FIG. 1.

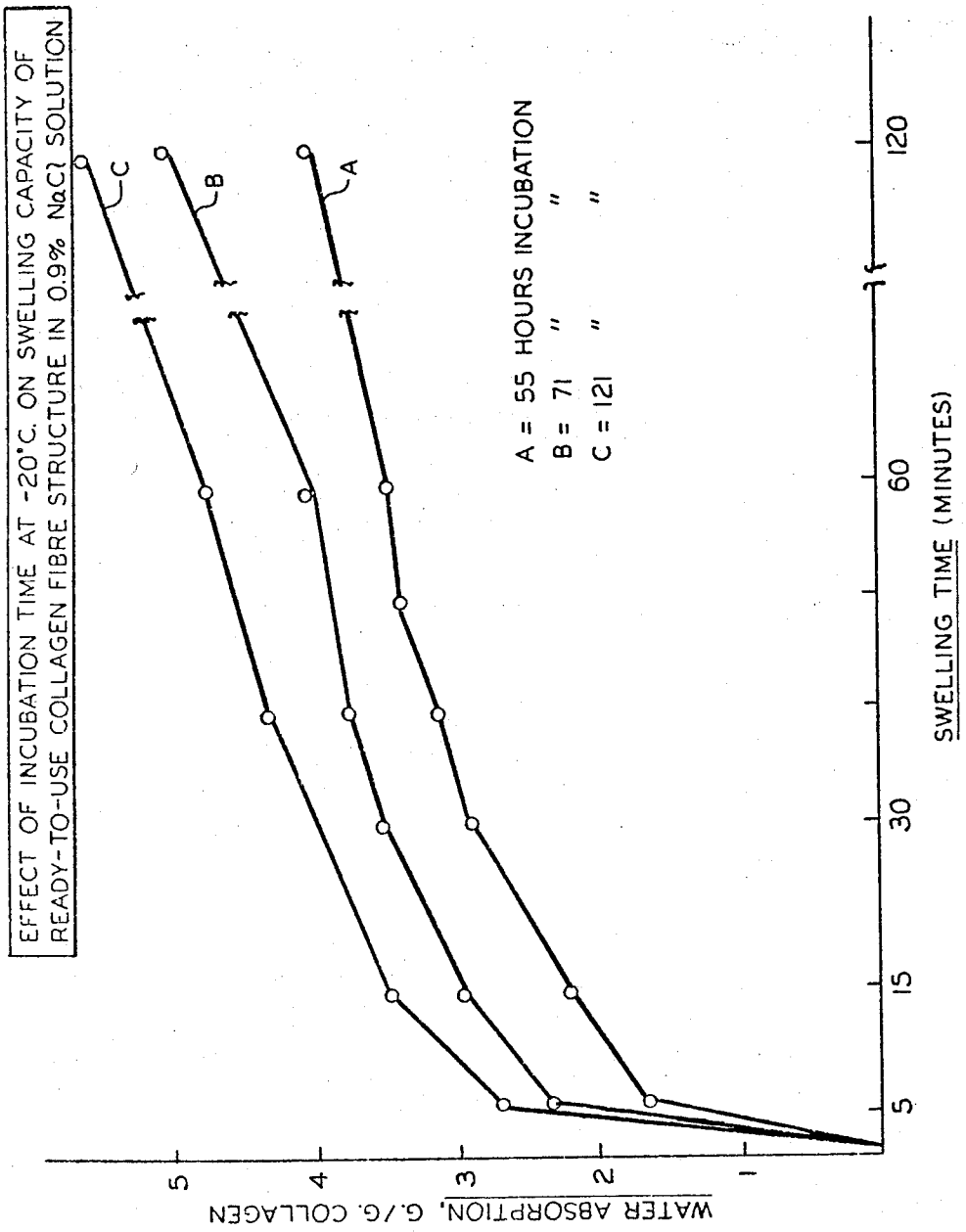
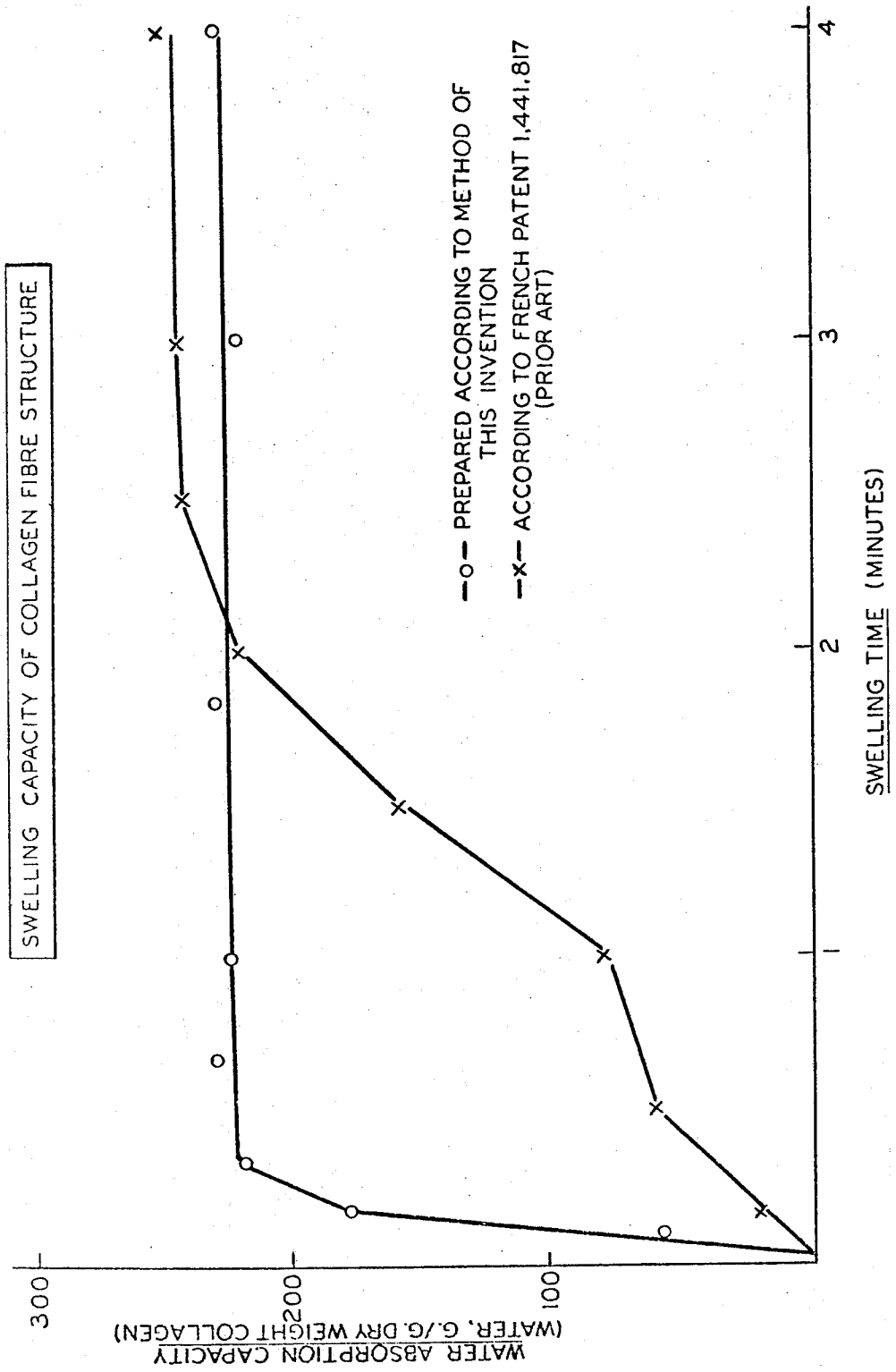

EFFECT OF INCUBATION TIME AT -20°C. ON SWELLING CAPACITY OF READY-TO-USE COLLAGEN FIBRE STRUCTURE IN 0.9% NaCl SOLUTION
A = 55 HOURS INCUBATION
B = 71 " "
C = 121 " "
A
B
C
WATER ABSORPTION, G./G. COLLAGEN
1
2
3
4
5
SWELLING TIME (MINUTES)
5
15
30
60
120

FIG. 2.


SWELLING CAPACITY OF COLLAGEN FIBRE STRUCTURE
—o— PREPARED ACCORDING TO METHOD OF THIS INVENTION
—x— ACCORDING TO FRENCH PATENT 1,441,817 (PRIOR ART)
WATER ABSORPTION CAPACITY (WATER, G./G. DRY WEIGHT COLLAGEN)
300
200
100
1
2
3
4
SWELLING TIME (MINUTES)

United States Patent Office 3,823,212
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3,823,212

PROCESS FOR THE PRODUCTION OF COLLAGEN FIBER FABRICS IN THE FORM OF FELT-LIKE MEMBRANES OR SPONGE-LIKE LAYERS

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Continuation-in-part of abandoned application Ser. No. 878,118, Nov. 19, 1969. This application May 28, 1971, Ser. No. 148,116
Claims priority, application Germany, Nov. 27, 1968, P 18 11 290.8–44
Int. Cl. B29d 27/03
U.S. Cl. 264—49 17 Claims

ABSTRACT OF THE DISCLOSURE

Production of felt-like membranes or sponge-like layers of collagen fibers by decomposing skin and/or tendons or other animal connective tissues rich in collagen under alkaline and/or acid conditions; mechanically comminuting the decomposition product; suspending the comminuted collagen stock obtained in water to form a homogeneous collagen slurry; adding a tanning or cross-linking agent to the slurry; foaming the collagen slurry; freezing the foamed collagen slurry so obtained in the form of a layer at −5 to −40° C.; incubating the frozen slurry for 1 to 30 days; and then freeing the bulk of the water therefrom by simple mechanical squeezing and/or evaporative drying.

This application is a continuation-in-part of Application Ser. No. 878,118, filed Nov. 19, 1969, now abandoned.

This invention relates to the production of collagen fiber structures. It more particularly refers to an improved process of producing such structures which are felt or foam-like and which are more economical than prior art processes.

It is known to use collagen fiber fabrics, referred to as collagen sponges, in medicine, in particular in surgery, as plugs and/or bandaging material for staunching bleeding. The production of such collagen sponges is described, for example, in German Patent Application S 97,055 and in French Pat. 1,441,817. As a rule, this known process for the production of collagen fiber fabrics comprise decomposing cattle skin and/or cattle tendons under alkaline and/or acid conditions; mechanically comminuting the decomposition product whereby the collagen stock is obtained; suspending such in water to form a homogeneous collagen slurry; freezing the collagen slurry obtained in the form of a layer after adjustment of the pH value to about 7.0; and removing the bulk of the water from the frozen collagen layer by lyophilisation or by extraction with organic solvents.

This process has the advantage that there can be incorporated into the collagen slurry compounds which, in an advantageous manner, affect the properties of the collagen fiber fabrics produced therefrom. For example, it is possible to incorporate plasticizers, as well as therapeutically and/or diagnostically active compounds, for instance compounds inhibiting or promoting clotting of the blood, analgetics, antibiotics or compounds containing isotopes, and in this way produce collagen fiber fabrics in the form of sponge-like fabrics which can be used for many purposes.

A disadvantage, however, is that this known process is relatively costly, more than 80% of the costs incurred being as a rule accounted for by the stage of the process in which the bulk of the water present is removed from the frozen collagen layer, i.e. the cost of energy for the lophilisation or the cost of solvent for the extraction makes

the process inordinately costly and has a very detrimental effect on the economy thereof.

A further disadvantage is that while the known process makes possible the production of collagen fiber fabrics in the form of sponge-like layers, it does not, however, permit the production thereof in the form of felt-like membranes of small thickness and adequate water absorption capacity. There is a great need for such membranes, however.

Membranes or films of this kind can find varied use, for example, in medicine, for instance for the treatment of skin burns and abrasions, where large wound areas must sometimes be covered, in order to protect the injured areas from additional external injury, prevent infection of the wound from occurring and prevent bandaging material from sticking to the wound. Further, such felts can promote healing of the wound by supplying therapeutically active compounds, and can also prevent maceration of the wound by slow absorption of the exudation formed.

Still further, such felt membranes may be employed as substitutes or replacements for certain body membranes, for example the dura mater. Heretofore, only the membranes obtained from newborn infants and constituting the allantois, the amnion and the chorion, as well as the dura mater recovered from corpses, have been available for this purpose, or alternatively physically foreign films or foils, for example tantalum foils, which are not absorbable and must be removed in a second operation, have had to be employed. Moreover, the membranes heretofore employed for the purposes indicated have the disadvantage that they cannot practically be charged with therapeutically active compounds for promoting the healing of wounds. Also, as a rule, they are not capable of absorbing the exudation that is formed by the wound. Additional disadvantages are that the production of such prior art membranes is time-consuming and costly and that it is difficult to produce such membranes which are free from antigenic effects and have reproducible properties.

One object of this invention is, therefore, to provide a process, which is simple and economically attractive, by which collagen fiber fabrics can be produced in the form of felt-like membranes or sponge-like layers having high mechanical strength and at the same time excellent flexibility.

Another object is to produce such fabrics in such a manner as to impart thereto a high capacity for absorbing liquids, a limited antigenic effect, a high binding capacity for solids and a fibrillogenesis stimulating action.

Still another object of this invention is to provide a novel process for producing collagen felts or sponges which is economically attractive.

Other and additional objects of this invention will become apparent from a consideration of this entire specification including the claims and drawings hereof.

In accord with and fulfilling these objects, one aspect of this invention lies in the surprising discovery that a foamed collegan slurry containing appropriate known tanning or cross-linking agents becomes cross-linked below a certain minimum temperature below the freezing point of the slurry with time to a felt-like or sponge-like structure. If, therefore, a foamed collagen slurry containing tanning agent is frozen in the form of a layer below a particular maximum temperature and is then incubated at such low temperatures for a given amount of time, a sponge or felt form product results. The structure of the product can be controlled by adjusting the incubation time and incubation temperature relative to one another so that felt-like membranes or sponge-like layers of only moderately swellable collagen fibers which are only partially cross-linked, or of greatly swellable collagen fibers which are three-dimensionally cross-linked

to a high degree respectively, are formed. The frozen mass is mostly water, e.g. up to about 99 volume percent. The bulk of this water frozen with the collagen fiber fabric is not removed from the structure in the frozen state as in the prior art, but rather the structure is thawed and the water removed by mechanical squeezing and/or conventional evaporation drying techniques at room temperature. This results in a marked economic advantage since it is not necessary to use lyopilization or freeze-drying or solvent exchange treatments as in the prior art.

The industrial utility of the simply and cheaply produced collagen fiber structures according to this invention extends not only to the medical fields which utility is generally known (see above), but also to numerous commercial fields where an absorbent and wear-resistant membrane or layer of good strength and flexibility, which can be combined if necessatry with plastics or textile materials, is desired, for instance as leather substitute material, for example in the manufacture of lining leather, or in other commercial fields, for example for making sanitary napkins and/or tampons.

The invention therefore encompasses an improved process for the production of collagen fiber structures, in the form of felt-like membranes or sponge-like layer with controlled structures and controlled degrees of swellability. In the process animal skin and/or tendons and/or other animal connective tissues rich in collagen is decomposed under alkaline and/or acid conditions; mechanically comminuted; the comminuted collagen stock obtained suspended in water to form a homogeneous collagen slurry with tanning agent added to such slurry and if necessary and/or desirable with the simultaneous addition of plasticizers and/or therapeutically or diagnostically active compounds; and the collagen slurry thus obtained is frozen, incubated, thawed and then freed from the main portion of water retained therein, which process improvement is characterized in that the comminuted collagen mass is made into a homogeneous collagen suspension in water and foamed in the presence of air and/or an inert gas, with the collagen suspension having a collagen content of about 0.3% to 3%, preferably from about 0.5 to 2% (dry weight basis), and an adjusted pH value of 3 to 5.5, preferably to 4 to 5; the foamed collagen suspension is frozen in the form of a layer at temperatures from —5 to —40° C., preferably between —10 to —30° C., and allowed to stand for about 1 to 30 days, preferably from about 2 to 8 days, under atmospheric pressure at the indicated temperatures, thereafter the frozen collagen layer is thawed at temperatures of about 10 to 30° C., preferably at room temperature, and the main portion of the water in this structure is removed therefrom by mechanical squeezing. If desired, the squeezed-out collagen fiber structure can be further dried at room temperature.

In the accompanying drawing:

FIG. 1 is a graphical representation showing the effect of incubation time on the welling (0.9% solution of sodium chloride in water absorption) capacity of a collagen fiber structure made according to this invention; and

FIG. 2 is a graphical representation showing the swelling (water absorption capacity) of collagen fiber structures made according to this invention compared with those made according to the prior art.

In accordance with a preferred embodiment of the process of the invention, first a collagen mass obtained through alkaline and/or acid decomposition of beefhide or beef-tendons is mechanically comminuted and the thus obtained collagen mass washed several times with a 10% sodium chloride solution in order to remove impurities which as a rule are responsible for the antigeneous effect of impure collagen. The washed collagen mass is then suspended in water and the pH value adjusted to above 4, preferably from 4 to 5. This exit collagen mass, with a collagen content of about 8 to 13% (dry weight basis), is then treated with so much water and with tanning materials, as well as plasticizers and/or therapeutically or diagnostically effective compounds, as necessary or desired, that the collagen content of the obtained mixture (dry weight basis), is 0.3 to 3%, preferably 0.5 to 2% whereupon the obtained mixture is foamed as aforesaid, preferably in a mixing apparatus running at high speed at about 500 to 3,000 r.p.m. and finally the foamed collagen slurry obtained is poured into containers of, for example, plastic, glass or metal in the form of layers about 0.5 to 5 cm. thick, preferably 0.5 to 3 cm. thick, and frozen in the manner indicated and incubated at said temperatures, after which incubation the frozen layers are thawed out, preferably at room temperature, and water mechanically squeezed thereout of. The squeezed-out collagen fiber structures are further dried by evaporation if necessary at room temperature or above and/or, possibly by blowing a stream of air on to one or both sides of the material to be dried. For preventing distortion, it is expedient to cover the collagen fiber structure to be dried on both sides with an absorbent web, for example filter paper, and arrange thereover a strong screen to preserve the dimensional stability of the material to be dried. The dried collagen fiber structure, which as a rule still contains about 10% of water, can then be cut into pieces of suitable size and shape, sealed in plastics films and sterilized.

As already mentioned, in order to carry the process of the invention into effect, it is important that the collagen slurry intended to be frozen is foamed, so as to obtain a slurry permeated by numerous gas bubbles. It has proven to be advantageous to homogenize the collagen slurry in a mixing apparatus operated at about 500 to 3000 r.p.m. while simultaneously introducing into the slurry air or inert gases, for example nitrogen or carbon dioxide. It has been found that a collagen slurry foam with a viscosity of about 200 to 600 cp., preferably about 300 to 500 cp., can be worked up in a particularly advantageous manner into high-grade collagen fiber structures. The viscosity can be controlled in an advantageous manner by adjusting the pH value or the collagen content, a collagen slurry with a comparatively low pH value exhibiting as a rule a comparative high viscosity.

The process of the invention can be controlled in such manner as to produce collagen fiber structures which are cross-linked to a greater or lesser degree and have a greater or lesser capacity for absorbing liquids or fluids. The fluid absorption capacity of the collagen fiber structures obtained depends, assuming a given proportion of tanning agent, primarily on the incubation time and incubation temperature.

It has proven to be advantageous to adjust the three indicated factors affecting the structure of the collagen fiber structures obtained, namely incubation time, incubation temperature and tanning agent content, relative to one another on the basis of preliminary tests. As a rule, a collagen slurry containing about 1% of glutaraldehyde, referred to total volume of the collagen mixture, as tanning agent produces, with incubation for about 8 days at —8° C., a collagen sponge having a three-dimensional net structure which is developed to such an extent that, after absorbing water, it assumes its original form again on being wrung out. In contrast, the same material otherwise treated in the same way but after incubation for about 8 days at —2° C. yields a collagen product in the form of a gel having a comparatively low degree of cross-linking and water absorbability.

In order to carry out the process of the invention, conventional tanning agents, known as hardening agents for proteins, may be incorporated into the collagen slurry. The use of glutaraldehyde or melamine formaldehyde resins, which are physiologically harmless, has proven advantageous. The tanning agents may be employed in various concentrations. Concentrations of about 0.1 to 3%, preferably 0.2 to 2% referred to the total volume of the collagen slurry, are suitable.

The addition of plasticizers to the collagen slurry, in particular when it is intended to produce collagen fiber structures in the form of felt-like membranes, is advantageous for controlling the elastic properties of the collagen fiber structures produced by the process of the invention. The known conventional plasticizers, for instance glycerine or sorbitol, may be incorporated into the collagen slurry. The plasticizers may be employed in various concentrations. Concentrations of about 0.1 to 3%, preferably 0.3 to 1%, referred to the total volume of the collagen slurry, are suitable. If the plasticizers are present in concentrations higher than those indicated, undesirably strongly hydrated collagen fibers of only low tensile strength may result.

If therapeutically active compounds are to be incorporated into the collagen slurry, these may be known conventional compounds usually employed in wound dressings and surgery, for example compounds useful in inhibiting or promoting blood clotting, disinfectants, analgesics, antibiotics, for instance bacitracin, neomycin or tetracyclin, and the like. Diagnostically active compounds may be added to the collagen slurry, such as compounds containing radioactive isotopes. The therapeutically and/or diagnostically active compounds may be employed in various concentrations. It has proven to be advantageous to determine the appropriate concentration by preliminary tests in the known matter, so as to prevent the collagen proteins being precipitated where such compounds are used in too high concentrations. When antibiotics are employed, concentrations of about 20 to 100%, preferably 30 to 60%, referred to the dry weight of the collagen present in the collagen slurry, are advantageous.

In order to carry out the process of the invention, it has been found to be advantageous to remove the bulk of the water from the final product mechanically by squeezing such out from the collagen layers thawed out at temperatures of about 10 to 30° C., preferably at room temperature. This squeezing may be effected by known conventional methods, for example by placing the collagen fiber fabric on a firm support and then pressing such with a rubber squeegee, or alternatively by pressing such between two rotatably arranged pressure rolls, having an adjustable nip. Depending on the thickness of the collagen fiber structure, a nip spacing of about 0.2 to 0.5 cm., preferably 0.3., between the two pressure rolls is suitable.

If the finished collagen fiber structures are to be sterilized, if necessary after cutting into pieces of suitable shape and size and after sealing in a plastic film, for example polyvinyl chloride film, sterilization may be by means of Co^{60} isotopes, for example in a dosage of 2 to 4 mrad., or by means of ethylene oxide gas, which have proved to be advantageous, since in this way the properties of the collagen fiber structures are not detrimentally affected.

According to one particularly advantageous method of carrying out the process of the invention, the collagen slurry, containing the indicated additions if necessary, is poured into containers into which a textile fabric of naturally occurring or synthetically produced staple fibers and/or substantially continuous mono or multifilaments has been previously introduced. This is then processed further as has been indicated above. The products produced in this way are combinations of collagen fibers and the other fibers utilized and are particularly advantageous for treating wounds to which bandaging materials stick easily.

The following non-limiting illustrative Examples are intended to illustrate the invention.

Example 1

500 ml. of cold water were added to 100 g. of a collagen slurry, having a collagen content of 8% (dry weight basis). The mixture thus obtained was homogenized in a mixing apparatus at 800 r.p.m. to form a collagen slurry foam while slowly adding 20 ml. of glycerine and 2 grams of glutaraldehyde. The foamed collagen slurry obtained was poured into trays in the form of layers 1 cm. thick, frozen at −20° C. and left for 3 days at the temperature indicated. The frozen collagen foam layers were thereafter allowed to thaw out at room temperature and then immediately freed from water by squeezing between two pressure rolls with a nip spacing of about 0.3 cm. The squeezed-out collagen fiber structure which resulted was dried at room temperature between two filter papers each having a metal screen placed thereon. The physical properties of the felt-like membrane obtained were tested.

The results obtained are given in the following Table:

TABLE 1

Thickness of the felt-like collagen membrane	0.3 cm.
Collagen content, (dry weight basis)	84.5%.
Swelling in 0.9% Na Cl after 24 hours	4.82 times the weight (original).
Swelling in 0.07 N HCl after 24 hours	8.60 times the original weight.
Tensile strength of a strip 0.2 x 1 x 4.5 cm.	4.2 kg.
Shrinking temperature (° C.) (for a shrinking temperature of 48 for the initial collagen	68± 2.

The felt-like membrane produced was sealed in a polyvinyl chloride film pouch and sterilized with Co^{60} isotopes at a dosage of 2.5 mrad.

Example 2

The procedure described in Example 1 was repeated in three test mixtures, with the difference being that 0.5 g. of the antibiotics bacitracin, neomycin or tetracyclin, respectively, was added to the collagen slurry during the homogenization. Correspondingly advantageous results were obtained.

Example 3

The procedure described in Example 1 was repeated in two test mixtures, except that a stream of air or nitrogen was introduced into the collagen slurry during the homogenization. Correspondingly advantageous results were obtained.

Example 4

The procedure described in Example 1 was repeated, except that the foamed collagen slurry was poured into containers on the bottom of which a textile fabric consisting of bandaging gauze had been placed. Correspondingly advantageous results were obtained.

Example 5

1 liter of water containing 6 ml. of N hydrochloric acid, 10 ml. of sorbitol and 3 ml. of 25% glutaraldehyde was added to 100 g. of an initial collagen slurry as shown in Example 1, having a collagen content of 13.5% (dry weight basis). The mixture obtained was homogenized in the manner indicated, after which the viscosity of the foamed collagen slurry was adjusted to 300 cp. by adding the necessary amount of concentrated sodium bicarbonate solution. The collagen slurry obtained was worked up further by the method described in Example 1. Correspondingly advantageous results were obtained.

Example 6

This Example shows the effect of the concentration of an added tanning substance on the collagen content and the swelling capacity of the resulting collagen fiber structure ready for use. In each case to 30 gms. of initial collagen slurry, having a collagen content of 8.5% (dry weight basis), there was added the amount of glutaraldehyde stated in Table 2 below and the mixture was worked up further as previously indicated. The foamed collagen

slurry was incubated for 6 days at −20° C. The results obtained are compiled in the following Table 2.

TABLE 2

Glutaraldehyde added as 25% solution (ml.)	Collagen content percent (dry weight basis)	Swelling after 24 hours in—	
		0.9% NaCl	0.01 N HCl
		Times the original weight	
0.5	81.4	5.04	7.97
1.0	75.5	13.1	12.6
1.5	84.5	17.6	20.3
2.0	83.5	25.2	22.2
2.5	82.0	24.8	28.0

Example 7

This Example shows the effect of the concentration of plasticizer on the water content to be calculated on the basis of the collagen content and on the swelling capacity of the product collagen fiber gauze ready for use.

1 ml. of 25% glutaraldehyde and the respective amounts of glycerine stated in Table 3 below were added to 30 g. in each case of the initial collagen slurry indicated by the method described in Example 1, the slurry having a collagen content of 8.5% (dry weight basis), after which processing was continued in the manner indicated above. The foamed collagen slurry was incubated for 6 days at −20° C. The results obtained are compiled in Table 3 hereunder.

TABLE 3

Glycerine added (ml.)	Collagen content, referred to dry substance (percent)	Swelling after 24 hours in—	
		0.9% NaCl	0.01 N HCl
		Times the original weight	
0	75.5	13.1	12.6
2	66.7	5.37	6.18
5	28.8	2.80	3.03
10	17.4	1.89	2.78

Example 8

This Example shows the effect of the incubation time at −20° C. on the swelling capacity of the produced collagen fiber structure, ready for use, in 0.9% NaCl solution.

100 g. in each case of the initial collagen slurry indicated were worked up in the manner stated by the method described in Example 1, except that the foamed collagen slurry was incubated for different lengths of time at −20° C. Thhe swelling capacities of the collagen fiber structure, obtained was tested. The results obtained were plotted in the graph of FIG. 1. The incubation at −20° C. was 55 hours for the sample marked A, 71 hours for the sample marked B and 121 hours for the sample marked C.

The results show that the swelling capacity of the finished collagen fiber structures increases with increasing incubation time.

Examples 9–20

In each example the collagen slurry was prepared in an identical manner as follows:

100 grams of aqueous acid swollen collagen containing 15% dry collagen solids was mixed with 500 ml. of water with continuous stirring. This slurry was placed in 5 liter capacity Waring Blender and another 500 ml. of ice cold water, containing 4 ml. of a 25% aqueous solution of glutaraldehyde, was added under stirring at 3000 r.p.m. A foamed collagen slurry having a pH of 3 to 3.5 resulted to which was added sufficient 2 normal NaOH to raise the pH to 4.5 to 5 (about 1.5 to 2 ml. of caustic added) to a gel-like consistency. The thus formed gel was transferred into 12 Petri dishes each of which was 2 cm. high and 15 cm. in diameter.

Six of the thus filled Petri dishes, marked A, were stored for varying lengths of time at 0 to −2° C. and the other six Petri dishes marked B, were stored for varying lengths of time at −20° C. At the end of the times specified below, each Petri dish was removed from the freezer, thawed at room temperature and then squeezed for 5 minutes under a 50 kg. load. Each product was dried at room temperature for a constant weight, cut into 50 mg. pieces and then immersed in water at 20° C. for 5 minutes. The average amount of water absorbed and the mean deviation was determined and is reported per gram of sample (dry weight).

Additionally, each sample was tested to determine the time in seconds it look for it to be completely wetted with such 20° C. water.

The results are set forth in the following table:

TABLE 4

Sample	Incubation time (days)	Product appearance	Water absorption	Wetting time
A (−2° C.)	1	Highly swollen gel	0	0
A (−2° C.)	3	do	0	0
A (−2° C.)	5	do	0	0
A (−2° C.)	7	do	0	0
A (−2° C.)	10	do	0	0
A (−2° C.)	15	do	0	0
B (−20° C.)	1	Sheet 2 mm. thick	5±0.8	120
B (−20° C.)	2	Felt 4 mm. thick	8±1.2	68±2.4
B (−20° C.)	3	Sponge 4 mm. thick	16±4	25±7
B (−20° C.)	4	Sponge 12 mm. thick	38±9	12±2.4
B (−20° C.)	5	Sponge 18 mm. thick	47±8	7±1.4
B (−20° C.)	6	do	48±6	5.2±1.2

Example 21

500 ml. of water, 2 ml. of sorbitol and 3 ml. of 25% glutaraldehyde were added to 100 g. of the initial collagen slurry indicated, having a collagen content of 13.5% (dry weight basis), and the mixture was suspended in a mixing apparatus at 800 r.p.m., with the formation of foam, to form a homogeneous collagen slurry foam. The pH value of the formed collagen slurry was adjusted to 5 by the addition of saturated sodium bicarbonate solution. The collagen slurry foam obtained was frozen at −8° C. in the form of a layer and was incubated for 8 days at this temperature. The collagen layer was thereupon allowed to thaw out at room temperature, after which the water was mechanically removed by squeezing it out of the thawed-out collagen fiber structure. The squeezed-out collagen fiber structure was dried between two sheets of filter paper and two screens at room temperature. About 1 sq. m. of collagen fiber structure in the form of a sponge-like layer 0.5 cm. thick and having a water content of about 10% was obtained.

Example 22

4.5 liters of water, 7.5 ml. of sorbitol, 10 ml. of 25% glutaraldehyde and 10 ml. of 1.5 N hydrochloric acid were added to 500 g. of the initial collagen slurry indicated, having a collagen content of 13% (dry weight basis), and the pH value of which was 4.5, after which the mixture obtained was mixed for 3 hours in a mixing apparatus at slow speed. 30 ml. of saturated sodium bicarbonate solution were added to the mixture obtained in order to lower the viscosity of the mixture. The mixture obtained was then homogenized in a mixing apparatus at a high speed of 600 r.p.m. with the formation of foam. The foamed collagen slurry was incubated for 14 days at −8° C. It was further worked up the method described in Example 9. Correspondingly advantageous results were obtained.

Example 23

The method described in Example 9 was repeated, except that 3 g. of bacitracin, in the form of a powder, were added to the collagen slurry during the homogenization. Correspondingly advantageous results were obtained. The blood-clotting action of the tanned collagen fiber fabric may be attributable to the fact that, on contact of the blood with the large sponge-like surface of the collagen fiber structure, the thrombocytes of the blood burst by reason of the local change in viscosity. It may also be attributable to the fact that the blood dissolves part of the collagen sponge with the irreversible formation of a gel,

so that a uniform collagen coating is formed over the bleeding wound which exerts a plug affect.

Example 24

This Example shows the effect that the water present in the frozen collagen layer exerts on the development of the net, cross-linked structure of the collagen fiber structure.

In comparison tests, a collagen slurry of the composition described in Example 9 was frozen at —20° C. in the form of a layer, following which the bulk of the water present was removed from the frozen collagen layer by the known prior art technique of lyophilization. In another series of tests, the collagen slurry was frozen at —20° C. by the process of the invention and incubated for 8 days at the temperature indicated without removal of the water present in the frozen layer. The collagen fiber structures obtained were tested for their water absorption capacity. The results obtained were plotted in the graph shown as FIG. 2.

The results show that the collagen fiber structures produced by the known prior art process has a lower initial water absorption capacity than the collagen fiber structures produced by the process of the invention, and also that after a longer time the water absorption capacity of the two samples is substantially equal.

Example 25

250 g. of initial collagen slurry (13% of collagen, dry weight) were mixed with 1.7 liters of water, 5 ml. of sorbitol (available commercially under the name "Karion F") and 8 ml. of 25% glutaraldehyde solution, the mixture was foamed after which the foamed collagen slurry was frozen at —8° C., in the form of a layer 1 cm. thick, and incubated for 3 days. After thawing out, the water was mechanically squeezed out. The collagen fiber structure obtained was a collagen sponge about 1 cm. thick which had the following properties:

Water content	77%.
Collagen content, referred to dry weight	23%.
Water absorption, referred to moist weight of the collagen	400 g. H_2 0/100 g. of collagen.
Water absorption, referred to dry weight of the collagen	2,000 g. H_2 0/100 g. of collagen.

The squeezed-out collagen fiber structure was mechanically squeezed out still further under a pressure of 250 atms. The membrane-like layer obtained was sterilized and stored under sterile conditions. When this membrane-like layer was wetted with water, it absorbed the water immediately and rapidly, reforming the original layer 1 cm. thick.

Certain comparative tests have been conducted for the purpose of establishing the criticality of both the freezing temperature and the incubation period on the physical properties of the products produced.

Examples 26–27

These Examples illustrate the fact that collagen sponge prepared by the procedure of this invention does not contain any free tanning agent as compared with collagen sponge made by prior art processes such as lyophilization processes.

A collagen slurry was prepared with stirring according to the technique described above containing 100 grams of collage, 1000 grams of water and 4 grams of 25% aqueous gluataraldehyde.

The slurry was foamed as aforesaid and separated into two (2) aliquots each of which was frozen at —20° C. One of the samples A was maintained frozen for 4 hours at —20° C. and then subjected to freeze drying in a virtis freeze drier by heating to 20° C. under a vacuum of 5 mm. Hg for 18 hours after which the sample was dry. The other same B was maintained frozen for 7 days at —20° C. after which it was thawed to room temperature washed in tap water, mechanically squeezed and dried.

1 gram of the dry collagen sponge resulting from each aliquot was extracted by vigorous shaking with 100 ml. of distilled water for 4 hours at 20° C. The amount of glutaraldehyde contained in an average of 6 extracts was determined by the method of Frigerio and Shaw (J. Histochem. Cytochem. *17*: 176, 1969).

TABLE 5

Sample	Mg. glutaraldehyde/9 g.
A	44±7.
B	None detected.

NOTE.—Maximum glutaraldehyde possible in sponge is 66 mg.

These last two Examples point up one very real advantage of the collagen sponge made according to this invention as compared to collagen sponges made by a lyophilization technique. Sponges made according to this invention can be water washed free of tanning agent in the dry sponge whereas sponges made by a lyophilization technique cannot be re-wet once they are dried.

What is claimed is:

1. In the production of a collagen fiber structure by forming an acidified slurry of collagen in water containing about 0.3 to 3% collagen on a dry basis and containing about 0.1 to 3% based on the dry weight of collagen of a tanning agent therefor; freezing said slurry; and removing the water from said frozen slurry; the improvement which comprises freezing said slurry at a temperature of up to about —5° C., maintaining said slurry in the frozen condition for at least about 1 day, thawing said frozen slurry, and removing the water from said thawed material.

2. The improved process claimed in claim **1** including freezing at about —5 to —40° C. for about 1 to 30 days, thawing at about room temperature and mechanically squeezing the water out of said thawed product.

3. The improved process claimed in claim **1** including foaming said slurry before freezing such and freezing such in foam form.

4. The improved process claimed in claim **1** including adjusting the pH of said slurry to about 3 to 5.5 prior to freezing.

5. The improved process claimed in claim **1** including beating a substantially inert gas into said slurry prior to freezing.

6. The improved process claimed in claim **1** including adjusting the viscosity of said slurry to about 300 to 500 cp. before freezing.

7. The improved process claimed in claim **1** where said tanning agent is a member selected from the group consisting of glutaraldehyde and melamine-formaldehyde resin.

8. The improved process claimed in claim **1** including adding about 0.1 to 3% of plasticizer, based on the total volume of collagen slurry, to said slurry.

9. The improved process claimed in claim **1** including adding about 10 to 100%, based on the dry weight of collagen, of at least one member selected from the group consisting of a blood clotting inhibitor, a blood clotting promotor, an analgesic agent, and an antibiotic to said slurry.

10. The improved process claimed in claim **1** including forming said slurry into a layer about 0.2 to 5 cm. thick prior to freezing such.

11. The improved process claimed in claim **2** wherein said mechanical squeezing is carried out between a pair of rolls having a nip of 0.2 to 0.5 cm.

12. The improved process claimed in claim **1** including admixing said slurry with a mass of preformed fibers prior to freezing.

13. The improved process claimed in claim 1 including freezing at —10 to —30° C. for 2 to 8 days and thawing at 10 to 30° C.

14. The improved process claimed in claim 1 wherein said collagen represents 0.5 to 2 weight percent of said slurry and said tanning agent represents 0.2 to 2 volume percent of said slurry.

15. A substantially tanning agent free collagen fiber structure made by the process of claim 1.

16. The improved process claimed in claim 1 wherein the collagen which is formed into an acidified slurry of collagen is in the form of fibers.

17. A collagen fiber structure formed by the process of claim 1.

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M. J. WELSH, Primary Examiner

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UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,823,212 Dated July 9, 1974

Inventor(XX Milos Chvapil

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 3, line 17

"necessatry" should be "necessary"

Column 3, line 57

"welling" should be "swelling"

Column 6, line 21

"0.07" should be "0.01"

Column 6, line 22

The line "Tensile strength of a strip" should be dropped down to go together with "0.2 x 1 x 4.5 cm."

Column 7, line 48

change "thhe" to "the"

Signed and sealed this 8th day of October 1974.

(SEAL)
Attest:

McCOY M. GIBSON JR.
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents