Studies towards the Influence of Syntans on the Assembly of Collagen

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Abstract

The tanning capacity of tanning agents is generally quantified on the basis of the shrinkage temperatures of pelts that are treated with these tanning agents. With differential scanning calorimetry, denaturing temperatures of collagen powder treated with a tanning agent are obtained. Both methods, however, lack qualitatively insights into the changed structure of collagen after the crosslinking. To achieve both objectives, we developed a method in which the interaction between a tanning agent and collagen is analyzed during and after the assembly of collagen to fibrils. Fibrillation in an acidic monomer solution is initiated by increasing the temperature and pH in the presence and absence of tanning agents. Data is created quantitatively by UV/Vis spectroscopy in the kinetic monitoring of the assembly of collagen and qualitatively, by microscopic analysis of the resulting fibrils by means of atomic force microscopy. A sulfone based syntan is compared with a phenolic based syntan to validate the use of this new method.

Keywords: Assembly of collagen, syntan, shrinkage temperature, polymer.

1 - Introduction

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. 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.

We wanted to develop a method to evaluate tanning agents by measuring their influence on the kinetics within the assembly of collagen. In addition to that, we wanted to gain insight into the change of the fibrils via atomic force microscopy, after they have assembled in the presence of a tanning agent.

2 – Material and Methods

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 figure 1

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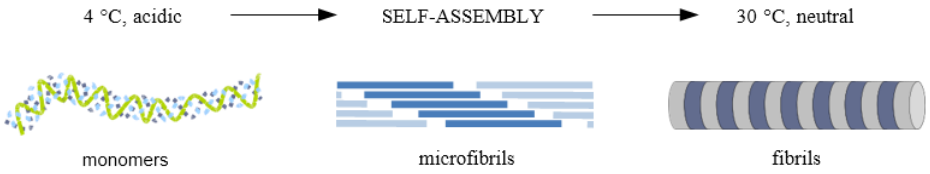


Figure 1: Representation of the self-assembly of collagen

The self-assembly process can be monitored via kinetic spectra taken by UV/Vis spectroscopy. This unspecific size effect is measurable at wavelengths between 200 nm and 400 nm. Monomers do not scatter light at 340 nm 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 figure 2:

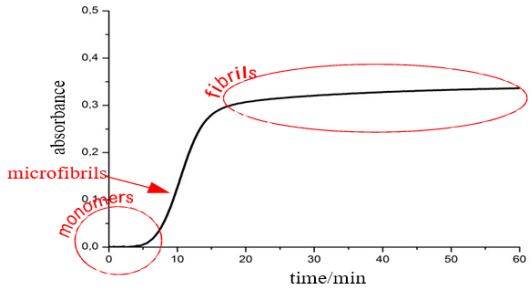


Figure 2. Time dependence of the assembly of collagen from monomers to fibrils

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 (Binning 1985). The resolution chosen for the pictures was between 256 x 256 and 512 x 512 pixels (Westra 1994).

Polycondensates based on formaldehyde and phenol (phenolic syntans) as well as polycondensates based on formaldehyde and dihydroxy diphenyl sulfone (sulfone synatns) 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 characterization, 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 pelt 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 degrees Celsius as recommended in the stated article.

3 – Results and Discussion

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

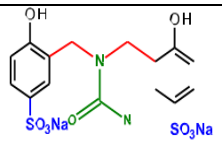
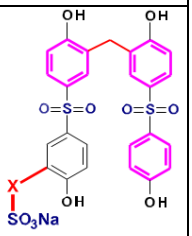
	Phenolic Syntan	Sulfone Syntan
Structure		
Average molecular	3.200	3.400
Shrinkage temperature after sole tanning	67°C	70°C
Leather quality after sole tanning	Soft and full	Softer and fuller than leather made with phenolic

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

Based on these results, the sulfone syntan is ranked as the better syntan with higher tanning capacity. Concerning their influence in the assembly of collagen, both syntans accelerate the assembly of collagen monomers to fibrils with increasing concentrations (0,001 – timeframe of 60 minutes, as depicted in figure 4.

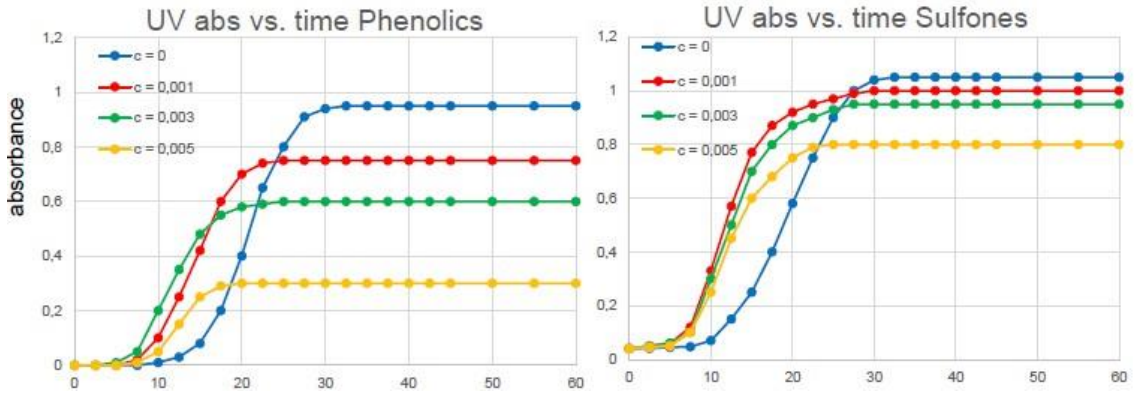


Figure 4. Time dependence of UV/Vis monitored assembly of collagen from monomers to fibrils, in the presence of a phenolic syntan and a sulfone syntan. Concentration is given in µmol.

With reference to figure 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 figure 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 than fibrils that have assembled without syntans.

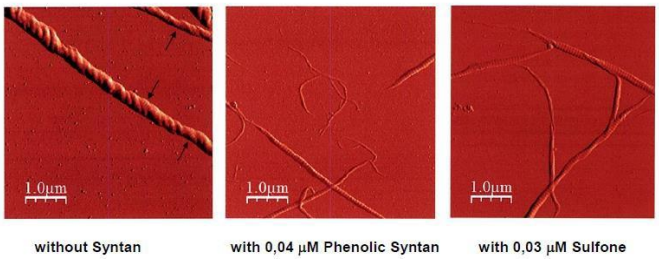


Figure 5: AFM images of collagen fibrils assembled without and with syntans

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 polymerization of acrylic acid) and a polymetacrylate (based on the polymerization of metacrylic acid) in concentrations between 0,1 and 1 µmol as shown in the graphs presented in figure 6.

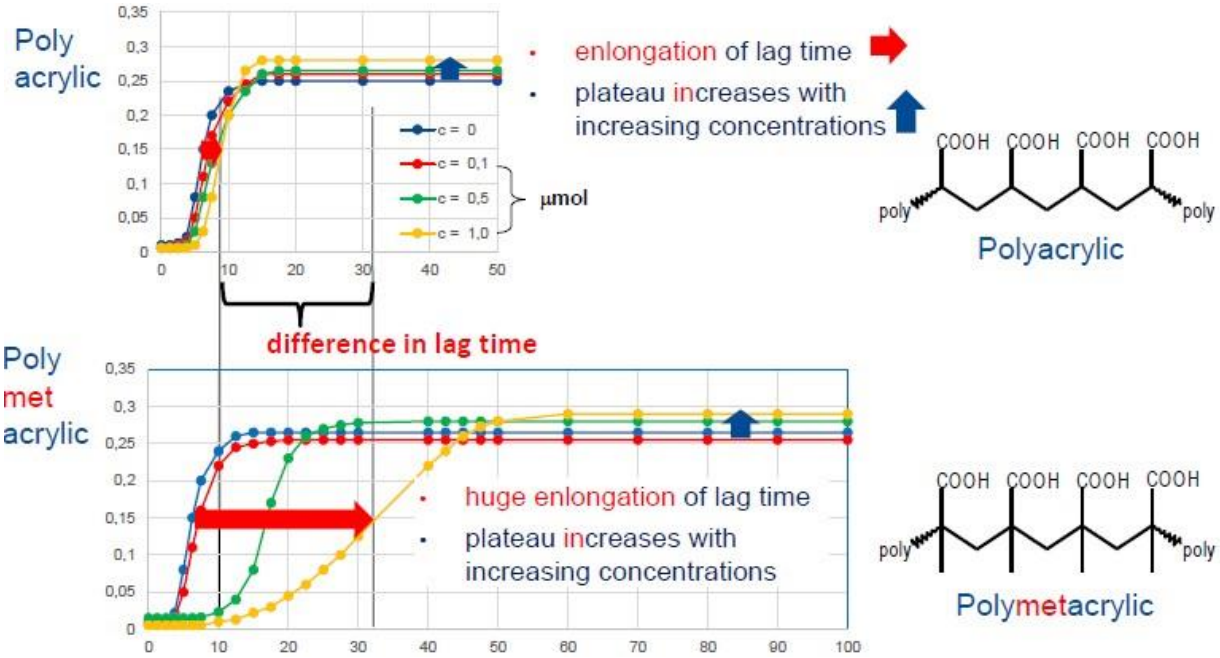


Figure 6: Time dependence of UV/Vis monitored assembly of collagen from monomers to fibrils in the presence of a polyacrylate (polyacrylic) and polymetacrylate (polymetacrylic)

With reference to figure 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 figure 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 polymetacrylate 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 analyzed via AFM. The result is presented in figure 7.

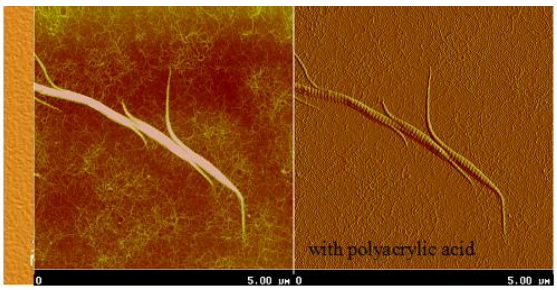


Figure 7: AFM images of collagen fibrils assembled without and with 1 µmol polyacrylate.

With reference to figure 7, the fibrils shown in the left image were assembled under standard 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.

3 – Conclusion

We established a method to monitor the assembly of collagen to fibrils with UV/Vis and analyzed the resulting morphology via AFM. With the two syntans of different chemistry, we found a decrease of lag time

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 polymethacylate, 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.

4 – Acknowledgements

This work was a joined effort between BASF and the group of Michael Mertig at the Technical University of Dresden. Doreen Naumburger had established the method. Nora Haufe conducted the experiments with the polymers, while Franziska Franke carried out the experiments with the syntans. Sole tanning experiments were done in the laboratories for the development of BASF Leather Chemicals in Ludwigshafen by Corinna Hübsch, Emil Schilling, and Bernd Dannheim. Valuable discussion partners for directing this research effort were Volker Bach from BASF and Günter Reich, former director of the FILK Institute in Freiberg, Germany.

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