United States Patent Office 3,055,790 Patented Sept. 25, 1962

3,055,790 HYDROXYLAMINE TREATMENT OF COLLAGEN FIBERS

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No Drawing. Filed Oct. 31, 1960, Ser. No. 65,911
8 Claims. (Cl. 162—2)

This invention relates to the disaggregation and delamination of collagenous structures by hydroxylamine, and to novel chemically modified and highly reactive fibers of collagen.

Collagen is the primary intercellular protein constituent of the white connective tissue in animal tissues. Skin, tendons, reticular tissue, bones and various other animal structures contain interwoven networks of collagen fibers which are highly organized in the native structures. Hydrolysis and other drastic chemical treatments normally melt or destroy the organized structure without releasing fibers in an intact form. Each fiber is ordinarily locked within the woven structure and tenaciously adheres to contiguous fibers.

Chemical modification, or tanning, of collagen to produce leather is traditionally performed on intact animal skins. During fabrication of leather goods, scrap leather resulting from the fabrication, plus defective skin areas which must be discarded, represents economic loss to the fabricator. It is therefore highly desirable to have available homogeneous collagenous raw material which may be reassembled into desirable structures without formation of waste material and which utilizes leather scrap. Because cost of fabrication is further increased by variations in thickness and shape of leather pieces, it is desirable to have available a method for reassembling collagen into skin-like structures of uniform thickness and shape.

Previously, attempts have been made to prepare collagen structures, such as sheets, from reconstituted collagen. Heretofore the most promising method of preparing reconstituted collagen involves dissolving the procollagen fractions of collagen and re-precipitating these dissolved fractions in the present of undissolved fibers to give the resulting mass strength and cohesiveness. Difficulties have been encountered in attempting to obtain strong and intact undissolved fibers. Previously, native collagen has been mechanically beaten to break fibers from the organized collagen structure. This mechanical beating results in cutting of the fibers, the shorter fibers in turn result in formation of lower strength reaggregated material.

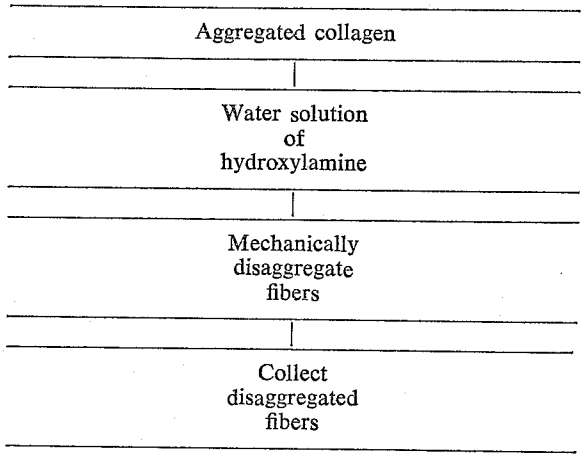
We have invented a method for delaminating, or slipping, and chemically modifying fibers in collagen aggregates in a manner whereby the individual fibers may be slipped from the network of the collagen structure without disintegration or degradation of the fibers to yield fibers having the basic physical properties of collagen, but the fibers being chemically modified to render them highly reactive.

An important object of this invention is to provide chemically modified fibers of collagen which are susceptible to reaggregation into desired skin-like structures. A second object is to provide a process for disaggregating aggregated collagen structures to thus prepare a homogeneous supply of collagenous fiber stock suitable for reaggregation. A further object is to provide collagen structures having the basic physical properties of native collagen but which are chemically modified so as to be highly susceptible to inner reaction with various chemical reagents.

Our method generally involves the reacting and conditioning of aggregated collagen with hydroxylamine solution, mechanically manipulating the conditioned collagen,

and collecting disaggregated and chemically modified fibers.

A simplified flow diagram of the process is as follows:



Suitable starting materials which we may use include collagen from various sources that has been exposed to various treatments. For example, traditional sources of leather stock such as calfskin, cattle hides, pig skins, etc., which have or have not been limed and/or enzymatically conditioned and the like may be used. Moreover we may use ordinarily less useful sources of collagen, such as tendons, bones, reticular tissue and detanned leather scrap as sources of our fibers.

Hydroxylamine, NH_2OH, interacts with the individual fibers of a collagen aggregate to so chemically modify them so that they are no longer capable of adhering to one another as they normally do, as a consequence of which the fibers slip from the structure without being markedly physically affected during the process.

We prefer to use a water solution of hydroxylamine having a concentration of about 20 to 80 percent hydroxylamine by weight in water. Collagen may be immersed in the solution of selected strength for varying lengths of time and at temperatures up to about 60° C. to give the desired conditioning effect. Hydroxylamine is somewhat unstable in solution, making it desirable to prepare the solution at the time of use rather than far in advance of the conditioning. Varying concentrations of the hydroxylamine solutions correspondingly varies the amount of chemical modification which the fibers undergo. At room temperatures a 20 percent water solution of NH_2OH which is allowed to contact the collagen for about 10 hours so modifies the fibers that about 10 moles of hydrazides are introduced per 10^5 gms. of collagen and about 10 moles of amides are removed from a like quantity of collagen. Increasing the hydroxylamine concentrations to about 70 percent by weight under like conditions causes about 24 moles of hydroxamates to be introduced into the fibers per 10^5 gms. of collagen, about 15 moles of amides to be removed, and about 12 moles of arginine to be converted to ornithine.

Depending on reaction conditions, the fibers of our invention consist of collagen fibers having the general physical properties of collagen and essentially consisting of collagen fibers containing about 10 to 46 moles of hydroxamates per 10^5 gms. of collagen, 10 to 24 moles of amides per 10^5 gms. of collagen, less than that contained in native collagen, and having 1 to 24 moles of arginine converted to ornithine.

Although we may use a temperature of from about 0 to 60° C., we prefer for convenience to use room temperatures. Lower temperatures usually require somewhat longer conditioning times and these lower temperatures are of no particular advantage. Higher tempera-

tures may shorten conditioning time, but careful control should be exercised to prevent excessive solubilization of collagen. Solubilized collagen is not readily susceptible to reaggregation and lower yields of fibers are therefore produced if solubilization occurs.

A conditioning time of about 10 hours is ordinarily preferred at room temperatures, although very high concentrations will ordinarily reduce the preferred conditioning time interval. Times widely varying from the preferred 10 hour time interval are satisfactory. Higher temperatures than room temperature will normally reduce required times by about 50 percent, keeping in mind that solubilization is optimally kept to a minimum. Optimal conditioning provides collagen which disaggregates easily during mechanical manipulation with no or only a small percentage of fibers being dissolved or transferred to a gel-like state. Ideal mechanical manipulation squeezes out the slipped or delaminated fibers from the aggregated collagen structure without appreciable occurrence of shearing. Although several types of mechanical means may be employed, a Hollander type paper pulp beater gives excellent results. We prefer mechanical means which do not cause excessive shearing of the fibers. Undue shearing causes formation of higher percentages of short length fibers which upon reaggregation may yield lower strength skin-like aggregates. The pulp beater causes the fiber slurry to be pushed over a bedplate by a rotating reel which crushes the weakened aggregate with little shearing.

Following mechanical manipulation, action of the hydroxylamine solution may be advantageously stopped. One method of stopping the action of the conditioning reagent involves the washing of the fibers with copious quantities of water to cause removal of substantial quantities of the reagent. Other suitable methods of neutralizing and removing the hydroxylamine may be used.

The wet fibers may be collected by any of several methods such as gravity drainage or mechanical separation such as centrifugation. The separated fibers may then be further dried, processed or stored for later use. If the fibers are to be held for long periods of time before further processing, they should be stabilized by well-known methods to inhibit the growth of microorganisms although fibers obtained from hydroxylamine disaggregation have more resistance to action of microorganisms. Following collection, we prefer to further dehydrate and slightly pre-tan the fibers. Contacting the wet fibers with a diluted solution of formaldehyde is one satisfactory method of pretanning. Various solvents such as ethers, acetone and alcohols may be used to solvent dehydrate the wet fibers. The collected fibers of our invention are susceptible to many uses. They retain the basic physical characteristics of native collagen, exhibiting the typical cross-striations having an axial periodicity of from about 600 to 640 A., but are rendered highly reactive by the chemical modification they undergo during hydroxylamine conditioning. They react readily with reagents used in various leather making process steps such as dyeing, tanning, fat liquoring and finishing. Subjecting them to the action of various cross-linking agents such as aliphatic and aromatic aldehydes causes reaggregation of the fibers, this method being used to prepare skin-like collagen structures of desired shape and size.

The detailed examples given below will serve to further illustrate various specific embodiments of our invention.

Example I

Defatted green hide pieces were placed into a room temperature water bath to which sufficient hydroxylamine had been added to obtain a 50 percent by weight solution. The hide was submerged into the hydroxylamine solution and vigorously mixed for six hours. The pieces were then removed from the bath, dehaired by mechanical means and replaced in the bath. Conditioning was continued for an additional eight hours without agitation.

After completion of conditioning, the hide was removed and washed with tap water to remove the bulk of the hydroxylamine. The conditioned hides were forced by an auger pump through progressively smaller sized screens through a tin mesh screen. The resulting fibrous slurry was then vacuum filtered through cloth with running water for about 30 minutes. After completion of washing, the fibers were finally washed with a 0.3 percent formaldehyde solution. The washed and treated fibers were pressed in a frame at 25 pounds' pressure for about 5 minutes. Pressure was released and the fibers sprayed with acetone. After 5 minutes additional pressing at 25 pounds' pressure, the resulting sheet was removed from the press.

Example II

Tannery hide scraps were solvent dehydrated with acetone and dried in a hot air stream to remove the solvent. The dried scraps were placed in a 70 percent hydroxylamine solution at room temperature for 8 hours. The hydroxylamine solution, after eight hours containing most of the dissolved hair, was drained and the dehaired hide pieces were transferred to another container which contained 40 percent hydroxylamine solution. The hide scraps were further conditioned for about 8 hours at room temperature. Washing and light pretannage with formaldehyde was accomplished as in Example I. The fibrous slurry may then be rolled continuously into sheets as by a Fourdrinier machine, sprayed with a quick cross-linking dialdehyde agent and dehydrated and tanned by usual and known methods to form leather.

Example III

Tanned leather scraps may be detanned by washing in a 0.5 percent calcium lactate solution at room temperature for about 48 hours. Following detanning additional washing will remove the lactate. The detanned collagen is then suitable material for hydroxylamine conditioning, mechanical slurrying, and reaggregation back to a skin-like material as described in Example I.

Example IV

Vegetable tanned leather pieces may be submerged in a room temperature urea solution having a concentration of about 2 percent for 48 hours. After detanning, the reagent may be removed by additional washing in clear water to render the pieces suitable for hydroxylamine conditioning and reaggregation back to a skin-like sheet or other structure as described in Example I.

While in the foregoing specification we have set out steps of process in considerable detail for the purpose of illustrating the various embodiments, it will be understood that such details of procedure may be varied widely by those skilled in the art without departing from the spirit and scope of our invention.

We claim:

1. A method of preparing collagen comprising the steps of conditioning aggregated collagen with a water solution of hydroxylamine until there is a substantial delamination of individual fibers from one another, mechanically disaggregating the fibers in said collagen, and collecting the disaggregated fibers.

2. The method of treating collagen comprising the steps of contacting aggregated collagen with hydroxylamine solution having a concentration of from about 20% to 80% hydroxylamine by weight in water, said contacting being continued until there is a substantial delamination of collagen fibers, mechanically manipulating the collagen to thereby slip individual fibers from the collagen aggregate, separating the fibers from the hydroxylamine solution and collecting the slipped fibers.

3. A method of treating collagen comprising the steps of contacting aggregated collagen with hydroxylamine solution having a concentration of from about 20% to

80% hydroxylamine by weight in water, said contacting being carried out at a temperature of not over 60° C. and being continued until there is a substantial delamination of collagen fibers, mechanically disaggregate the collagen so as to slip individual fibers from the collagen aggregate, collecting the disaggregated fibers.

4. A method for treating collagen comprising the steps of contacting aggregated collagen with hydroxylamine solution having a concentration from about 20% to 80% by weight, maintaining temperature of said solution from about 0° to 60° C., said contacting being continued until there is a substantial delamination of collagen fibers, mechanically manipulating the collagen to slip individual fibers from the collagen aggregate, separating the fibers from the hydroxylamine solution, and collecting the said fibers.

5. A method for treating collagen comprising the steps of contacting aggregated collagen with hydroxylamine solution having a concentration of from 20% to 80% by weight, maintaining temperatures of said solution at between about 0° to 60° C., said contacting being continued until there is a substantial delamination of collagen fibers, separating the fibers from the hydroxylamine solution, collecting said fibers, and reaggregating the collected fibers to form a collagen structure.

6. A process for treating collagen animal skin material comprising contacting the animal skin material with hydroxylamine solution having a concentration of about

20% to 80% by weight in water, maintaining temperatures of said solution during conditioning at between 0° to 60° C., said contacting being continued until there is a substantial delamination of collagen fibers, mechanically manipulating the animal skin material to slip fibers therefrom, separating the fibers from the hydroxylamine solution, and collecting said fibers.

7. Collagen fibers having the general physical properties of native collagen and being chemically modified by the introduction of from about 10 to 46 moles of hydroxamates per 10^5 gms. of native collagen, the removal of 10 to 24 moles of amides, and the conversion of 1 to 24 moles of arginine to ornithine.

8. Collagen structures consisting essentially of cross-woven structured collagen fibers, a substantial portion of said fibers being chemically modified by the introduction of from about 10 to 46 moles of hydroxamates per 10^5 gms. of native collagen, the removal of 10 to 24 moles of amides and the conversion of 1 to 24 moles of arginine to ornithine.

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