

UNITED STATES PATENT OFFICE

2,535,684

RECOVERY OF PROTEIN FROM
KERATINOUS MATERIALS

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No Drawing. Application February 26, 1949,
Serial No. 78,663

11 Claims. (Cl. 260—123.7)

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This invention relates to new and useful improvements in the recovery of protein from keratinous materials.

Keratinous materials such as hair, hoofs, horns, feathers, wool and the like, may be subjected to a cystine reducing treatment to thereby obtain a desirable protein. The cystine reduction involves the conversion of the cystine linkage to cysteine. Sodium sulfide (Na_2S) at an alkaline pH has been successfully used in the past for the cystine reducing treatment. Normally, keratinous material, however, will, as such, not readily disperse in the sodium sulfide treating medium so that the desirable intimate contact between the reactants is not obtained resulting in an incomplete reaction and low yields. For the purpose of improving the dispersibility of the keratinous materials, their acid pre-treatment has been proposed. Such pre-treatment may, for instance, comprise the use of concentrated sulfuric acid or of formic or hydrochloric acid.

Both the hitherto proposed acid pre-treatment of keratinous materials and the sodium sulfide cystine reduction are subject to certain disadvantages and drawbacks. If, in the acid pre-treatment, a concentrated acid is used, relatively stable salts of the acids with a keratin are formed and a partial deterioration of the peptide linkages occurs resulting in an appreciable loss of material available for cystine reduction. Furthermore, the protein salts have different solubility properties and indicate per se an alteration of the protein. On the other hand if relatively dilute acids, such as formic acid and hydrochloric acid, are used, although no appreciable salt formation may take place under certain conditions and although they tend to "soften" difficultly soluble parts of the keratinous material as, for instance, the epidermal layer and cortical fibrils of wool, they do exercise a considerable solvent action on the keratinous material resulting in a loss thereof and thus of ultimate yield of protein upon cystine reduction.

The sodium sulfide cystine reducing treatment requires relatively large amounts of this chemical and an excess thereof is normally necessary to effect a satisfactory reduction of the cystine grouping to cysteine. On the other hand, sodium sulfide possesses an appreciable hydrolytic and otherwise destructive influence upon protein so that the required excess of this material is reflected in impaired yields of protein resulting from the cystine reduction.

One object of the instant invention comprises an improved method for the recovery of protein from keratinous materials.

Another object of the invention comprises an improved acid pre-treatment of keratinous materials as part of the processing thereof for the recovery of protein.

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Still another object of the invention is an improved cystine reduction of acid pre-treated keratinous materials as part of the processing thereof for the recovery of protein.

The foregoing and still further objects of the invention will be apparent from the following description.

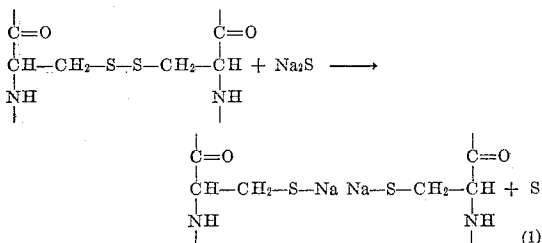
I have discovered that, when treating a keratinous material such as hair, hoofs, horns, feathers, wool, etc., with a polyvalent acid being one of a 12–14 N phosphoric acid and 8–9 N sulfuric acid at room temperature, for a period of time and using an amount of acid sufficient to render the keratinous material readily dispersible in an alkaline sodium sulfide solution, only a negligible loss of keratinous material occurs. Whereas, for instance, the use of a 4–7 N hydrochloric acid gives rise to a loss of approximately 15% by weight of keratinous material (degreased wool) the treatment with either a 12 to 14 N phosphoric acid or 8 to 9 N sulfuric acid shows a loss of usually not more than about 2–3% by weight of the same material treated. Furthermore, analysis of the keratinous material treated with either the phosphoric acid or the sulfuric acid of the strength indicated showed the complete absence of salt formation between the acid and the keratin.

When applying the acid pre-treatment in accordance with the invention, the range of normality of the acid medium is critical. The normality of the treating medium should be checked after the addition of the acid to the keratinous material. In many cases by reason of alkaline residues or impurities the normality of the medium may become reduced from that of the acid added. In such cases, the normality should be readjusted and be brought back to within the range of 12–14 N for phosphoric acid or 8–9 N for sulfuric acid. The amount of acid should be sufficient to completely submerge the keratinous material in the same. An excess of acid is normally not harmful. For most purposes, and particularly for wool, a ratio by weight of 8 parts of the acid to one part of the keratinous material will suffice for proper submergence. The temperature should be room temperature, i. e., a temperature within a broad range of from about 18–25° C. The completion of the acid pre-treatment is best determined for any given keratinous substance by removing samples from time to time and checking these samples for ready dispersibility in a sodium sulfide cystine reducing medium. In the majority of cases, however, a period of 60 hours will suffice for the completion of the acid pre-treatment, i. e., the conversion of the keratinous material into a form readily dispersible in a cystine reducing agent containing sodium sulfide.

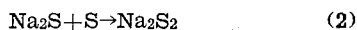
In the practice of the cystine reducing treat-

ment in accordance with the invention a suitably acid pre-treated keratinous material, and preferably a keratinous material subjected to acid pre-treatment with one of 12 to 14 N phosphoric acid and 8 to 9 N sulfuric acid, is subjected to the action of a mixture comprising 3 to 5% by weight of dry keratinous material sodium sulfide (Na_2S) and 3 to 6% by weight of dry keratinous material sodium sulfoxylate ($\text{Na}_2\text{S}_2\text{O}_4$) at an alkaline pH of about 9 to 10 and at a temperature of less than 60°C . and preferably of from 20 – 60°C . Heating is continued until the keratinous material is substantially completely dissolved, which is, in most cases, accomplished after a period of heating of from 45–60 minutes. The hot solution is then filtered and/or centrifuged and permitted to cool. Thereupon, the cold protein solution is precipitated with acid while constantly stirred. Best results are obtained by adding the acid through the hollow stem of a stirrer. The addition of acid is continued until the solution becomes substantially a rigid mass which normally occurs when the pH reaches about a value of 5 to 5.2. The end point of acidification may be visually determined by the appearance of curdling in the protein mass. Within the preferred practice of the treatment in accordance with the invention, I find it of advantage to add the sodium sulfide-sodium sulfoxylate solution to the washed, acid pre-treated keratinous material, stirring the cold mass until gelatinous and thereafter warm with constant stirring to the desired reaction temperature. In the event that the sodium sulfide is used in the form of the usual commercial hydrate ($9\text{H}_2\text{O}$) this should be taken into account and calculated within the above set forth percentage range for sodium sulfide as water free.

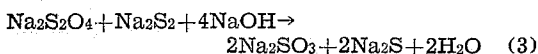
When the proceeding with the cystine reducing treatment in accordance with the invention using the combination of sodium sulfide and sodium sulfoxylate as hereinabove set forth, appreciably lesser amounts of sodium sulfide are required than would be the case if sodium sulfide alone or in conjunction with some chemical other than sodium sulfoxylate were used. The reaction mechanism involved in the cystine reduction when proceeding in accordance with the instant invention may be exemplified as follows. The sodium sulfide (Na_2S) in the mixture reduces the cystine in wool to cysteine, liberating sulfur:



The liberated sulfur is immediately taken up by the remaining Na_2S to form the disulfide:

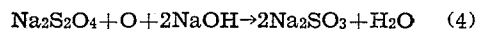


Sodium sulfoxylate reacts spontaneously with the disulfide:



Thus, the sulfur liberated in the cystine to cysteine reduction is continuously used for the regeneration of sodium sulfide (Na_2S) which is again ready to reduce the cystine to cysteine. In

this manner, appreciably lesser amounts of sodium sulfide can be used to effectuate the same result and at the same time avoid all the disadvantages attending the use of sodium sulfide in the normally large quantities. Furthermore, in addition to materially reducing the deteriorating and hydrolytic influence of Na_2S on protein, savings per se in amount of material used are accomplished. Still further, oxidation by oxygen either originally in the dispersing bath or entering the open bath during dispersion, is also beneficially influenced by the sulfoxylate:



Towards the end of the cystine reduction, most of the sulfoxylate has been used up and there is a tendency for the liberation of free sulfur in the solution. The presence, however, of the sodium sulfite (Na_2SO_3) (see 3 and 4 above) produced in the regeneration of the sodium sulfide (see 3 above) functions to absorb any free sulfur readily forming therewith sodium hyposulfite ($\text{Na}_2\text{S}_2\text{O}_3$):



Thus, when proceeding in accordance with the instant invention, the protein precipitated from the solution with acid (at a pH of about 5–5.2) is substantially free of salts and sulfur and is recovered in exceptionally high yields. All of the reaction products that finally result from the cystine reduction treatment in accordance with the invention are substantially water soluble and can be easily removed from the acid precipitated protein by washing with water or other aqueous media at a substantially neutral pH at which keratinous proteins are substantially insoluble.

Analysis further showed that there was no evidence of any deterioration or modification of any of the amino acids that are normally a constituent part of keratinous substances.

The following examples are furnished by way of illustration but not of limitation.

Example I

Take 900 cc. of phosphoric acid having a strength of 13.0 N. The temperature of this solution should be less than 25°C .

Into this solution immerse 110–120 grams of wool (containing approximately 10% moisture); assure good wetting of the material and mixing of the acid by evacuation or by some kind of stirring motion. Take care that all wool is covered by the liquid. Check again normality which should not be below 12.0 N. If necessary, the normality should be readjusted and preferably to its original strength of 13.0 N. Finally close container and let stand. A sample drawn after 60 hours and thoroughly washed with water was readily dispersible in an aqueous solution containing about 3.4% $\text{Na}_2\text{S}_2\text{O}_4$, 9.4% $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ and 2.4% NaOH .

Now drain acid as completely as possible, add approximately 200 cc. of cold water (preferably by means of a spray) and drain again; both drainings should total approximately 900 cc. of liquid. After readjustment to 13 N strength, this acid may be reused again for acid pre-treatment. The wool is then washed with cold water removing thus most of the acid left in the material. For complete neutralization add slowly preferably in small portions, 1–2 grams of soda ash in form of a 10% solution; the pH of neutralization bath should not even temporarily be greater than 8. Let stand for at least one hour, then rinse thor-

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oughly with cold water; the material may then be hydro-extracted and dried if desired.

Example II

Take 200 cc. of concentrated sulfuric acid and make up by proper procedure to a total volume of 900 cc., adjust strength of acid to 8 N, temperature should be less than 25° C.

Into this solution immerse 110-120 grams of wool (containing approximately 10% moisture); assure good wetting of the material and mixing of the acid by evacuation or by some kind of stirring motion. Take care that all wool is covered by the liquid. Check again normality which should not be much below 8 N. If necessary, the normality should be readjusted. Finally, let stand. A sample drawn after 60 hours and thoroughly washed with water was readily dispersible in an aqueous solution containing about 3.4% $\text{Na}_2\text{S}_2\text{O}_4$, 9.4% $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ and 2.4% NaOH.

Now drain acid as completely as possible, add approximately 290 cc. of cold water (preferably by means of a spray) and drain again; both drainings should total approximately 900 cc. of liquid. After readjustment to 8 N strength, this acid may be reused again for acid pretreatment. The wool is then washed with cold water removing thus most of the acid left in the material. For complete neutralization add slowly preferably in small portions 1-2 grams of soda ash in form of a 10% solution; the pH of neutralization bath should not even temporarily be greater than 8. Let stand for at least one hour, then rinse thoroughly with cold water; the material may then be hydro-extracted and dried if desired.

Example III

80 grams of acid pre-treated and neutralized wool (containing approximately 10% moisture) and obtained in the manner set forth in either Example I or II, are placed in a 2000 cc. beaker whereupon 900 cc. of cold water is added and the material wetted out as well as possible by suitable stirring. To this is then added 100 cc. of an aqueous solution containing about 2.4 grams NaOH, 3.4 grams $\text{Na}_2\text{S}_2\text{O}_4$ (4.7% per weight of dry wool) and 9.3 grams $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ (4.2% Na_2S per dry weight of wool). The cold mass is stirred until gelatinous and is thereupon warmed with constant stirring to a temperature of about 50° C. (the temperature should be guarded against exceeding 60° C.). The pH of the solution should be approximately between 9 and 10 and preferably at about 9.5. Temperature and stirring are maintained until the wool is dissolved. Substantial dissolution will be effected after about 45-60 minutes. Thereafter, the solution is filtered and/or centrifuged while hot.

After cooling, the cold centrifuged and/or filtered protein solution is now precipitated in the conventional manner with, for instance, sulfuric acid. In order to achieve best results, it is preferred to add the acid through the hollow stem of the stirrer. Acid addition is continued until the solution becomes a substantially rigid mass which is normally achieved at a pH of about 5.1. The end point of acid addition may be easily observed by the appearance of curdling in the protein mass. It is recommended that once this point is attained, a few ccs. of extra acid solution be added under stirring to assure complete and uniform precipitation.

The further working up of the precipitated protein is also carried on in the conventional manner by separating the liquid from the protein,

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draining, centrifuging or filtering the liquid, and washing the precipitated protein with minimum amounts of cold water having a pH of approximately 5.1 until a sample of cold wash water gives only a slight discoloration with silver nitrate solution (as test for hyposulfite ion). The protein is now ready for drying either in the air, in an oven or in vacuo. The preferred method is drying in vacuo over caustic as it will remove from the protein any trace of possibly unpleasant odor.

Example IV

Chicken feathers cleaned and defatted and acid pre-treated in accordance with the procedure set forth in Examples I or II are used. 86 grams of these acid pre-treated chicken feathers and containing about 80 grams of dry material are placed in 900 cc. of cold water and wetted out as well as possible by suitable stirring. To this is then added 100 cc. of an aqueous solution containing 9.3 grams of $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ (3.8% Na_2S per dry weight of keratin), 3.4 grams $\text{Na}_2\text{S}_2\text{O}_4$ (4.2% per dry weight of keratin) and 2.5 grams NaOH (3.1% per dry weight of keratin).

The cold mass is then stirred until gelatinous and thereafter heated with constant stirring to a temperature of about 50° C. avoiding at any time that the temperature exceeds 60° C., the pH substantially maintained around 9.5 and heating and stirring continued until the feathers are dissolved which takes place in about 45 to 60 minutes. The solution is then filtered or centrifuged while hot and the protein precipitated, washed and recovered as set forth in the preceding example.

Example V

Cleaned and defatted filings from cow horn, and acid pre-treated in accordance with Examples I or II, are used. 88 grams of such acid pre-treated filings and containing about 10% moisture are placed in 900 cc. of cold water and wetted out as well as possible by suitable stirring. There is then added 100 cc. of an aqueous solution containing approximately 11.4 grams $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ (4.6% Na_2S per dry weight of keratin), 4.2 grams $\text{Na}_2\text{S}_2\text{O}_4$ (5.2% $\text{Na}_2\text{S}_2\text{O}_4$ per dry weight of keratin), and 3 grams NaOH (3.7% NaOH per dry weight of keratin). The cold mass of the horn material is then stirred until gelatinous and thereafter heated with constant stirring to a temperature of about 50° C. taking care that the temperature does not at any time exceed substantially 60° C. The pH is substantially maintained around 9.5. The heating and stirring are continued until the material is dissolved, which will take place in about 45 to 60 minutes. The solution is then filtered or centrifuged while hot, and the protein precipitated is further worked up for the recovery of the protein as set forth in Example III.

Within the preferred embodiment of my invention, best results are obtained particularly with respect to yield, quality and purity when using the combination of my novel acid pre-treatment in conjunction with the herein set forth sodium sulfide-sodium sulfoxylate cystine reduction. It is to be understood, however, that my novel cystine reduction treatment may be used with the realization of the therein inherent sodium sulfide regeneration independent of the particular pre-treatment to which a given keratinous material may have been subjected to adapt the same to the $\text{Na}_2\text{S}-\text{Na}_2\text{S}_2\text{O}_4$ cystine reduction. Thus, for

instance, a suitable keratinous material may be subjected to acid pre-treatment with, for example, a 4 to 7 N HCl solution. Though such hydrochloric acid treatment involves the loss of approximately 15% of keratin, as against a loss of not more than about 2% of keratin when utilizing the acid pre-treatment in accordance with my invention, the product resulting from the HCl treatment is dispersible in the sulfide-sulfoxylate solution in accordance with the invention and may be subjected to such treatment in accordance with any one of the above Examples III to V. In such cases, although the ultimate yield of undeteriorated protein (calculated on the keratinous material used in the acid pre-treatment) is appreciably less than when proceeding in accordance with the sulfuric acid or phosphoric acid treatment herein specified, the regeneration of sodium sulfide with its attendant advantages permits the highly beneficial results of the treatment to assert themselves even with such HCl pre-treated material.

As will be seen from the foregoing, my invention, within the broad scope thereof, essentially embraces in the recovery of protein from keratinous materials, the improvement which comprises subjecting a keratinous material, pre-treated for Na₂S cystine reduction, to the action of an aqueous solution, containing per dry weight of keratinous material present about 3 to 5% Na₂S and about 3 to 6% Na₂S₂O₄, at a pH of about 9 to 10 and preferably about 9.5 and at a temperature of less than 60° C. and preferably of from 20 to 60° C., the amount of aqueous medium being sufficient to substantially cover the keratinous material present, and the combined Na₂S—Na₂S₂O₄ concentration in said medium being sufficient for cystine reduction, preferably from 0.2–1% and for best results from about 0.5–0.8%, maintaining the reaction mass at said pH and at said temperature until the keratinous material is substantially dissolved and recovering protein from said solution.

Within the broad preferred embodiment of the invention, the pre-treated keratinous material is an acid pre-treated keratinous material and preferably a keratinous material pre-treated for Na₂S cystine reduction with one of about 12 N–14 N H₃PO₄ and about 8 N–9 N H₂SO₄ and preferably one of about 13 N H₃PO₄ and about 8 N H₂SO₄ at room temperature, and preferably between 18–25° C., the amount of acid used being sufficient to substantially submerge the keratinous material therein, and maintaining the reaction mass at said acid normality until the keratinous material is substantially readily dispersible in an aqueous solution containing by weight thereof .25–.35% Na₂S and .3–.4% Na₂S₂O₄.

The remarkable results obtained in the cystine reduction in accordance with the invention using the sodium sulfide-sulfoxylate combination are particularly unexpected in view of the fact that normally mixtures of sodium sulfoxylate and sodium sulfide outside of the range of percentage specified therefor in accordance with the instant invention possess a synergistic damaging effect upon keratinous materials and particularly wool fibers which is considerably in excess of the individual damaging effect exercised upon such materials by either one of these chemicals alone even in their higher concentrations. It is within the very specific ranges and percentages herein recited that this damaging effect is substantially eliminated, thus giving rise to the highest possible yields of the reducing treatment of cystine to cysteine.

Although for the sake of convenience and simplicity, NaOH has been specified in the above formulae as participating in the reaction involving the regeneration of Na₂S, it is understood that the pH of the reaction is the controlling factor in that at the alkaline pH of about 9 to 10 specified for the cystine reducing treatment in accordance with the invention, dissociated cations and OH ions are present in the solution and they react as such irrespective of the specific nature of the cation present. Where reference, therefore, is made herein to the pH range of 9 to 10 in connection with the cystine reducing treatment in accordance with the invention, it is understood that it involves the presence of the requisite amount of dissociated cations and OH ions controlling the particular pH and participating in the reaction mechanism as hereinabove exemplified.

I claim:

1. In the recovery of protein from keratinous materials the improvement which comprises subjecting a keratinous material, acid pre-treated for Na₂S cystine reduction, to the action of an aqueous solution, containing per dry weight of keratinous material present about 3–5% Na₂S and about 3–6% Na₂S₂O₄, at a pH of about 9 to 10 and at a temperature of less than 60° C., the amount of aqueous medium being sufficient to substantially cover the keratinous material present and the combined Na₂S—Na₂S₂O₄ concentration in said medium being sufficient for cystine reduction of said keratinous material, maintaining the reaction mass at said pH and at said temperature until the keratinous material is substantially dissolved and recovering protein from said solution.

2. Improvement in accordance with claim 1 in which said keratinous material is subjected to the action of said aqueous solution at a temperature substantially from 20–60° C.

3. Improvement in accordance with claim 2 in which said combined Na₂S—Na₂S₂O₄ concentration in said medium is substantially from 0.2 to 1%.

4. Improvement in accordance with claim 3 in which said keratinous material is subjected to the action of said aqueous solution at a pH of about 9.5 and in which said combined

Na₂S—Na₂S₂O₄

concentration in said medium is from about 0.5 to 0.8%.

5. In the recovery of protein from keratinous materials the improvement which comprises subjecting such material to the action of one member of the group consisting of about 12 N–14 N H₃PO₄ and about 8 N–9 N H₂SO₄, the amount of acid used being sufficient to substantially submerge the keratinous material therein, and maintaining the reaction mass at said acid normality until the keratinous material is substantially readily dispersible in an aqueous solution containing by weight thereof .25–.35% Na₂S and .3–.4% Na₂S₂O₄.

6. Improvement in accordance with claim 5 in which said keratinous material is subjected to said acid treatment at a temperature from about 18–25° C. and in which the reaction mass is maintained substantially at said temperature.

7. Improvement in accordance with claim 6 in which said keratinous material is subjected to the action of one member of the group consisting of about 13 N H₃PO₄ and about 8 N H₂SO₄.

8. In the recovery protein from keratinous ma-

terials the improvement which comprises subjecting keratinous material to the action of one member of the group consisting of about 12 N-14 N H_3PO_4 and about 8 N-9 N H_2SO_4 the amount of acid used being sufficient to substantially submerge the keratinous material therein, maintaining the reaction mass at said acid normality until the keratinous material is substantially readily dispersible in an aqueous solution containing by weight thereof .25-.35% Na_2S and .3-.4% $\text{Na}_2\text{S}_2\text{O}_4$, thereafter subjecting the neutralized and washed thusly pre-treated keratinous material to the action of an aqueous solution, containing per dry weight of keratinous material present about 3-5% Na_2S and about 3-6% $\text{Na}_2\text{S}_2\text{O}_4$ at a pH of about 9 to 10 and at a temperature of less than 60° C., the amount of said last mentioned aqueous medium being sufficient to substantially cover the keratinous material present and the combined Na_2S - $\text{Na}_2\text{S}_2\text{O}_4$ concentration in said medium being sufficient for cystine reduction, maintaining the reaction mass at said pH and at said temperature until the keratinous material is substantially dissolved, and recovering protein from said solution.

9. Improvement in accordance with claim 8 in which said keratinous material is subjected to said acid action at a temperature of about 18-25° C., in which said acid treatment is substantially maintained at said temperature of about 18-25° C. in which said acid treated keratinous material is subjected to the action of said aqueous solution containing said Na_2S and said $\text{Na}_2\text{S}_2\text{O}_4$ at a temperature of about 20-60° C., and in which

said Na_2S - $\text{Na}_2\text{S}_2\text{O}_4$ reaction mass is substantially maintained at said last mentioned temperature.

10. Improvement in accordance with claim 9 in which the combined Na_2S - $\text{Na}_2\text{S}_2\text{O}_4$ concentration in said aqueous medium is from about 0.2 to 1%.

11. Improvement in accordance with claim 10 in which said keratinous material is subjected to the action of one member of the group consisting of about 13 N H_3PO_4 and about 8 N H_2SO_4 , in which said acid treated keratinous material is subjected to the action of said aqueous Na_2S - $\text{Na}_2\text{S}_2\text{O}_4$ solution at a pH of about 9.5, in which the said last mentioned pH is substantially maintained in the Na_2S - $\text{Na}_2\text{S}_2\text{O}_4$ reaction mass until the keratinous material is substantially dissolved, and in which the combined Na_2S - $\text{Na}_2\text{S}_2\text{O}_4$ concentration in said medium is sufficient for cystine reduction.

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