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Review

John D. Ferry's Patents

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Patented Oct. 2, 1945 2,385,802

UNITED STATES PATENT OFFICE

2,385,802

PROCESS FOR THE MANUFACTURE OF PLASTICS

John D. Ferry, Woods Hole, Mass., assignor to Research Corporation, New York, N. Y., a corporation of New York

No Drawing. Application February 9, 1942, Serial No. 430,077

4 Claims. (Cl. 106—124)

The present invention relates to a novel plastic and to processes for the manufacture thereof, and has for its object the provision of improvements in products and processes of this nature.

Many plastics and rubberlike materials are water-resistant but are not resistant to hydrocarbons. I have found that proteins, which are constituted largely of polar groups, are well suited to the production of plastics which have an excellent resistance to non-polar solvents such as hydrocarbons, so that these plastics do not absorb or swell in oils or other petroleum fractions. Furthermore, unlike many rubberlike materials, proteins contain but few of the unsaturated bonds which are prone to oxidation and consequent deterioration.

Proteins of the type of albumin, however, are not well adapted to the manufacture of molded plastics. Plastics and molded resins made from blood albumin and similar materials tend to be brittle and do not have favorable molding qualities. It is frequently difficult to incorporate plasticizers with such proteins in more than small amounts. Products made from blood albumin and like materials dispersed in a substantial amount of plasticizer lack coherence and strength, probably because the globular molecules which are characteristic of this type of protein do not attract each other unless they are in close contact.

I have found fibrinogen, a protein made up of long chain molecules which are capable of interlocking and forming a coherent structure even when separated by a plasticizing medium, to be an admirable material for the manufacture of a novel plastic product.

Fibrinogen suitable for the manufacture of my improved plastic may be obtained from blood in various ways, of which the following is an example:

The corpuscles are first removed from the blood by centrifugation, clotting of the fibrinogen being prevented by the addition of citrates or like agents. The plasma remaining after the separation of the corpuscles may then be treated for the precipitation therefrom of commercial amounts of fibrinogen by cooling the same to 0° C. and adding thereto an alcohol such as ethanol, in amount sufficient to constitute 10% by volume of the plasma. The hydrogen ion concentration and the ionic strength of the plasma are also preferably adjusted. The pH of the solution may be controlled by the addition of acids or alkalis and the ionic strength by the addition of a salt, for example sodium chloride, ammonium sulfate, sodium sulfate, sodium, ammonium or potassium phosphate, acetate, carbonate or the like. Phosphates, acetates, carbonates or borates are particularly suitable salts because they have a buffer action and thus control both the ionic strength and the hydrogen ion concentration. For the precipitation of fibrinogen the pH may initially be adjusted in the neighborhood of 6.0 to 7.8. An ionic strength of 0.05 is adequate for effecting electrical discharge and flocculation; higher ionic strengths (e. g., 0.15 or more) are sometimes desirable for buffering.

Other precipitants and other procedures may be employed in obtaining fibrinogen from blood plasma, as more fully set forth in a copending application of Edwin J. Cohn, Ser. No. 430,401, filed February 9, 1942.

As stated in said copending application, the conditions selected for the fractionation depend upon the solubilities of the various protein components of the system and are determined by the five variables: temperature, pH, ionic strength, concentration of precipitant and concentration of the protein components. The latter factor is of most importance when the concentration of the various proteins in the system is high and diminishes in importance in dilute protein solutions. These effects of protein concentration often protect labile proteins from denaturation. The other four variables are important under all conditions and must always be under control if reproducible separations are to be carried out in protein systems. In sufficiently dilute protein solutions they alone suffice to define the separations.

Other precipitants include the water-miscible alcohols, such as methanol, butanol, etc., and the water-miscible acetones.

The precipitant may be added to the protein solution by diffusion through a semi-permeable membrane (for example, Cellophane) or it may be added directly to said solution. Fibrinogen has been precipitated from plasma by spraying the plasma into ethanol-water mixtures, or vice versa. A flowing junction has also been employed for this addition, by supplying ethanol-water mixtures through one end of the top of a T-shaped tube, and plasma through the other end of the top, the two solutions being thoroughly mixed and passing together down the stem of the T.

In an alternate procedure, any desired protein, for example fibrinogen, may be obtained by extraction from a solid protein mixture. Thus

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UNITED STATES PATENT OFFICE

2,385,803

PROCESS FOR THE MANUFACTURE OF PLASTIC COMPOSITIONS

Edwin J. Cohn, Cambridge, and John D. Ferry, Woods Hole, Mass., assignors to Research Corporation, New York, N. Y., a corporation of New York

No Drawing. Application February 9, 1942, Serial No. 430,076

3 Claims. (Cl. 106—124)

The present invention relates to a novel plastic and to processes for the manufacture thereof. It is the object of the invention to provide improvements in plastic products and processes.

It has been found that protein materials, such as are contained for example in animal fluids and in animal and vegetable extracts, are suitable for use in the production of novel plastics. Proteins contain but few of the unsaturated bonds which are prone to oxidation and consequent deterioration. In addition, since proteins are constituted largely of polar groups, plastics made therefrom are resistant to non-polar solvents such as hydrocarbons.

Various protein components, principally fibrinogen, globulins and albumins, are found in blood. These differ considerably from each other in molecular form and structure, in solubility and in other respects. Fibrinogen, for example, is made up of long chain molecules, while the globulin and albumin molecules are more nearly globular in shape. Perhaps for this reason, fibrinogen forms a coherent structure, well adapted to the manufacture of molded plastics, even when considerable amounts of plasticizer are present. The globulins and albumins, on the other hand, are not by themselves so well adapted to the manufacture of molded plastics. Plastics made from albumin tend to be brittle or, especially if a substantial amount of plasticizer is present, to lack coherence and strength. These latter types of proteins, however, when added to fibrinogen in the manufacture of plastics, impart to the plastic highly desirable properties not possessed by like products containing only fibrinogen.

Fibrinogen plastics tend to be rubbery and flexible. Although this property is desirable in plastics for some uses, it is disadvantageous in other cases. We have found that by mixing albumin or globulin with the fibrinogen, the amount of plasticizer remaining the same, greater rigidity is imparted to the plastic product.

The whole properly dried blood plasma is a mixture of albumins, globulins and fibrinogen, and may be made into a plastic by our methods. The variety of properties obtainable is far greater, however, if the ratio of albumin to globulin and to fibrinogen, as well as the amount and nature of the plasticizer and the conditions of manufacture, are subject to control and to variation. Thus, for certain purposes the albumin may be omitted and for others the globulins. Certain of the globulins are also constituted of rod-shaped proteins and they can be substituted, in part at least, for fibrinogen. Other rod-shaped proteins, such as collagen, could be substituted for the fibrinogen, and other more nearly globular proteins for the albumins, and some of these may be derived from vegetable as well as animal sources. The conditions for making the plastic will differ somewhat, however, if rod-shaped or globular proteins of other natures are substituted.

If a protein poor in hydrophilic but rich in hydrophobic groups, or vice versa, is chosen, the properties of the plastic product, especially its swelling in polar or non-polar solvents, may be modified. This can also be accomplished by chemically modifying the protein by transforming certain of its reactive groups into either polar or non-polar groups.

Proteins for use in the manufacture of our novel plastic may be obtained, for example, from blood, by various methods. One such method is as follows:

The corpuscles are first removed from the blood by centrifugation, clotting of the fibrinogen being prevented by the addition of citrates or like agents. The plasma remaining after the separation of the corpuscles is then treated for the precipitation therefrom of fibrinogen by cooling it to 0° C. or lower and adding an alcohol, such as ethanol, in amount sufficient to constitute 10% by volume of the plasma. The hydrogen ion concentration and the ionic strength of the plasma are also preferably adjusted. The pH of the solution may be controlled by the addition of acids or alkalis and the ionic strength by the addition of a salt, for example sodium chloride, ammonium sulfate, sodium sulfate, sodium, ammonius or potassium phosphate, acetate, carbonate or the like. Phosphates, acetates, carbonates, borates, or citrates are particularly suitable salts because they have a buffer action and thus control both the ionic strength and the hydrogen ion concentration. Citrates are especially advantageous for the precipitation of fibrinogen because they not only control the ionic strength and the hydrogen ion concentration, but also prevent the transformation of fibrinogen into fibrin. For the precipitation of fibrinogen the pH may initially be adjusted in the neighborhood of 6.0 to 7.8. An ionic strength of 0.05 is adequate for effecting electrical discharge and flocculation; higher ionic strengths (e. g., 0.15 or more) are sometimes desirable for increasing the buffering and the solubility.

Under these conditions fibrinogen is precipitated from the plasma.

If the remaining plasma is now cooled to —5° C. and the alcohol concentration increased, for example to 25%, the pH remaining as before, gamma globulin will be precipitated. Alpha and beta globulins may be obtained by increasing the alcohol concentration to 40%, the temperature remaining at —5° C. and the pH being adjusted to 5.5–6.0.

By lowering the temperature to —15° C. and the pH to 4.7, the alcohol concentration remaining the same, the albumins are precipitated from the plasma.

Shaped plasticized polymers formed with proteins like fibrinogen that were prepared by processes described in Ferry patents 2385802 and 2385803. Nowadays we would probably call them 'shaped biopolymers'

Patented Dec. 5, 1950 2,533,004

UNITED STATES PATENT OFFICE

2,533,004

FIBRIN CLOTS AND METHODS FOR PREPARING THE SAME

John D. Ferry, Woods Hole, Mass., and Peter R. Morrison, Washington, D. C., assignors to United States of America as represented by the Secretary of War

No Drawing. Application October 27, 1943, Serial No. 507,904

5 Claims. (Cl. 260—112)

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This invention relates to fibrin clots and to methods for preparing the same.

As is well known, fibrin is derived from fibrinogen which is present in solution in the plasma of blood. Blood fibrin is usually prepared either in stringy masses by whipping freshly drawn blood or in the form of weak clots by inhibiting the normal clotting process with citrate or oxalate, removing the red corpuscles by centrifuging and adding to the remaining plasma an excess of calcium salt. However, the fibrin clots obtained by either of these processes do not lend themselves readily to any practical application, nor can their properties readily be varied.

One object of this invention is therefore to provide a method for producing fibrin clots susceptible of practical application in various fields now unavailable to clots of this character prepared in accordance with prevailing methods.

Another object of this invention is to provide a method for effecting, under controlled physical-chemical conditions, various types of fibrin clots, the respective types possessing different properties relative to clotting time, friability, adherence to surfaces, tensile strength and the like.

Another object of the invention is to provide a fibrin clot from which may be prepared a wide variety of fibrin products not heretofore known to the art and which are readily adapted to numerous practical uses such as burn and wound coverings, nerve, intestinal and artery sutures, artificial catgut, preventing adhesions in brain surgery, and for protecting vulnerable areas from irritants.

By the present invention, instead of clotting fibrin from whole blood or whole plasma, the plasma is first fractionated to obtain a more concentrated fibrinogen solution or one in which the fibrinogen is not associated with as large a relative amount of other proteins as in whole blood. This may be accomplished, for instance, by employing fractionated purified materials prepared by the methods described in the copending patent applications of Edwin J. Cohn, Serial Nos. 371,401; 430,075; and 460,121, filed December 23, 1940; February 9, 1942; and September 29, 1942, respectively, Serial No. 371,401 has been forfeited, Serial No. 430,075 is now Patent No. 2,390,074, and Serial No. 460,121 is abandoned.

The desired fibrinogen solution having been obtained, the fibrinogen is clotted with a fibrin ferment such as purified thrombin, which may be prepared by procedures known to those skilled in the art.

The physical-chemical conditions under which

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the fibrin clot is formed are carefully controlled, particularly the concentration of fibrinogen in the solution, the concentration with respect to thrombin, the pH, the ionic strength, and the temperature of the solution in which the clot is formed.

In this connection, it has been found that by varying these conditions the properties of the final clot can be altered with respect to clotting time, friability or ease of crushing, amount of spontaneous syneresis, tensile strength, elongation at break, modulus of elasticity, permanent set, etc.

It has also been found that the properties of the clot can be changed markedly by the addition of certain other substances such as polyhydric alcohols and starches.

The properties of our novel fibrin clots may be graded between two extremes. One extreme type, which we will refer to as type A, is transparent, gelatinous, friable, and easily crushed, with relatively low tensile strength and low permanent set. This type of clot does not synerize and adheres well to surfaces on which it is formed.

The other extreme type, which we shall refer to as type B, is opaque, plastic, non-friable, impossible to crush, with relatively high tensile strength and considerable permanent set. This type of clot synerizes enormously, contracting to 5% or less of its original volume and forming a compacted structure. It does not adhere well to surfaces on which it is formed.

Intermediate types can be prepared.

The conditions which favor type A clots are high pH, high ionic strength, high fibrinogen concentration, high thrombin concentration, and high temperature (e. g. 37° C.). The converse conditions favor type B clots.

Addition of polyhydric alcohols, for example glycols or glycerol, to the solution before clotting changes the properties toward those of a type A clot (except that the clotting time is increased). Addition of soluble starch changes the properties toward those of a type B clot.

The clotting time is generally decreased by changing conditions in the direction of formation of a type A clot, except in the case of addition of a polyhydric alcohol, which increases the clotting time.

As an example of the preparation of fibrinogen suitable for use in the formation of these novel clots, the following may be given:

The corpuscles are first removed from the blood by centrifugation, clotting of the fibrinogen being prevented by the addition of citrates or like agents.

Patented Nov. 20, 1951 2,576,006

UNITED STATES PATENT OFFICE

2,576,006

METHODS OF FORMING SHAPED FIBRIN PRODUCTS

John D. Ferry and Peter R. Morrison, Madison, Wis., assignors to Research Corporation, New York, N. Y., a corporation of New York

No Drawing. Application November 29, 1947, Serial No. 788,854

6 Claims. (Cl. 154—83)

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This invention relates to fibrin products having novel characteristics and to methods of preparing the same.

In our co-pending applications, Serial Nos. 507,903, now abandoned, and 507,904, now Patent No. 2,533,004, both filed on October 27, 1943, we have described and claimed novel fibrin clots and products, and methods for their preparation. The present application relates to further treatments of the films described in said application Serial No. 507,903, and to improvements and modifications in the treatments described in said application Serial No. 507,904 to produce fibrin products having highly valuable and improved properties.

One object of the present invention is the provision of fibrin products having improved water-equilibrated mechanical properties and to methods for producing the same. One valuable use of these fibrin products involves their implantation in animal body tissue either in the form of films or in some other physical form or shape. Since, as thus implanted, the products are in a state of equilibrium with tissue fluids (which is substantially equivalent to a state of equilibrium with water) their water-equilibrated mechanical properties are of fundamental interest and importance. The invention is particularly concerned with such water-equilibrated mechanical properties as tensile strength and modulus of elasticity.

A further object of the invention is the production of steam-sterilized fibrin products which have survived a treatment comparable in bactericidal action to that of standard sterilization procedures, with the result that, in the manufacture of products of this invention designed for surgical use, a final steam-sterilization step may be included. Such a step is much more easily performed and bactericidally more reliable than manufacture by aseptic technique.

The fibrin films of our co-pending application, Serial No. 507,903, cannot be heat-sterilized without substantial degradation of the fibrin accompanied by materially lowered tensile strength and loss of elasticity. Where a plasticizer such as glycerol displaces the water in the fibrin film, as described in our application Serial No. 507,904, the film withstands heat treatment somewhat better but again, as stated in said application,

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"protracted heating decreases the tensile strength and maximum elongation." Glycerol still has a degrading influence on the protein at elevated temperature though it is not as pronounced as that of water.

If then the materials of said prior applications are subjected to conventional sterilization techniques involving, for example, autoclaving at 120° C.± for twenty minutes more or less, their pre-sterilization physical properties are substantially impaired, if not wholly lost, as is the case with protein materials generally. On the contrary, the steam-sterilized products of this invention display improved water-equilibrated physical properties over the pre-sterilization properties, increasing their usefulness.

A fresh or intermediate stage fibrin clot comprising the product described in our application, Serial No. 507,903, may be prepared by the methods therein set forth from whole blood by separating the corpuscles by centrifugation, precipitation from the resulting plasma of fibrinogen, as by cooling the plasma to 0° C. to —3° C. and adding thereto an organic precipitant such as ethanol at controlled hydrogen ion concentration, ionic strength and pH, drying of the fibrinogen from a frozen state, and dissolution of the dried powder to a concentration of about 0.5% to 2% at 25° C., and clotting by addition to the fibrinogen solution at controlled pH and ionic strength of a clotting agent such as purified thrombin. The clots are then turned out on sheets of fine muslin and pressed between pieces of plate glass at a pressure of about 0.4 pounds per square inch to expel the synergetic fluid. The compaction time is dependent upon the concentration of thrombin solution previously added. In general the thrombin solution should be added in an amount to make a final concentration of about 0.3 units per cc. for optimal compaction time.

The thrombin unit referred to is that amount of thrombin which will clot one cc. of 1% fibrinogen solution at a pH of 7 and an ionic strength of 0.3 at 25° C. in fifteen seconds.

Fibrin film, as thus prepared in accordance with the disclosure of Serial No. 507,903 comprises a rubbery white opaque sheet containing about 30% fibrin and 70% water; its tensile strength in equilibrium with water may be in about the range—160–220 g./mm.² and it may

United States Patent [19]
Müller et al.

[11] **Patent Number: 4,548,736**
[45] **Date of Patent: Oct. 22, 1985**

[54] **PREPARATION OF PROTEIN FILMS**

[75] Inventors: **Michael F. Müller**, Münster, Fed. Rep. of Germany; **John D. Ferry**, Madison, Wis.

[73] Assignee: **Wisconsin Alumni Research Foundation**, Madison, Wis.

[21] Appl. No.: **527,345**

[22] Filed: **Aug. 29, 1983**

[51] **Int. Cl.**[4] **B01J 13/00**
[52] **U.S. Cl.** ..... **252/315.1**; 260/112 B; 424/101; 424/107
[58] **Field of Search** ..... 428/294, 293; 260/112 B, 117, 121, 112.5; 424/101, 107; 252/315.1

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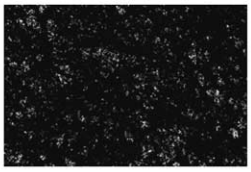
Primary Examiner—Edith Buffalow
Attorney, Agent, or Firm—Isaksen, Lathrop, Esch, Hart & Clark

[57] **ABSTRACT**

A method to prepare protein films from protein gels is disclosed which utilizes the process of osmosis to remove excess fluid from protein gels without destroying the structural integrity of the protein. The method is conducted by first preparing a protein gel in a solvent, then contacting the gel with one side of a semipermeable membrane permeable to the solvent in which the gel was prepared, next adding a concentrated polymer solution to the other side of the membrane which membrane is impermeable to the polymer so as to cause movement of solvent from the gel, through the membrane, and into the polymer solution, and thus removing solvent from the gel for a period of time sufficient to contract the gel to a desired thickness.

20 Claims, 2 Drawing Figures



A photograph of fibrin film that was found in John Ferry's files.