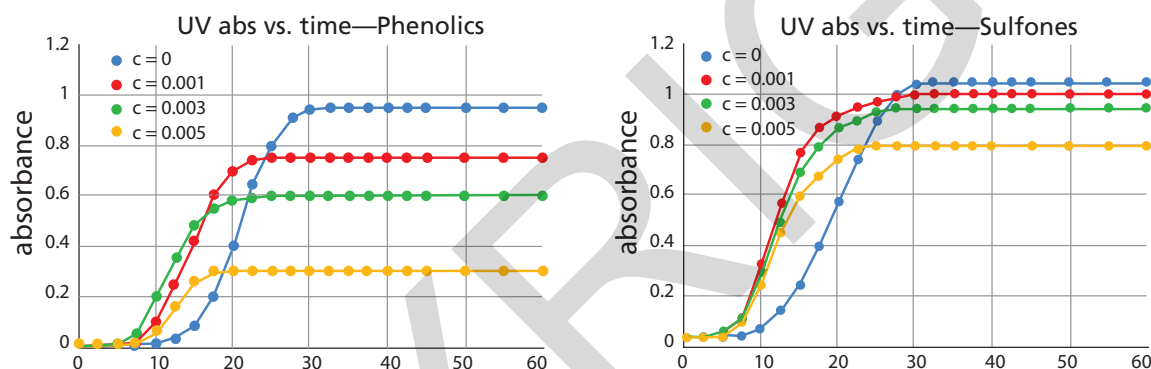
	Phenolic Syntan	Sulfone Syntan
Structure		
Average molecular weight	3.200	3.400
Shrinkage temperature after sole tanning	67°C	70°C
Leather quality after sole tanning	Soft and full	Softer and fuller than leather made with phenolic syntan

influence in the assembly of collagen, both syntans accelerate the assembly of collagen monomers to fibrils with increasing concentrations (0.001 – 0.005 mol) in a monitored timeframe of 60 minutes, as depicted in *Panel 4*.

With reference to *Panel 4*, the standard curve for collagen assembly is shown in blue. The shorter lag time caused by both syntans can be observed, which means that the assembly of the collagen is accelerated with both syntans to a comparable degree. A notable difference is that the level of absorbance decreases more drastically with increasing concentrations of the phenolic syntan.

As can be seen in *Panel 5*, no significant differences were found in the AFM analysis of assembly experiments with the two syntans. In the presence of both syntans, the fibrils assembled in a similar manner, with a marginally finer pattern

Panel 4: Time dependence of UV/Vis monitored assembly of collagen from monomers to fibrils, in the presence of a phenolic syntan and a sulfone syntan. Concentration is given in μmol .



Findings and discussion

A polycondensate based on formaldehyde and phenol (phenolic syntan) and a polycondensate based on formaldehyde and dihydroxy diphenyl sulfone (sulfone syntan) were synthesised. Their molecular weight was comparable, as revealed by results using gel permeation chromatography, depicted in *Panel 3*. After application in sole tanning, the shrinkage temperature of leather obtained with the sulfone syntan was three degrees higher and the quality of the leather was improved in aspects of softness and fullness, when compared to the phenolic syntan.

Based on these results, the sulfone syntan is ranked as the better syntan with higher tanning capacity. Concerning their

than fibrils that have assembled without syntans.

The results that were found for these two syntans were significantly different to experiments that were carried out with two polymers and presented during the IULTCS conference in 2011 (Ammenn 2011). Those assembly studies were carried out in the presence of a polyacrylate (based on the polymerisation of acrylic acid) and a polymethacrylate (based on the polymerisation of methacrylic acid) in concentrations between 0.1 and 1 μmol as shown in the graphs presented in *Panel 6*.

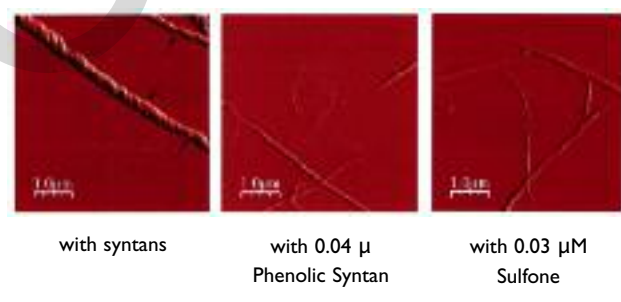
With reference to *Panel 6*, the standard curve for collagen assembly is shown in blue. The longer lag times caused by both polyacrylate and polymethacrylate can be observed, and this effect is indicated by the red arrows. When adding the polymethacrylate, this time lag effect is much more pronounced. Another observation is the fact that the plateau of absorbance increases marginally with increasing concentration, as indicated by the blue arrows in *Panel 6*. This could be caused by a scattering effect.

Unlike the syntans that accelerate the assembly of collagen, the two polymers decreased the speed of assembly of collagen, with the polymethacrylate doing this in a very pronounced way. It should be noted, however, that the concentration of both polymers was significantly higher than the concentration within the syntan trials.

The fibrils assembled in the presence of a polyacrylate were analysed via AFM. The result is presented in *Panel 7*.

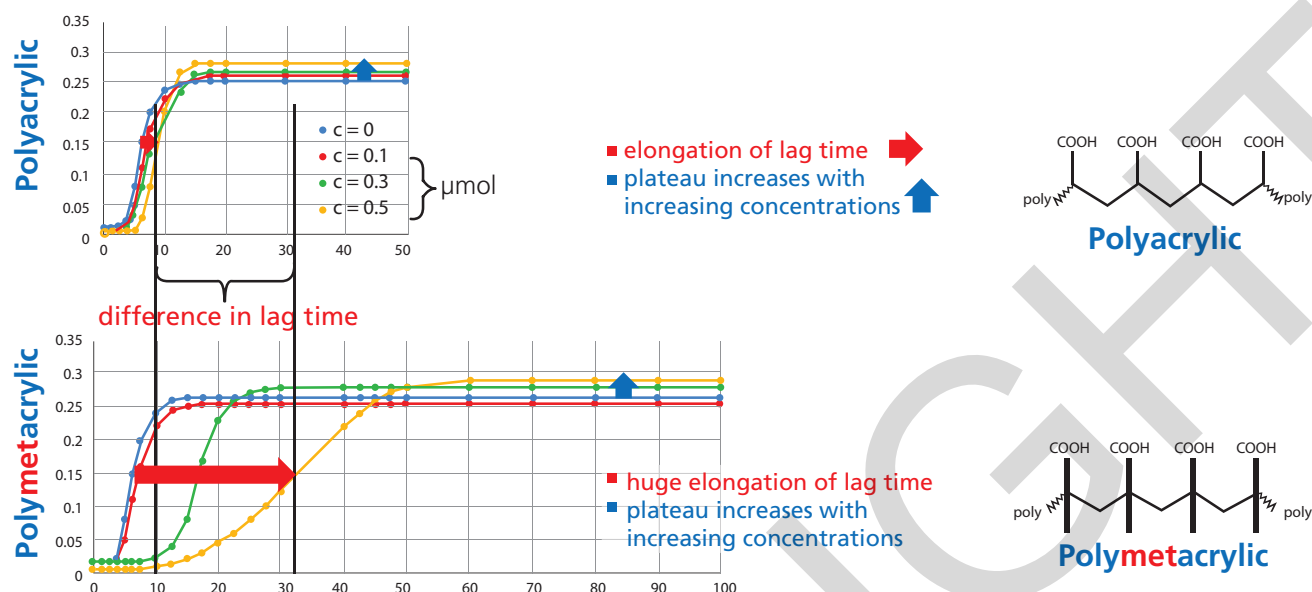
With reference to *Panel 7*, the fibrils shown in the left image were assembled under standard conditions. The fibrils

Panel 5: AFM images of collagen fibrils assembled without and with syntans

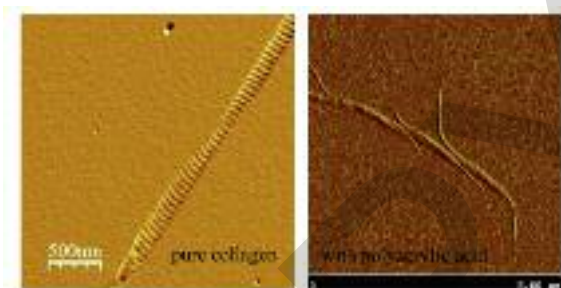




Panel 6: Time dependence of UV/Vis monitored assembly of collagen from monomers to fibrils in the presence of a polyacrylate (polyacrylic) and polymetacrylate (polymetacrylic)



Panel 7: AFM images of collagen fibrils assembled without and with 1 μmol polyacrylate



shown in the right image were formed in the presence of 1 mol polyacrylate, and show tails branching off the fibril.

Polymethacrylates and polyacrylates acid only differ by one methyl side chain in their chemical structure, but this influences their effect on the speed of the assembly of collagen. The polymethacrylate has a large impact on the kinetics, whereas the use of polyacrylate leads to considerable changes in the morphology of the assembled fibrils.

Conclusion

A method was established to monitor the assembly of collagen to fibrils with UV/Vis and analysed the resulting morphology via AFM. With the two syntans of different chemistry, a decrease of time lag was found which implied a faster assembly for both syntans, but with no differences in atomic force microscopy.

With the two polymers of different chemistry, we found an increase in lag time, especially with the polymethacrylate, which also meant a slower assembly. Significant differences were observed in AFM with collagen assembled in the presence of a polyacrylate compared to collagen assembled without additive.

Acknowledgements

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