

DESTABILIZATION OF COLLAGEN IN HIDE AND LEATHER BY ANIONIC SURFACTANTS. I. DIFFERENTIAL SCANNING CALORIMETRY OF COMPLEXES WITH SULFATES*

by

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ABSTRACT

It is shown that surfactants in sulfated fatliquor oils destabilize collagen in leather, in untanned fibers, and in solution. The melting point of the collagen measured by differential scanning calorimetry was lowered by 11°C, to 85°C, by fatliquor applied to chrome-tanned crust leather in a conventional process; the lowering was as much as 26°C, to 72°C, when more surfactant was used. The results explain why leather cannot be dried at temperatures within 50°C of the shrinkage temperature as usually determined, and might also explain some cases of shrinkage after drying and of stress-cracking.

INTRODUCTION

The surfactants in most anionic fatliquors chemically resemble the detergent sodium dodecyl sulfate (SDS), which is used to denature collagen and most other proteins in the biochemistry laboratory (Fig. 1). It works on warmed collagen fibers by unraveling them and so causing them to shrink. If this happens to the collagen fibers in leather, even to a small degree, it might explain many of the properties of the products, including grain cracking and the shrinkage we see after leather has been toggled and dried.

There seems to be a lack of information about how fatliquoring relates to collagen stability, so we examined the products of the reactions of different forms of collagen with

various anionic surfactants using a scanning calorimeter. In a parallel study, to be published, we used an isothermal calorimeter to monitor the reactions.

MATERIALS AND METHODS

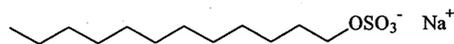
Soluble collagen was enzyme-digested bovine dermal collagen obtained in 1 mM hydrochloric acid at a concentration of 3 mg/ml (Vitrogen-100, Collagen Corp., Palo Alto, CA) (95-98% type I; rest, type III).

Surfactants from anionic fatliquors: The surfactants were extracted from whole fatliquors by partition between water and ether.¹ The equivalent concentration of surfactant was determined by a two-phase titration against the quaternary ammonium salt diisobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride (Hyamine 1622, Rohm and Haas, Philadelphia, PA).²

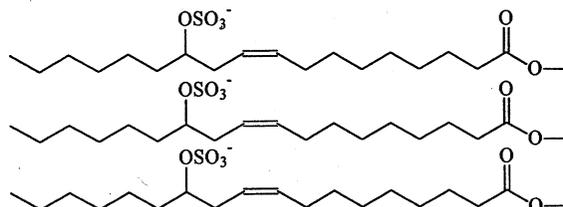
Sodium dodecyl sulfate-collagen complex: Five milligrams of dissolved collagen were titrated into 0.67 mg SDS (66% dodecyl, 26% myristyl, 6% cetyl, Sigma Chemical Corp., St. Louis, MO), using cessation of precipitation as the end-point. The reaction, in 7 ml citrate buffer, pH = 4.5, was continued at room temperature overnight. The product was centrifuged and washed with deionized water.

Synthetic sulfated oil-collagen complex: Two milliliters of 3 mg/ml collagen solution were diluted with 4 ml of 0.05 M citrate buffer and adjusted to pH = 5.3. Seventy microliters of the 142 mM synthetic sulfated oil solution (extracted from Chemol-57, ChemTan Co., Exeter, NH)

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sodium dodecyl sulfate



sulfated glyceryl triricinoleate (sulfated castor oil)

FIGURE 1. — Chemical formulas of sulfated anionic surfactants.

were titrated in, until precipitation stopped. This suspension was degassed and used for microcalorimetry.

Complex of sulfated castor oil with fibrous hide collagen: Ten milliliters of 0.2 M sulfated castor oil (extracted as described above from Hydrolene C-75 sulfated castor oil, Reilly-Whiteman, Inc. Conshohocken, PA) were added to 0.2 g comminuted collagen³ in 0.05 M citrate at pH = 5.3. The slurry was tumbled for three hours at room temperature, then filtered and washed with water. The control sample was prepared the same way, without the surfactant.

Complex of sulfated castor oil with chrome-tanned collagen: Dry blue leather prepared by the standardized ERRC process,⁴ containing neither retan nor fatliquor, was comminuted with a Wiley mill. The same preparative procedure was used as for fibrous hide collagen described above. The control sample was prepared the same way, without the surfactant. The content of surfactant of the product, determined on three samples by means of a detailed mass balance, was $52.4 \pm 2.0\%$.

A different leather was prepared from the same wet blue by adding fatliquor according to the manufacturer's recommendations — a conventional crust leather with fatliquor, but no retan. The fatliquor was a solvent oil containing 2.0% organic sulfate (Product X76-31, Reilly-Whiteman, Inc. Conshohocken, PA). After tanning, the 2.5-kg hide quarter (head end) was washed, covered with water at 50°C containing 250 g fatliquor, drummed for 1.25 hours, and hot-air dried.

Differential scanning calorimetry: The suspensions made from soluble collagen were scanned in a MC-2 microcalorimeter (Microcal Inc., Northampton, MA) at 30°C/hour. The solid preparations were scanned in hermetically sealed capsules in a DSC-7 calorimeter (Perkin-Elmer, Stamford, CT) at 5°C/min. Although the different scanning rates affect the apparent melting temperatures, the control samples were always scanned at the same rate as the samples, and the changes due to the surfactants were observed with the melting points of the controls as references.

Shrinkage temperature: Unloaded strips of leather were heated at 2°C/min in water, and the temperature for 10% shrinkage was recorded.

RESULTS

Sodium dodecyl sulfate and collagen in solution: A portion of the solid product, containing 1.7 mg collagen, was placed in the solid-state cell of the microcalorimeter with 0.1 ml water and scanned at 30°C/hour from 25°C to 60°C (Fig. 2, curve a). There was no thermal activity — therefore the collagen in the complex had already been denatured by the SDS at room temperature.

Sulfated synthetic oil and collagen in solution: The calorimeter scan of the reaction product of collagen with the sulfated synthetic oil at pH = 5.3 is shown in Fig. 2, curve b. Collagen in solution at this pH has an endotherm at 39.5°C. Solid collagen, for example in fresh bovine hide,

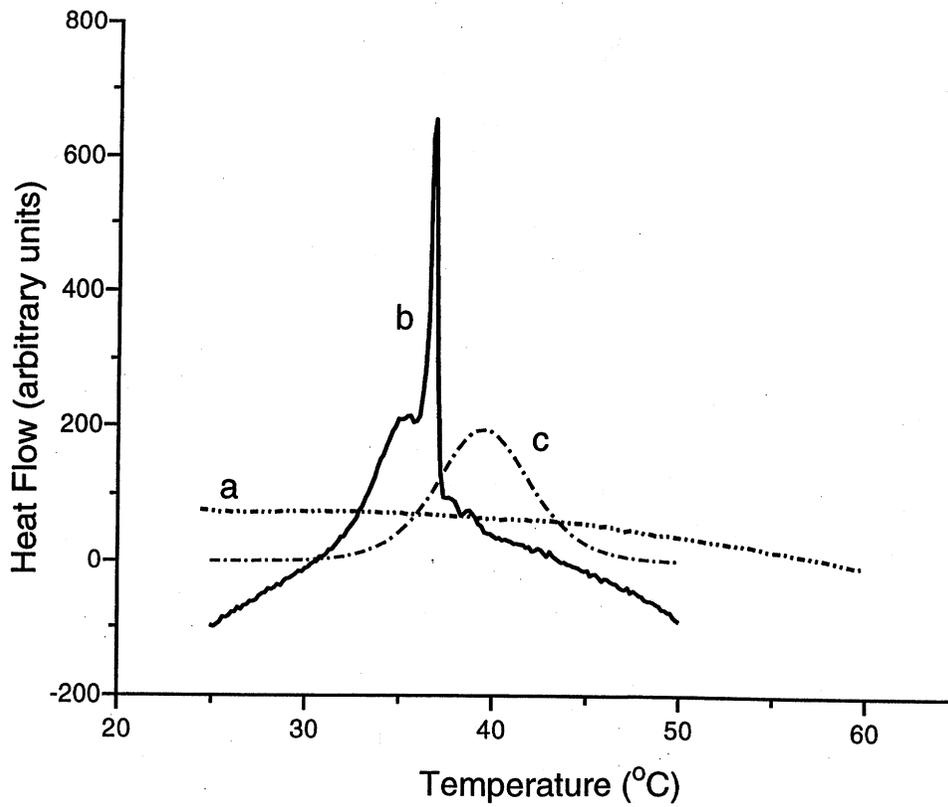


FIGURE 2. — Melting endotherms of soluble collagen in solid complexes with organic sulfates. (a) sodium dodecyl sulfate; (b) synthetic fatliquor extract; (c) uncomplexed collagen in solution. Curve (a) was not corrected for instrument baseline.

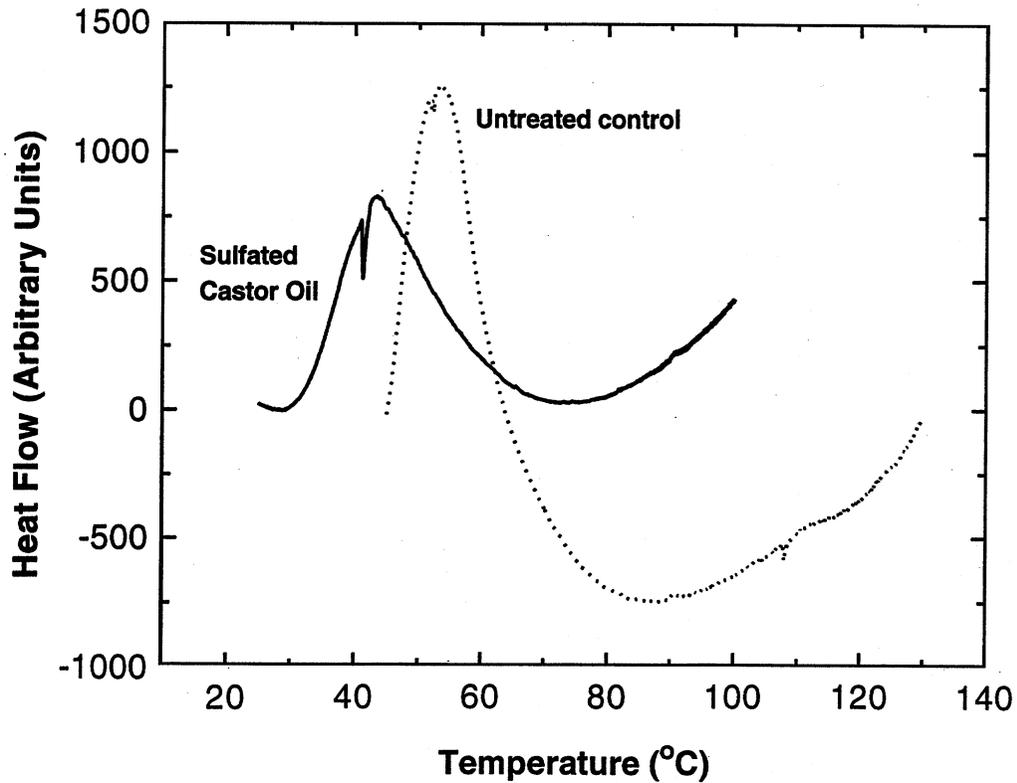


FIGURE 3. — Effect of sulfated castor oil on melting endotherm of fibrous collagen.

melts at a much higher temperature, about 60°C; fibrils reconstituted from solution, at 46°C. As seen in Fig. 2, curve b, the collagen in the complex melts at 35°C, 4.5°C lower than that free in solution. There is a reproducible endothermic spike superposed on the wider peak, which is probably due to a denaturation cascade under the influence of the surfactant. Therefore the collagen at pH = 5 is not denatured by the sulfated oil, but is destabilized by it.

Sulfated castor oil and solid fibrous collagen: The endotherms of comminuted solid collagen prepared from limed bovine hide, with or without sulfated castor oil, are compared in Fig. 3. The untreated collagen melted at 53.3°C; the treated collagen, $9.7 \pm 0.2^\circ\text{C}$ lower. This shift is double that displayed by the complex formed in solution with the synthetic oil, 4.5°C, and again shows the destabilizing effect of fatliquor surfactant, this time on solid collagen fibers.

Sulfated castor oil and solid collagen crosslinked with sulfated chromic oxide (chrome-tanned): The effect of the surfactant on chrome-tanned hide is shown in Fig. 4. Before the treatment with fatliquor surfactant, the crosslinked collagen melts near the temperature of boiling water, at 98°C; in the presence of the surfactant, 25°C lower, at 72°C. Incipient melting appears as low as 64°C. The small endothermic peaks in the scan of the treated leather, near the endotherm of the untreated, are not accurately reproducible. These could be due to the remnants of untreated collagen in non-uniform samples, or to some reaction product formed between the surfactant and the sulfated chromium oxide.

The leather treated conventionally with fatliquor was found to have a shrinkage temperature of 85°C, compared with 96°C for the untreated wet blue. This coincides with the low-temperature shoulder on the DSC endotherm in Fig. 5. Clearly, conventional fatliquor also destabilizes the collagen in leather. In this case, however, the product is not uniform, since there are at least two melting populations indicated by the data in Fig. 5. When the less stable population melts, the sample shrinks as a whole, even though the main endotherm is at higher temperature. Even this high-temperature endotherm occurs at lower temperature than that of the leather without fatliquor.

DISCUSSION

A number of steps are used in which anionic surfactants are introduced into hide being made into leather, including soaking, fatliquoring, and impregnation.⁵ This study,

concerned with a particular class of surfactant, sulfated oil used in fatliquors, showed that all of these steps could destabilize the collagen of the hide or the final leather, and that fatliquor definitely does so, at pH's to which leather is finally neutralized.

The data in Fig. 5, supplemented by the shrinkage measurement, shows that the destabilization is not merely due to unusually high amounts of fatliquor. This material contains only the usual amount, about 10% oil, of which only a portion is sulfated; yet it shrinks at 85°C. "Fatliquoring" destabilizes leather, reversing the advantage of chrome tanning. This shrinkage temperature is easily achieved with organic tans. In fact, the high-fatliquor containing leather described in Fig. 4 begins to melt at 64°C, not much above the melting temperature of collagen in raw hide.

Such destabilization, which must mean that the structure of the fibrils is disrupted, occurs throughout the leather, since whole endothermic peaks were shifted downward in temperature. The strength of leather, both wet and dry, depends on the integrity of the fibrils. Therefore, our observations imply that leather with anionic fatliquor has less than optimal strength. The effect on the collagen would be expected to show itself as grain cracking during lasting, slow delayed shrinking after leather is dried, and generally weaker leather. Further, the lower shrinkage temperature would affect the appearance of leather that has undergone plating and hot gluing. A contemporary study⁶ compares the denaturation of collagen by various detergents, including a homologous series of aliphatic sulfates. At pH = 7.2, the melting point of collagen in solution was lowered as much as 12.7°C by 4 mM SDS. We can say that the melting-point lowering that we observed at pH = 4.5 was greater than that, since all the collagen, ordinarily melting at 39°C, was denatured at 25°C, a lowering of 14°C. It is not surprising that the denaturing effect of SDS increases at lower pH; the important observation is the effect of fatliquor surfactant at the pH of leather.

Nandi et al.⁶ also found that the denaturing effect of the sulfate series increased with chain length n for $n \leq 12$, but neither they nor we could examine higher pure homologues, because of their insolubility. The SDS that we used for most of our experiments was contaminated with 28% of higher homologues (q.v. Material and Methods), which do dissolve in mixtures. Experiments with pure SDS gave results similar to those of the mixture, however. Fatliquors are based on mixtures of sulfated oils with higher molecular weights than that of SDS or the contaminants (Fig. 1), so should be even more effective denaturants than they.

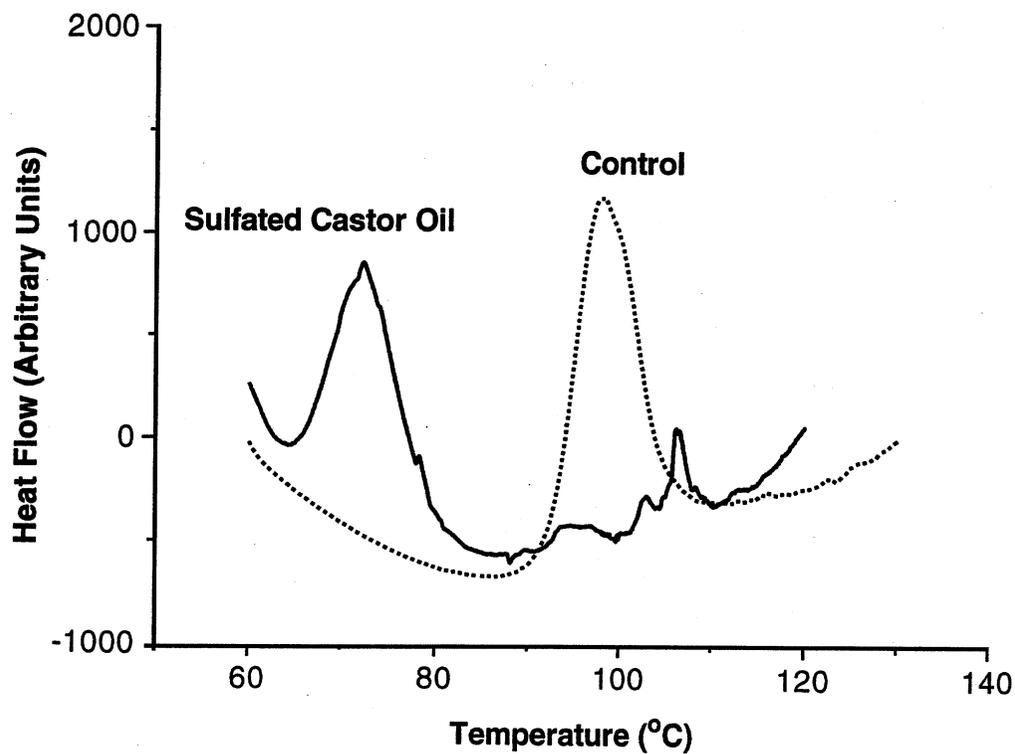


FIGURE 4. — Effect of sulfated castor oil on melting endotherm of collagen in chrome-tanned leather. Surfactant content of treated leather is 52.4%.

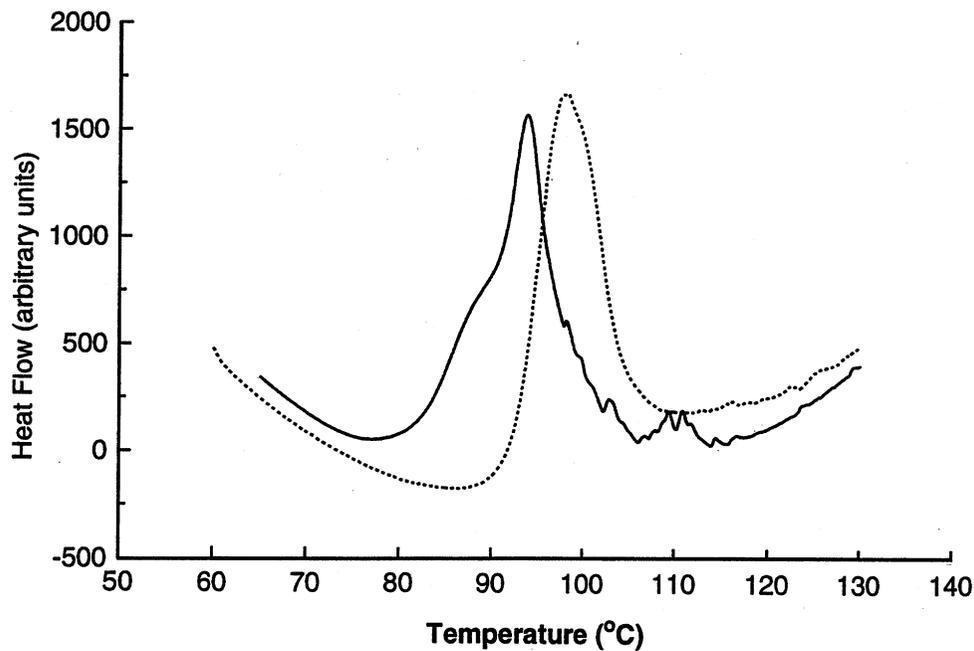


FIGURE 5. — Effect of solvent fatliquor (10% offer) on melting endotherm of collagen in chrome-tanned leather. Solid line: treated; dotted line: no fatliquor.

The compositions of the complexes of collagen with the organic sulfates in our work was determined by titration, using the precipitation of the coacervate as the end point. The SDS complex formed from soluble collagen contained 0.134 g SDS/g protein, lower by an order of magnitude than the ratio determined for a series of globular proteins by Tanford by means of optical and viscometric measurements.⁷ The difference is probably due to the low concentration of SDS that we used, below the critical micelle concentration (cmc), and peculiarities of the fibrous macromolecule. Precipitation of positively charged protein molecules with low concentrations of SDS, below cmc, with redissolution at higher concentration, has been described.⁸

The composition of the complex of collagen with the synthetic sulfated oil was 497 mol sulfate/mol collagen. Since the end-point that was used to prepare the complexes, the cessation of precipitation, was not sharply defined, it is not certain that the collagen was saturated with surfactant. A more definitive titration using isothermal calorimetry will be described in paper II of this series.

We conclude that fatliquoring significantly lowers the stability of collagen in both hide and in tanned leather. This effect has not been reported before. It indicates an important reason why high molecular weight fatliquors and retans, which are restricted from diffusing into collagen fibrils, are superior to and should eventually displace conventional anionic fatliquors from the leathermaking process.

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REFERENCES

1. Hart, R.; *Ind. and Eng. Chem.* **9**, 177-181, 1937.
2. Longman, G. F.; **The Analysis of Detergents and Detergent Products**, John Wiley and Sons, New York, 1978.
3. Whitmore, R., Jones, H., Windus, W., and Naghski, J.; *J. Food Sci.* **37**, 302-305, 1972.
4. Taylor, M. M., Diefendorf, E. J., Hannigan, M. V., Artymyshyn, B., Phillips, J. G., Fearheller, S. H., and Bailey, D. G.; *JALCA* **81**, 43-61, 1986.
5. Kronick, P. L.; in **Bailey's Industrial Oil and Fat Products**, Vol. 5, 5th Edition, Y. H. Hui, ed., pp. 309-316, New York, 1996.
6. Nandi, P. K., Grant, M. E. and Robinson, D. R.; *Int. J. Peptide Prot. Res.* **25**, 206-212, 1985.
7. Reynolds, R. and Tanford, C.; *J. Biol. Chem.* **245**, 5161-5165, 1970.
8. Bigelow, C. C. and Sonenberg, M.; *Biochemistry* **1**, 197-204, 1962.