

fact, it will be seen that while collagen can be solubilized by pepsin in acid solutions, the rate of solubilization of the collagen is significantly promoted by the addition of calcium chloride or other neutral salt.

Example 14

Example 13 was repeated employing a 0.4% solution of dodecylamine in place of the calcium chloride solution. The dodecylamine was acidified to a pH of 3.0 employing acetic acid. After adding the enzyme, the digestion mass was agitated for 48 hours. 100% of the collagen was solubilized.

When the solubilization process of this example is carried out in the absence of dodecylamine, only 60% of the collagen is solubilized after 48 hours.

From the foregoing examples, it will be understood that collagen previously considered difficult to solubilize can be easily solubilized with the procedures of the present invention.

To ascertain the characteristics of solubilized collagen prepared in accordance with the present invention, the filtrate obtained in Examples 2 through 14 was dialyzed against 0.01 N in acetic acids and measurements were made of the specific optical rotatory power, viscosity, flow birefringence and sedimentation constant of the dialysate. Almost the same values were observed as those of a solution obtained by conventional methods, for example, by treating acid-soluble collagen with pepsin in the absence of a neutral bivalent salt or cationic surfactant. These results indicate that the insoluble collagen fibers can be converted into a collagen which exhibits the same properties as monomeric acid-soluble collagen. From the resulting solution obtained by the above method, collagen fibers can, of course, be reconstituted by conventional methods with a yield of 100%. Electron microscopic study demonstrates that the collagen fibers obtained with the procedures of the present invention have a striated structure having a period of about 640 A.-700 A. and are the same as native collagen fibers.

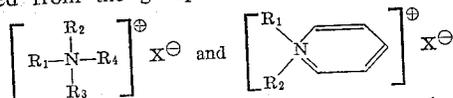
Ultracentrifuge study on the denatured state of the collagen obtained shows that the enzymes digest the telopeptide groups, in which covalent inter- and intramolecular crosslinks exist, and that the collagen molecules consist of almost non-crosslinked three polypeptide chains.

From the collagen solution thus obtained, preparations of film, yarn, textile, sponge, sheet, casing of collagen and the like can be prepared. After desalting and inactivating the enzyme, if the suspension of reconstituted collagen in water is heated to a temperature of 40° to 70° C., gelatin can be made in an extremely short period of time in comparison with other conventional methods of making gelatin.

The invention has been described in detail with particular reference to preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention as described hereinabove and as defined in the appended claims.

I claim:

1. A method for converting insoluble collagen into a fiber reconstitutable form in an aqueous solution which comprises treating insoluble collagen with a proteolytic enzyme other than collagenase which will hydrolyze the telopeptide group of collagen and which is active in a pH range of about 2 to 10 in the presence of an aqueous solution of at least one water-soluble cationic surfactant selected from the group consisting of

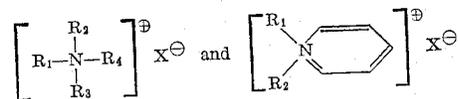


each of R_1 , R_2 , R_3 and R_4 being selected from the group consisting of hydrogen, lower alkyl radicals containing from 1 to 3 carbon atoms in the alkyl group, and long-

chain alkyl, aryl, and arylalkyl hydrocarbons containing from 6 to 20 carbon atoms, there being in said cationic surfactant from 1 to 3 hydrogen and lower alkyl radicals, and from 1 to 3 long-chain alkyl, aryl and arylalkyl radicals and X is a water-solubilizing anion, said cationic surfactant being present in a concentration between about 0.01 M and 0.1 M, said reaction being carried out at a pH between about 2 and 10 at which said enzyme is active and at a temperature below the denaturing temperature of said collagen.

2. A method for converting insoluble collagen into a fiber reconstitutable form in an aqueous solution which comprises treating insoluble collagen with a proteolytic enzyme other than collagenase which will hydrolyze the telopeptide group of collagen and which is active in pH range of about 2 to 10 in the presence of an aqueous solution of at least one water-soluble divalent metal salt of a metal selected from the group consisting of calcium, magnesium, barium, strontium, zinc, cadmium, and manganese with an acid selected from the group consisting of hydrochloric, sulfuric and acetic acid, said divalent metal salt having a concentration between about 0.01 and 1.5 molar, said reaction being carried out at a pH, between about 2 and 10, at which said enzyme is active and at a temperature below the denaturing temperature of the collagen.

3. A method for converting insoluble collagen into a fiber reconstitutable form in an aqueous solution which comprises treating an insoluble collagen with a proteolytic enzyme selected from the group consisting of trypsin, pepsin, ficin, bromelin, papain and proteolytic enzymes other than collagenase which will hydrolyze the telopeptide group of collagen and which are produced by microorganisms selected from the group consisting of *Bacillus subtilis*, *Streptomyces griseus*, *Streptomyces caespitosus*, *Aspergillus niger*, *Aspergillus saitoi*, *Aspergillus oryzae*, *Aspergillus niger van Tieghem*, *Trametes sanguinea* and *Paecilomyces varioti*, the maximum activity of said enzyme occurring at a pH between about 2 and about 10, in the presence of an aqueous solution consisting of at least one compound selected from the group consisting of cationic surfactants and which have a formula selected from the group consisting of



each of R_1 , R_2 , R_3 and R_4 being selected from the group consisting of hydrogen, lower alkyl radicals containing from 1 to 3 carbon atoms in the alkyl group, and long-chain alkyl, aryl, and arylalkyl hydrocarbons containing from 6 to 20 carbon atoms, there being in said cationic surfactants from 1 to 3 hydrogen and lower alkyl radicals, and from 1 to 3 long-chain alkyl, aryl and arylalkyl radicals and X is a water-solubilizing anion, and water-soluble divalent metal salts of metals selected from the group consisting of calcium, magnesium, barium, strontium, zinc, cadmium, and manganese with an acid selected from the group consisting of hydrochloric, sulfuric and acetic acid, the concentration of said cationic surfactant, when present, being between about 0.01 M and 0.1 M by weight and the concentration of said salt, when present, being between about 0.01 M and 1.5 M, said reaction being carried out at a pH between about 2 and about 10 at which said enzyme is active and at a temperature below the denaturing temperature of the collagen.

4. A process according to claim 3, wherein said enzyme is the enzyme produced by *Bacillus subtilis*.

5. A process according to claim 3, wherein said enzyme is produced by *Streptomyces griseus*.

6. A process according to claim 3, wherein said enzyme is pepsin.