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3,014,024

## COLLAGEN FILM

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2 Claims. (Cl. 260-117)

The present invention relates to a highly oriented collagen film of uniform thickness, characterized by an exceptional tensile strength in the direction of orientation and to a method of preparing the collagen film.

Films of collagen are customarily prepared by casting (pouring) a fluid dispersion of collagen fibers on a flat or screened surface and permitting the dispersing media to evaporate. In general, one or two days are required to completely evaporate the dispersing media at room temperature, the residual film being uneven and varying widely in cross-section. The prior art collagen films have not been uniform in thickness because of the viscous nature of the collagen dispersions and the non-uniform manner of drying. Moreover, since the collagen dispersion is laid down in a flat surface without the application of any external force, the individual collagen fibers in the dried film are in a random and disoriented state.

Attempts to speed up the process described above by elevating the temperature results in denaturation and degradation of the collagen fibers to such a degree that gelatin films are obtained in place of the desired collagen films. Removal of the liquid phase in a vacuum is also impractical as the viscous dispersion foams and the resulting film has a rough and uneven surface. Blowing air over the collagen dries the surface rapidly, but the dried surface film then interferes with evaporation of the solvent trapped beneath this surface layer.

It is an object, therefore, of the present invention to substantially reduce the time required in the manufacture of collagen films.

It is a further object of the present invention to produce a clear collagen film that is extremely uniform in thickness.

It is also an object of the present invention to produce a dry uniform collagen film at least 5 mills in thickness.

Still another object of the present invention is to manufacture a collagen film characterized by a substantial orientation of the collagen fibers in a single direction and an improved tensile strength in the direction of orientation.

Another object of the present invention is to provide a process that will enable the operator to add plasticizers and/or tanning agents to the collagen film after it has been formed.

It has now been discovered that superior collagen films may be obtained by centrifuging a uniform collagen dispersion of comparatively low viscosity. A basket centrifuge is particularly well adapted to the present process, and for ready removal of the dried collagen film, a filter cloth is placed into the basket centrifuge prior to addition of the collagen dispersion.

The collagen dispersion that is suitable for the present invention should contain collagen fibers in a pure and undenatured state. Such a dispersion may be obtained by swelling collagen in aqueous acid solutions and re-

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moving the associated impurities that do not swell by filtration. The preparation of a collagen dispersion that is satisfactory for the practice of the present invention has been described in the Salo Patent No. 2,598,608.

The concentration of the collagen fibers in the dispersion should preferably be maintained below about 1% as higher concentrations in the neighborhood of 2% or above are too viscous to give satisfactory films unless a special type of centrifuge is employed that has an interior blade to distribute the dispersion. Excellent results have been obtained with a dispersion containing 0.5% by weight collagen fibers.

The following examples illustrate procedures suitable for the production of collagen films from collagen fiber dispersions, but are not intended to limit the scope of this invention.

*Example I*

Beef tendon from freshly killed steers is trimmed free of fat and other extraneous tissues. The cleaned tendon is sliced and the slices are added to about 20 volumes of 2.5-5.0% acetic acid. The mixture is stirred for approximately four hours, during which time the collagen is swollen. The mixture is then diluted with an additional 160 volumes of water to facilitate removal of unswollen particles by filtration. The diluted dispersion is filtered under pressure through a 20-mesh stainless steel screen. The filtered liquid at this stage contains approximately 0.1% collagen and small unswollen particles that are not removed by the first filtration step. These impurities may be removed by additional filtration through successively finer screens or by centrifugation.

The dilute dispersions of pure collagen fibers is next treated with Normal ammonium hydroxide to neutralize all of the acetic acid (pH 7.0). The collagen fibers that are precipitated by the ammonium hydroxide are thoroughly washed in distilled water to remove all traces of salts and the washed fibers are redispersed in an aqueous acid solution containing 6 grams of malonic acid per liter. The dispersion so obtained contains 0.5% by weight collagen fibers.

*Example II*

A canvas filter cloth is wetted down with concentrated ammonia and placed into a basket centrifuge  $4\frac{1}{2}$  inches in diameter. Two hundred and fifty cubic centimeters of the 0.5% aqueous collagen dispersion described in Example I above is added to the centrifuge, which is rotated at just sufficient speed to bring the dispersion evenly up the sides of the basket onto the canvas cloth. The film so formed is then sprayed with about 20 cc. of ammonia to precipitate the collagen fibers and the basket is allowed to spin at a speed of 2500 r.p.m. for  $1\frac{1}{2}$  hours. The centrifuge is then operated at a speed of 3500 r.p.m. for an additional half hour. Finally, a draft of warm air is directed into the basket for an additional half hour to completely dry the film. The dried film is readily removed from the canvas cloth and is completely free of bubbles. The thickness of this film is  $3.0 \pm 0.1$  mills. The homogeneity of the dispersion described in Example I above is such that the final film is clear and transparent.

The tensile strength of this film is measured perpendicular to and parallel with the direction of fiber orientation. Strips of the film exactly one-half inch in

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width are tested on an Instron tester and compared with a control film of the same width obtained by casting the dispersion of Example I on a fine wire mesh base. The results indicate a distinct orientation of collagen fibers as a result of the centrifugal force on the collagen dispersion and the subsequently precipitated fibers.

| Type             | Thickness, mils | Tensile Strength, Pounds |          |
|------------------|-----------------|--------------------------|----------|
|                  |                 | Perpendicular            | Parallel |
| Centrifugal..... | 3.0             | 16                       | 25.5     |
| Cast.....        | 3.0             | 20                       | 20       |

#### Example III

The procedure of Example II is repeated using an 0.5% aqueous dispersion prepared from the sheath tendon of cattle swollen in an 0.1% malonic acid solution. The dispersion is added to the centrifuge basket and the speed immediately brought to 1750 r.p.m. After two minutes, concentrated ammonia is sprayed on the dispersion and the film is sprayed again after ten minutes. The speed is maintained at 1750 r.p.m. for 35 minutes, raised to 2600 r.p.m. for 40 minutes and finally to 3500 r.p.m. for 25 minutes. The speed is reduced to 2600 r.p.m. for 20 minutes, during which time the film is dried by means of a draft of warm air. This film has a thickness of 2.5 mills with a perpendicular tensile strength of 9.5 pounds and a parallel tensile strength of 19.9 pounds.

#### Example IV

The perforated basket of a large centrifuge 20 inches in diameter is lined with an Orion filter cloth saturated with concentrated ammonium hydroxide. This centrifuge is rotated at 800 r.p.m. while 4 liters of an 0.47% collagen dispersion prepared as described in Example I above, is added slowly to the centrifuge over a period of ten seconds. The surface of the dispersion layer is then sprayed immediately with concentrated ammonia, and is sprayed two more times at one-hour intervals. The speed of the centrifuge is then increased to 1500 r.p.m. and the centrifuge is allowed to spin at this speed for an additional three hours. Finally, a current of air at 35° C., is passed into the centrifuge for half an hour. A very thin film is obtained, approximately 1.0 mil in thickness. The tensile strength of this film is indicated on the Instron tensile tester is 5.5 pounds in a direction perpendicular to the fiber orientation and 11.0 pounds in a direction parallel to the fiber orientation.

#### Example V

The procedure of Example II is repeated and the film, after spraying with 20 cc. of ammonia and spinning for 1½ hours at 2500 r.p.m., is sprayed with 20 cc. of a 2% formaldehyde solution. The basket is allowed to

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spin at a speed of 2500 r.p.m. for half an hour and then sprayed again with an additional 25 cc. of 2% formaldehyde solution. One-half hour after the second addition of formaldehyde, the speed of the centrifuge is increased to 3500 r.p.m. and a draft of warm air is directed into the basket until the collagen film is completely dry. The dried film is easily removed from the canvas cloth and has properties similar to those obtained when the film is tanned in a formaldehyde tanning bath. In a similar manner, aqueous solutions of known plasticizers such as glycerol or sorbitol can be sprayed directly into the centrifuge to produce a plasticized collagen film.

Strips of collagen film prepared by the procedure described above may be sterilized and used as a surgical suture or ligature for the purposes described in United States Patent No. 2,039,145. Such films may be used in surgery for hernia repair and may be formed into tubes for arterial graphs.

The collagen films of the present invention are also useful in wrapping meat products such as ground sausage.

While the invention has been described with particular reference to specific embodiments, it is to be understood that it is not to be limited thereto, but it is to be construed broadly and restricted solely by the scope of the appended claims.

What is claimed is:

1. A collagen film of uniform thickness characterized by a substantial orientation of collagen fibers in a single direction and a tensile strength in the direction of orientation at least about 1.6 times the tensile strength in a direction perpendicular to the direction of orientation; produced by centrifuging an acid dispersion of collagen fibers to form a film, spraying the film with an alkaline reagent to neutralize the acid and continuing to centrifuge until the dispersing liquid has been substantially removed from the film and removing the film so obtained from the centrifuge.

2. A collagen film as described in claim 1, the thickness of which is at least 5 mills.

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