

[54] **KERATIN POLYPEPTIDE HYDROLYZATES AS HAIR TREATING AGENTS**

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[58] Field of Search **424/71, 72; 132/7; 8/127.51, 128**

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[57] **ABSTRACT**

The invention relates to compositions for the treatment of filamentous keratins, for example, to cosmetic compositions for the treatment of human hair and compositions for modifying animal hair or fur. The compositions are made up of water soluble peptide products of partial hydrolysis of keratinaceous materials, such as hog hair, resulting from hydrolysis using acids under conditions which, while breaking down the complex proteins, will leave a substantial portion of the disulfide linkages present in the keratinaceous materials intact. Filamentous keratins have the peptide products chemically bonded thereto by a two step process wherein disulfide linkages of both the peptide products and the filamentous keratins are split by the action of a reducing agent and disulfide linkages are then reformed by action of an oxidizing agent whereby at least some of the sulfhydryl groups of the peptide products formed by the action of the reducing agent are bonded to sulfhydryl groups of the filamentous keratins.

2 Claims, No Drawings

KERATIN POLYPEPTIDE HYDROLYZATES AS HAIR TREATING AGENTS

This invention relates to materials for modification of filamentous keratins such as human hair, animal hair and similar filamentous keratin products by being chemically bonded thereto so as to become an integral portion thereof. More particularly, it relates to a method for the coupling of the product of partial hydrolysis of a keratinaceous material to filamentous keratins by cross-linking of sulfhydryl groups to improve such characteristics as strength and manageability and to impart gloss.

In accordance with this invention, compositions derived from natural sources of keratin by hydrolysis under conditions to preserve a substantial portion of the disulfide linkages of the peptide or amino acid such as cystine intact, are produced which have utility in aqueous media for treatment and protection, during treatment of filamentous type keratin materials, such as human hair, animal hair such as wool, fur, etc.

Hair, including human hair, and the animal hairs such as wool and fur, consists of strands of keratin fibers surmounted by a scaly cuticle of keratin protein. Keratin is unique in its content of sulfur-containing amino acids, in particular the amino acid cystine. The disulfide linkages of cystine can be broken to produce at least one free sulfhydryl group by means of reducing agents, and the linkages can be reclosed to recover the original cystine by use of an oxidizing agent. If hair strands are placed under stress, many of the cystine disulfide units are in a state of tension. If the strands are treated with a reducing agent while under stress, the disulfide units are broken. If, while still under stress, after the disulfide units have been opened up, the strands are subjected to an oxidizing agent, the disulfide units are reformed, but now they are reformed with different half-cystine units in a way that the stress now leaves the strands in their new position, since the geometry of the strands is "locked in" to the new configuration. This is the basis of the well-known permanent wave process in which the hair is treated after curling with ammonium thioglycolate to open up the disulfide bonds, after which the curled hair is oxidized with sodium bromate or other oxidizing agents to form the permanent curl.

It is well known that proteinaceous materials can be produced of a character which can be adsorbed or absorbed or both when applied to hair. The disadvantage of the hydrolyzed proteins such as gelatin, etc., is the non-uniformity in hydrolysis, the salt content present due to neutralizing the hydrolyzing agents, and the loss of significant amounts of the hydrolyzates when the treated hair is subjected to rinses, etc.

It is also well known that treatment of filamentous keratin materials such as human hair with reducing agents will effect cleavage of the disulfide linkages. Where the hair, wool, etc., are treated with reducing and oxidizing agents such as when treating with hair-waving preparations, the action is damaging and results in a marked loss in weight from virgin hair strands, and the hair becomes weak and brittle. Such damage is accentuated when such treatments are applied to bleached hair.

In keratin fiber dyeing procedures, such as those used in the treatment of wool, it is generally necessary to heat the keratin fiber with the dye in water at the boil-

ing point to insure fastness of the dye. The results of such treatment are that with a fast dye the color can no longer be removed readily and the keratin fibers become tenderized by the treatments to add or remove the dye.

Reducing and oxidizing agents are commonly used in the creating of permanent wave sets for human hair. The hair is put under stress as by winding on rollers and a reducing agent applied, such as ammonium thioglycolate, after which the reagent is drained off or rinsed out and the hair treated with an oxidizing agent such as sodium bromate or hydrogen peroxide. During treatment with the reducing agent, the stress on the hair is minimized by the opening up of the cystine disulfide linkages. Upon oxidation, cystine disulfide linkages are reformed but the closure occurs between not only a portion of the original sulfhydryl groups which developed during the reduction splitting but primarily between sulfhydryl groups which have been brought into close proximity as a result of distortion of the keratin filaments due to the applied stress. By such action, the hair strand is reformed but with a new waved structure. This remains as a permanent feature of the hair strands until the hair is subjected to another waving procedure or until the hair strands grow out.

Now it has been discovered that if keratin-containing material, such as hog hair, is partially hydrolyzed through the use of dilute phosphoric acid or of multifunctional organic acids in a manner such that a substantial proportion of the cystine disulfide units are retained intact, the keratin polypeptides obtained have interesting properties.

Since the chemical composition of the keratin polypeptide has considerable similarity to that of the hair or wool, there is no direct chemical proof that a reaction occurs to link the polypeptide directly to the hair other than by the use of keratin polypeptides containing radioactive elements. However, after treatment of the hair or wool with the reducing agent containing the keratin polypeptide, there is generally a gain in weight of the hair or wool swatch, while with the reducing agent alone there is a sharp loss in weight. The hair also retains its structure, form and sheen in the presence of the polypeptide, while keratins treated with the reducing agent alone show brittleness, shrinkage and loss in strength.

A more definitive proof that linkage has occurred is demonstrated when the keratin polypeptides are coupled through their free amino groups to a diazotized dye intermediate by known means, and the dye-coupled keratin polypeptide is isolated. When human hair or wool is treated in pH 9.2 buffer with the dye-coupled polypeptide and washed with water, all of the dye-coupled polypeptide washes out, since there has been no chemical combination of the two components and there is little or no physical sorption of the dye-coupled derivative upon the hair or wool. If, however, the dye-coupled polypeptide is dissolved in ammonium thioglycolate at pH 9.2, and the human hair or wool is treated with this solution, drained and reoxidized with a mild oxidant, the hair strands or wool swatches are permanently dyed, and the dye cannot be washed out with water, detergents, acids or alkalis, or organic solvents.

Coupling of the dye-keratin polypeptide derivative with the hair or wool occurs at room temperature, and there is no necessity for heating the reaction mixture in

boiling water. The extent of linkage of the dye-keratin polypeptide complex to the hair or wool is a function of the concentration of the complex in the reducing solution, the concentration of added unmodified keratin polypeptides, if any, and the reaction time used. Thus, by modifying the conditions, any shade or tint of the dye can be obtained.

Wool can be modified in the form of yarn, or after weaving. Permanent prints can be made on wool if, for example, dry virgin wool is treated with solutions of the keratin-dye complex in a reducing solution by means of a printing roller in which the design is transferred to the flannel, dried and subjected to a flow of air to obtain atmospheric oxidation, or passed through a dilute solution of mild oxidant, washed, and dried by normal means.

As has been pointed out above, the modification impressed on the hair or wool is permanent and is not removed by the ordinary rinse or shampoo treatments. There is one method, however, by which the modified keratin polypeptide can be substantially removed from the hair or wool product. This method is to treat the hair or wool product with thioglycolate alone, in order to reopen the disulfide linkages, wash the product well with water, and reoxidize with a mild oxidant. However, since repeated reductant and oxidant treatments tend to degrade the hair or wool, it is preferred to inhibit the degradation by adding unmodified keratin polypeptide to the reductant, so the modified derivative is replaced substantially by unmodified keratin polypeptide which has the ability to minimize the damage to hair of such chemical actions.

When preparing the keratin polypeptide having utility as hair modifiers, the number of intact disulfide linkages remaining in the keratin polypeptides is dependent upon the purity and cleanliness of the initial hog hair, and the processing conditions for hydrolysis. The cleaner the hair, the higher is the amount of intact disulfide linkage. The more drastic the digestion conditions generally the lower the amount of intact disulfide linkages. Since, in a partial hydrolysis, the product will be made up of a heterogeneous mixture of substances of different molecular weights, the measure of disulfide units is an average one. On separation of a hydrolysis mixture by reverse osmosis, it was found that the lowest molecular weight fraction, below 1,000, had the smallest number of intact disulfide linkages, while the fraction of molecular weight 1,000 to 10,000 had a larger number, and the fraction with a molecular weight over 10,000 had the largest number of intact disulfide linkages.

The extent of disulfide units is measured by known means, using a polarograph and a rotating platinum electrode, with titration of the sulfhydryl groups amperometrically with methyl mercuric iodide. Under optimum conditions, hydrolysis of clean hog hair with 85 percent phosphoric acid for 10 to 15 minutes at 135°C. gives a product which shows approximately 50 moles of disulfide linkage per 100,000 grams of hair. This value approximates the value for the total sulfur content of the hair. Under plant operating conditions, however, where it is uneconomical to purify the hair completely and the use of highly purified reagents is impractical, intact disulfide values of 15 to 49 moles per 100,000 grams are normally found, which is the product of subsection of keratin-containing material to heat for period of 1 to 24 hours at temperatures in the range between

100°C. and 160°C. in the presence of acid having a concentration in the range between 4 and 85 percent and in quantities to maintain a pH of less than 4 throughout the hydrolysis reaction, said period varying inversely with the temperature level. All keratin polypeptides, for example, dipeptides, tripeptides, tetrapeptides, etc. provided the peptides still contain one or more intact cystine units, are applicable for use in this invention.

In Table I are listed a few of the results obtained by the use of this invention. These results are based on weight changes, so relative differences in one series are comparable. However, it is not always possible to compare the results in one series with those in another, since the weight changes are occasionally modified by changes in moisture content due to changes in relative humidity.

After treatment with keratin polypeptide, the samples were washed thoroughly with water and detergent, dehydrated with acetone, and finally air-dried.

After treatment with ammonium thioglycolate the samples were washed with water, oxidized for 5 minutes with 1.5 percent sodium bromate solution, washed again with water and detergent, dehydrated with acetone, followed by air-drying.

After treatment with mixtures of keratin polypeptides and ammonium thioglycolate, the samples were drained a short time to remove the excess solution, after which they were oxidized with 1.5 percent sodium bromate solution for 5 minutes, and washed and dried as above.

The wool swatches were 100 percent worsted flannel, and the human hair samples were of white virgin hair and a medium bleached hair obtained from commercial sources.

The invention will be further understood from the following examples which are given for the purposes of illustration and without any intention that the invention be limited thereto.

The method of preparing a water soluble product by partial hydrolysis of keratinaceous materials is as follows:

EXAMPLE I

To 100 grams of 75% H_3PO_4 , heated in a large test tube to 125°C. to 130°C. in an oil bath, was added portions of hair over a period of 5 hours. A total of 56 grams of hair was added, and this amount appeared to be about the maximum which could be added under these conditions. The mixture was heated for another 1.5 hours at this temperature, and cooled. No unchanged hair particles were observed. The mixture was then diluted with 4 to 5 volumes of water, centrifuged to remove dark insoluble material, and the supernatant, at pH 1.7, was brought up to pH 6.7 with solid $CaCO_3$. The light yellow filtrate was concentrated to approximately 50 percent polypeptide solids by vacuum evaporation.

The method of treating hair to incorporate the products of hydrolysis as an integral part of filamentous keratins is illustrated by the following examples.

EXAMPLE II

50 grams of the dry hydrolyzate product of Example I were dissolved in 1,000 grams of aqueous solution containing 6 percent by weight of ammonium thioglycolate to form a 5 percent by weight solution. Coils of medium bleached hair strands were placed in the solu-

tion for 15 minutes, the solution drained off and then the coils are oxidized by treatment for 5 minutes with an aqueous solution containing 1.5 percent by weight of sodium bromate. The hair coils were then washed with water, detergent, acetone, alcohol and finally ether. Additional coils of the medium bleached hair were similarly treated with the 6 percent ammonium thioglycolate solution not containing any hydrolyzate. Similar coupling operations were carried out on white virgin hair using 6 percent ammonium thioglycolate solutions.

EXAMPLE III

250 grams of the dry hydrolyzate product of Example I were dissolved in 1,000 grams of aqueous solution containing 6 percent by weight of ammonium thioglycolate to form a 25 percent by weight solution. Coils of medium bleached hair strands were placed in the solution for 15 minutes, the solution drained off and then oxidized by treatment for 5 minutes with an aqueous solution containing 1.5 percent by weight of sodium bromate. The hair coils were then washed with water, detergent, acetone, alcohol and finally ether. Additional coils of the medium bleached hair were similarly treated with the 4 percent ammonium thioglycolate solution not containing any hydrolyzate. Similar coupling operations were carried out on white virgin hair using 4 percent ammonium thioglycolate solutions.

bromate. The wool segment was then washed with water, detergent, acetone, alcohol and finally ether.

Additional segments of wool flannel were similarly treated with 8 percent ammonium thioglycolate solution not containing any hydrolyzate.

Additional segments of wool flannel were treated in a similar manner with an 8 percent thioglycolate solution containing 10 percent and 20 percent by weight of the hydrolyzate product of Example I.

Details of the additional wool treatment and the results thereof are set forth in Examples VI and VII.

EXAMPLE V

200 grams of the dry hydrolyzate product of Example I were dissolved in 1,000 grams of aqueous solution containing 4 percent by weight of ammonium thioglycolate solution to form a 20 percent by weight solution. Segments of wool flannel were placed in the solution for one half hour, the solution drained off and then oxidized by treatment for 5 minutes with an aqueous solution containing 1.5 percent by weight of sodium bromate. The wool segment was then washed with water, detergent, acetone, alcohol and finally ether.

The weight changes effected by the treatments described in Examples II through V are set forth hereinafter in Table I. Table I also shows the result of subsequently treating the products with thioglycolate solutions with and without the presence of the polypeptide product of Example I.

TABLE I

Sample and Reaction Time	Initial Treatment			Subsequent Treatment		
	Keratin Polypeptide	Thioglycolate	Weight Change	Keratin Polypeptide	Thioglycolate	Weight Change
Wool Flannel	20%	0%	+0.02%			
½ hour	20%	4%	+0.8 %			
	20%	4%	+0.65%	0	4%	-2.6%
Medium Bleached Hair	25%	4%	+2.4 %			
½ hour	25%	4%	+3.6 %	0	4%	-9.3%
	0	4%	-11.0%			
Medium Bleached Hair	5%	0	-1.0 %			
15 minutes	0	6%	-8.5 %			
	0	6%	-10.0%	5%	6%	+0.97%
	5%	6%	-6.1 %	0	6%	-3.0 %
	5%	6%	-6.1 %			
White Virgin Hair	5%	0	+0.3%			
1 hour	0	6%	-0.9%			
	0	6%	-0.54%	5%	6%	+1.45%
	5%	6%	0			
	5%	6%	+0.15%	0	6%	-0.23%

EXAMPLE IV

50 grams of the dry hydrolyzate product of Example I were dissolved in 1,000 grams of aqueous solution

containing 8 percent by weight of ammonium thioglycolate to form a 5 percent by weight solution. Segments of wool flannel were placed in the solution for one half hour, the solution drained off and the wool flannel then oxidized by treatment for 5 minutes with an aqueous solution containing 1.5 percent by weight of sodium

EXAMPLE VI

Three swatches of 100 percent worsted wool test flannel were treated as follows:

Sample No.	1	2	3
Weight of sample	99.52 mg	87.86 mg	88.82 mg
Treatment	1 hr.	1 hr.	1 hr.
% Keratin polypeptide in pH 9.2 buffer	10% solution	10% solution	10% solution
% Ammonium thioglycolate			
pH 9.2	0	8%	8%

Sample 1 was washed 4 times with water, while samples 2 and 3 were drained for a short time, oxidized with 1.5 percent sodium bromate, and washed four times in water, after which all samples were air dried overnight.

Weight of Sample	98.87 mg	90.58 mg	92.26 mg
Weight change	-0.65 mg	+2.72 mg	+3.44 mg
% Weight change	-.65%	+3.1%	+3.9%

Sample 2 was immersed for one hour in 8 percent ammonium thioglycolate, washed well with water, oxidized with 1.5 percent sodium bromate solution, washed again with water, and air dried.

Final weight of sample	86.98 mg
Weight change	-3.60 mg
% Change	-4.14%

Thus there is an increase in pickup of the peptide with increase in concentration. The ammonium thioglycolate concentration was double that in Example V and Sample 5 indicates that the 5 percent level of peptide under these conditions essentially balances out the normal weight loss to be expected from thioglycolate treatment. An increase in color occurs from Sample 5 to 7 since the peptide is darker than the flannel swatch.

10 The effect of the keratin peptides on samples of medium bleached human hair is shown in the following example. In this case, all of the original samples showed weight losses as a result of humidity changes on standing overnight. However, the relative weight changes are of greater importance than the absolute changes.

EXAMPLE VIII

Sample No.	8	9	10	11	12
Weight sample	19.28 mg.	37.52 mg.	41.24 mg.	30.12 mg.	87.84 mg.
Treatment time	15 min.	15 min.	15 min.	15 min.	15 min.
% Keratin peptide in pH 9.2 buffer	5% sol.	0	0	5% sol.	5% sol.
% Ammonium thioglycolate pH 9.2	0	6%	6%	6%	6%

Treatment of human hair or wool with ammonium thioglycolate always results in a loss in weight of the sample, but in the presence of keratin polypeptides there is a gain in weight, or, at the most, a smaller loss

Sample 8 was washed in water and dried, while samples 9, 10, 11 and 12 were immersed in 1.5 percent sodium bromate for 5 minutes, washed well with water, and air dried.

Weight after drying	19.08 mg.	34.32 mg.	37.12 mg.	28.28 mg.	73.12 mg.
Weight change	-0.20 mg.	-3.20 mg.	-4.12 mg.	-1.84 mg.	-4.72 mg.
% Weight change	-1.0%	-8.5%	-10%	-6.1%	-6.1%

in weight than when ammonium thioglycolate is used alone without keratin peptides. This is shown in Sample 3 which showed a sharp loss in weight when treated with the reducing agent alone. The insignificant change in weight in Sample 1, treated with the keratin polypeptides alone at pH 9.2, shows that the increase in weight is not due to sorption of the polypeptide.

EXAMPLE VII

The effect of increasing concentration of keratin polypeptides was shown when four swatches of the test flannel were treated as follows:

Sample No.	4	5	6	7
Weight of sample	100.32 mg	101.52 mg	98.56 mg	94.12 mg
Treatment time	½ hr.	½ hr.	½ hr.	½ hr.
% Keratin polypeptide in pH 0.2 buffer	20%	5%	10%	20%
% Ammonium thioglycolate pH 9.2	0	8%	8%	8%

Sample 4 was drained, washed well with water, and air dried. Samples 5, 6 and 7 were drained, treated for 5 minutes with 1.5 percent sodium bromate, washed with water and air dried.

Weight of Sample	100.44 mg.	101.44 mg.	96.16 mg.	95.68 mg.
Weight change	+0.16 mg.	+0.03 mg.	+0.60 mg.	+1.5 mg.
% Change	+0.16%	-0.079%	+0.61%	+1.65%

There is thus less weight loss in the presence of keratin peptide. The hair samples 11 and 12 were smooth, soft and silky, very similar to the initial samples and Sample 1.

Samples 10 and 11 were treated as follows:

	Sample 10	Sample 11
Treatment time	15 minutes	15 minutes
% Keratin peptide in pH 9.2 buffer	5% solution	0
% Ammonium thioglycolate pH 9.2	6%	6%

Sample 10 was subjected to the same treatment as the initial treatment of samples 11 and 12, while sample 11 was given the same treatment as that of samples 10 and 11.

The samples were then allowed to dry.

	Sample 10	Sample 11
Weight after drying	37.48 mg.	27.44 mg.
Weight change	+0.36 mg.	-0.84 mg.
% Weight change	+0.97%	-2.97%

Thus, this example again illustrates the protective action of keratin polypeptide during treatment of human hair with reducing and oxidizing agents.

Since the weight changes which occur in the above examples are small, and at times may be overshadowed by weight changes due to changes in relative humidity, a more positive demonstration is necessary to show that the effect observed is actually due to a chemical reaction, with the formation of stable covalent bonds which are much stronger than the bonds associated normally with sorption of peptides or protein by hair strands. One indication is that shown in Example VII, in which the color of the flannel swatches increases with increase in peptide concentration.

Additional proof of the bonding of disulfide linkage containing polypeptides to filamentous keratins is shown by the following example.

EXAMPLE IX

Cystine was converted to dinitrophenylcystine by a known reaction with fluorodinitrobenzene. The product was crystallized from alcohol, dissolved in ammonium thioglycolate, and swatches of wool flannel and coils of hair were immersed in the solution for a short time, drained, and the keratin samples oxidized with dilute sodium bromate, washed with water, detergent, and organic solvents. The bright golden color in the

samples was impervious to all solvents which did not destroy the wool or hair swatches.

Similar results were obtained when keratin polypeptides, prepared as described in Example I, were converted to dinitrophenyl derivatives by the same reaction procedure.

Although the best mode contemplated for carrying out the present invention has been herein shown and described, it will be apparent that modification and variation may be made without departing from what is regarded to be the subject matter of the invention as set forth in the appended claims.

I claim:

1. A method of treating hair fibers to provide a permanently bonded protective and conditioning content of disulfide-containing proteinaceous agent comprising contacting the hair with an effective amount of an aqueous composition containing water having dissolved therein from 4 percent to 8 percent by weight of ammonium thioglycolate reducing agent and from 5 percent to 25 percent by weight of a water soluble keratin polypeptide hydrolyzate having an intact disulfide unit content in the range between 15 and 49 moles of disulfide linkages per 100,000 grams of hair, said hydrolyzate being produced by hydrolyzing keratin-containing hair at 100°-160°C. in the presence of phosphoric acid having a concentration of 4 percent to 85 percent until the disulfide content is in the range specified while maintaining a pH less than 4 throughout the hydrolysis reaction, draining off said aqueous composition, and then contacting said hair with an effective amount of an aqueous solution of an oxidizing agent.

2. The method as recited in claim 1 in which said oxidizing agent is selected from the group consisting of sodium bromate and hydrogen peroxide.

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UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,842,848 Dated October 22, 1974

Inventor(s) Sulo A. Karjala

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 10, after "wool" insert --flannel--.

Column 7, in the first column of the first Table under Example VII, before "buffer", "0.2" should be --9.2--.

Signed and sealed this 4th day of February 1975.

(SEAL)
Attest:

McCOY M. GIBSON JR.
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents