

Application of the theory of hydrophobic bonds to hair treatments

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Synopsis

A novel technique of HAIR TREATMENT via introduction of nonpolar residues into hair structure in HYDROALCOHOLIC MEDIA is described. Hair modified in this manner exhibits greatly ENHANCED SETTABILITY and HIGH SET RETENTION, even at high levels of ambient humidity. The setting behavior can be manipulated by utilizing the differential swelling response of treated hair to water and aqueous alcohols.

I. INTRODUCTION

Conformational stability of a protein, and thus, its response to external mechanical or chemical forces, depends on the type and number of stabilizing bonds present within the protein structure. In the case of α -keratin, this stability is primarily derived from covalent crosslinking by cystine and intrachain hydrogen bonding. Some contribution also comes from the electrostatic interaction of basic and acidic sidechains, as well as from the hydrophobic bonding of nonpolar residues such as proline, leucine, and valine (1). However, the contribution of the latter is small, and in the intact fiber, the covalent and polar interactions greatly overshadow the nonpolar ones.

In an earlier work reported by Harris (2), it was shown that the wet mechanical properties of reduced keratin fibers could be restored without crosslinking by alkylating the fibers with high molecular weight monohalides. Alkylation with alkyl halides of low molecular weight produced permanently weakened fibers. Successful mechanical recovery of the alkylated fibers was ascribed to the interaction of secondary forces, arising from the high molecular weight residues incorporated into the fiber structure during alkylation. In this respect, it is of particular interest to note that the introduction of apolar residues creates an environment favoring the formation of hydrophobic bonds, and that the strength of these bonds depends on the size and shape of the introduced alkyl groups.

The term "hydrophobic interaction" describes the tendency of nonpolar groups to associate in aqueous solution. This interaction results in an increased ordering of the water molecules into a quasi-crystalline structure in which there is improved hydrogen bonding surrounding the nonpolar groups. These hydrophobic regions are disrupted in nonpolar solvents because stronger solute-solvent interactions are thermodynamically favored. Thus, a unique property of the hydrophobic bond is its dependence on water for its existence.

The important practical point raised by these considerations is the utility of this type of bonding for setting hair. If the hydrophobic bonds could resist the swelling pressure generated within the keratin which is exposed to high humidity, then the set-conformation would be maintained and a novel process for hair manipulation would be feasible.

An investigation of the properties of S-alkylated keratin was therefore undertaken, and this report is an account of such study.

II. EXPERIMENTAL

A. MATERIALS AND METHODS

1. *Reagents*: The chemicals utilized in this study were commercially available American Chemical Society grade reagents and were used without further purification.

2. *Caucasian hair*: Brown Caucasian hair as supplied* was used without further cleansing.

3. *Mechanical properties*: The mechanical properties of hair were determined on a table model Instron.† The fibers were mounted on plastic tabs at 2 in. gauge length, equilibrated under the desired conditions, and stretched to break at a rate of 1 in./min. The broken ends were conditioned at 65 per cent RH, cut off the tabs, weighed, and the denier of the tested fibers calculated.

In some cases, the calibration technique (3) was used to follow the change in the fiber performance. Intact fibers were mounted as above, equilibrated in the desired solvent, and then stretched to 30 per cent extension at a rate of 1 in./min, using the table model Instron. After a 24-h relaxation period in water, the calibrated fibers were given the proposed chemical treatment. The ratio of the energy required to stretch the fibers (30 per cent extension) the second time to that required initially, was expressed as the 30 per cent index.

4. *Amino acid analysis*: Hair/wool samples (~10 mg) were hydrolyzed at 105°C for 24 h in 6 N HCl followed by lyophilization for removal of HCl. The hydrolyzates were analyzed for cystine on a Phoenix‡ model M-7800 Micro Analyzer.

5. *Liquid retention measurements*: The swelling of hair was determined by the liquid retention technique as described by Valco and Barnett (4). This involved measuring

*De Meo Brothers, New York, N.Y.

†Instron Corp., Canton, MA.

‡Phoenix Instrument Co.

the liquid retained by the hair after a 30 min equilibration in water or other specified solvent.

6. *Setting*: One gram (7 in.) tresses were set on one-half in. rollers with water or aqueous alcohol as specified in the text. The set tresses were allowed to dry overnight at ambient temperature and humidity. After removing the tress from the roller, the hair was combed, being careful to maintain the alignment of the hair fibers.

The set stability of treated hair was assessed by measuring the hanging length of the tresses after various relaxation times, while exposing them to maintained conditions of humidity and temperature (85 per cent RH, 85°F).

B. RESULTS AND DISCUSSION

1. *The reaction of reduced hair with alkyl iodides*: Earlier investigations conducted by Harris (2) on wool suggested that the wet mechanical properties of reduced wool could be restored following alkylation with long chain alkyl halides. Both the magnitude of the restorative effect and the simplicity of the alkylation step suggested this approach as being particularly attractive for application to hair. An attempt was, therefore, made to evaluate the efficacy of the alkylation reaction.

Calibrated hair fibers were treated with 0.25 *N* potassium thioglycolate at pH 5 (3 h at 50°C, 25:1 bath ratio) to cleave approximately 50 per cent of the disulfide bonds. Samples of the reduced fibers were then alkylated with 0.02 *M* alkylating agent suspended in 0.1 *M* pH 8 phosphate buffer utilizing 100:1 bath ratio. The alkyl halides used as blocking agents were methyl, hexyl, and decyl iodides, respectively. After 20 h, at 35°C, the fibers were thoroughly rinsed with running tap water, and dried. Bulk samples were treated simultaneously in order to determine the weight changes following alkylation. A small weight increase (1.9 per cent) was observed only in the case of the sample treated with decyl iodide. This weight increase corresponded to less than 20 per cent yield of the alkylation reaction.

The alkylation treatment also had a negligible effect on the mechanical properties of the reduced hair (Table I). These results were in sharp contrast with the data reported for wool by Harris (2). To ascertain whether the reactivity of the substrate (hair versus wool) contributes to these large differences in behavior, it was decided to re-examine the reaction system using wool fibers. The reduction-alkylation cycle was run under conditions identical to those described by Harris.

2. *Alkylation of wool with alkyl iodides*: New Zealand wool samples were reduced with 0.2 *N* potassium thioglycolate at pH 4.5 for 21 h at 35°C (25:1 bath ratio). The subsequent alkylation was performed using 0.02 *M* alkylating agent, methyl, hexyl, or decyl iodides suspended in 1 *M*, pH 8 phosphate buffer, 35°C at 100:1 bath ratio. The alkylation proceeded very slowly in the presence of the longer chain halides, as was evident by the persistence of thioglycolic acid after 18 h reaction time. To insure that an excess of alkylation agent was present, fresh solutions of the hexyl and decyl iodides, respectively, were added to the wool samples, and the alkylation continued for an additional 7 h. These reactions were monitored by measuring the weight changes, as well as examining the properties of the treated wool.

Table I
30 Per Cent Indices of Hair Samples Following Treatment with Alkyl Iodides

Treatment	30 Per Cent Index
Intact	0.98
Reduced	0.54
Reduced—CH ₃ I	0.66
Reduced—C ₆ H ₁₃ I	0.66
Reduced—C ₁₀ H ₂₁ I	0.64

Table II
30 Per Cent Indices of Wool Samples Following Treatment with Alkyl Iodides

Treatment	30 Per Cent Index
Intact	1.02
Reduced—CH ₃ I	0.63
Reduced—C ₆ H ₁₃ I	0.85
Reduced—C ₁₀ H ₂₁ I	0.92

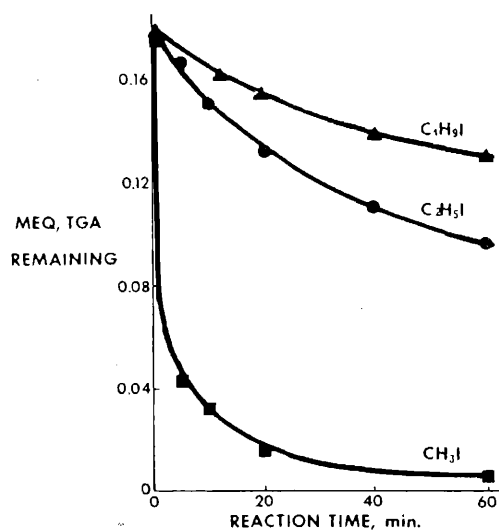


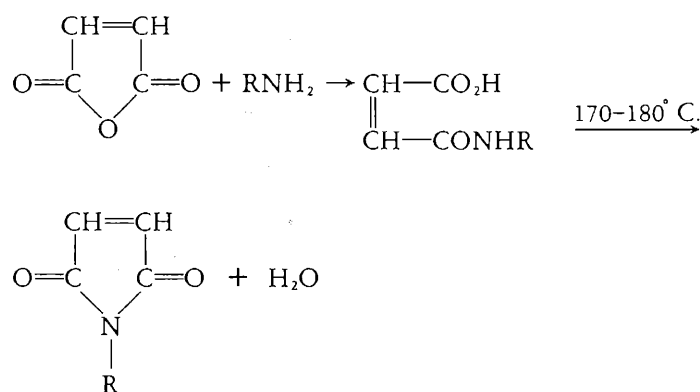
Figure 1. Reactivity of thioglycolic acid with methyl, ethyl and butyl iodides in 40 per cent ethanol, pH 9 with respect to time

The alkylation of the reduced wool with hexyl and decyl iodides resulted in weight gains of 1.7 and 2.5 per cent, respectively. It was obvious that the extent of alkylation was again low. On the basis of the weight gain, only 0.18 to 0.20 m moles at most, of SH per gram of wool had been alkylated. Yet, the mechanical performance of the alkylated wool (Table II) conformed with the earlier data published by Harris. There was a steady improvement in the mechanical recovery of the fibers with the increasing

chain length of the alkylating agent. However, this unexpected discrepancy between the weight gain values and the 30 per cent work indices was resolved satisfactorily by the amino acid analyses of the treated wools. The cystine contents of both hexyl iodide and decyl iodide treated samples were almost identical with those of the untreated unreduced wool (860 $\mu\text{mol/g}$). The methyl iodide alkylated sample had a cystine content of 470 $\mu\text{mol/g}$. It is, thus, obvious that the mechanical recovery of the alkylated wool fibers had been brought about by reformation of the keratin disulfide and not by the residue reinforcement effect. The mechanism of the disulfide rebuilding is not yet known. Most likely, the alkyl iodides undergo some secondary reactions involving formation of iodine, which acts as an oxidant for the protein sulfhydryl. This secondary reaction is unimportant in the case of methyl iodide, which reacts with mercaptans very rapidly. An increase in chain length of the alkyl group causes a precipitous drop in the rate of the alkylation reaction (Fig. 1), and thus, may set a stage for the secondary process.

3. *Synthesis of N-alkyl maleimides:* A more dependable method for introducing apolar residues into the keratin was clearly required. N-ethyl maleimide is often used as a standard blocking agent for protein sulfhydryl, and it was thought that its higher homologues might be of value in this respect.

Although, the N-alkyl maleimides are not commercially available, they were easily prepared by pyrolysis of the corresponding N-alkyl maleamic acids (5, 6). N-hexyl, N-heptyl, and N-dodecyl maleamic acids were prepared by reacting maleic anhydride with the appropriate amine in glacial acetic acid. The acids were isolated in good yields (ca. 85 per cent) as white crystalline solids and pyrolyzed without further purification. The properties of the maleamic acids and the corresponding maleimides are given in Table III. The low yields of final product (26 per cent) were due to a concurrent polymerization reaction leading to a resinous by product. In the course of our work, a one step synthesis was also utilized for the preparation of N-alkyl (aryl) maleimides. The overall yield continued, however, to be low (~30 per cent). The overall reaction is shown below



4. *Reduction of hair with dithiothreitol (DTT):* DTT was used as an alternate reductant in our studies. This reagent (8) causes a specific and symmetric scission of the

Table III
Properties of N-Alkyl Maleamic and N-Alkyl Maleimides

Derivative	Maleamic Acid ^b	Maleimide ^b
	Melting Point, °C	Melting Point, °C
Hexyl	78°	125° @ 5.5 mm ^a
Heptyl	75–77°	43–44°
Dodecyl	—	56–59°
Benzyl	140–142°	73–75°

^aBoiling point.

^bObserved melting points were within the range reported by Coleman *et al* (7).

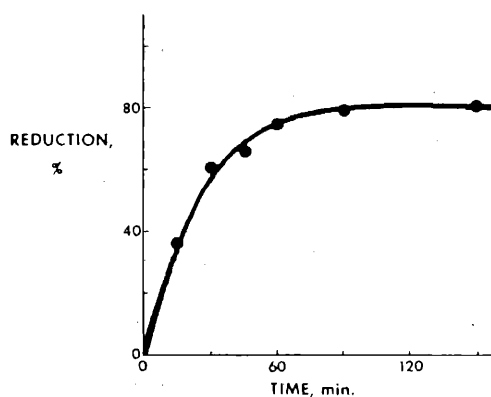


Figure 2. Effect of time on reduction of hair by 0.1 M DTT at self-pH, 35°C

disulfide bonds without producing any byproducts such as mixed disulfides. Its efficacy as a reductant allowed us to perform the reduction swiftly at neutral pH and a temperature of 35°C.

Hair samples were treated with unbuffered solutions of 0.1 M DTT (self-pH, 5.4) at 25:1 bath ratio, 35°C for various times. Levels of reduction were calculated on the basis of the SH content determined via mersalyl acid titration (9). These data agreed with the reduction levels determined from residual disulfide analyses (via amino acid analysis) after cyanoethylation of the free SH groups. A plot of the reduction levels against time is shown in Fig. 2.

5. Alkylation of hair with N-alkyl maleimides: N-hexyl maleimide was used to alkylate reduced hair containing 0.6 m moles of SH per gram of hair. The extent of reaction was monitored by determining the residual SH following the alkylation. Under the conditions employed, the alkylation was complete within 2 h. The alkylation was carried out in 20 per cent n-propanol/0.04 M phosphate buffer, pH 7 at 35°C, 100:1 bath ratio; under these conditions the extent of hydrolysis of the N-alkyl maleimides is negligible.

In using the next higher homolog, i.e., N-heptyl maleimide, the alkylation reaction could also be completed within 2 h. Following the alkylation with N-ethyl maleimide,

Table IV
Effect of Time on the Alkylation of Hair with N-Ethyl and N-Hexyl Maleimide^a

Time, Minutes	Degree of Alkylation, Per Cent	
	N-Ethyl Maleimide	N-Hexyl Maleimide
30	75	82
60	81	94
120	86	100

^a[Maleimide] = 0.01 M; Solvent = 20 per cent n-propanol/0.04 M phosphate (pH 7) buffer; temperature = 35°C; bath Ratio = 100:1.

approximately 15 per cent of the SH remained unreacted after 2 h, while a corresponding sample of hair treated with the N-hexyl maleimide contained no residual SH (Table IV). This finding was in accord with the data presented by Heitz (6), who determined the second-order rate constants for the binding of N-heptyl maleimide to yeast alcohol dehydrogenase. There, it was shown that the reaction rate for the N-heptyl maleimide reaction was approximately 8.4 times that observed with N-ethyl maleimide. This was somewhat unexpected, since a chainlength effect was not observed in the reactions of these maleimides with cysteine and glutathione (6). We observed similar enhancement of the rate of alkylation in the case of reduced hair. Following reduction with thioglycolic acid, the sample was rinsed only briefly prior to the alkylation, and thus, residual thioglycolic acid remained in the fiber. At the end of a 24-h treatment, the cysteinyl residues were completely blocked by N-heptyl maleimide, while free thioglycolic acid was still detected in the alkylating solution. This observation and the previous results on the alkylation of reduced keratin indicate that an increase in the alkyl sidechain of the maleimide leads to faster rates of alkylation in spite of the unfavorable diffusion factor. Such an enhancement in the reactivity may be tentatively ascribed to the interaction between the alkyl sidechain of the reactant and the nonpolar residues of the keratin, which apparently provide an effective hydrophobic environment for the combined cystine.

6. *Swelling properties of the alkylated hair:* The extent of internal modification of keratin often can be readily assessed from the change in the swelling characteristics of this protein. Thus, fission of the disulfide bonds is accompanied by an increase in water imbibition, which is almost directly proportional to the number of crosslinks severed. According to our hypothesis of hydrophobic modification of hair, the introduction of apolar residues should compensate for at least some of the disulfide bond breakdown. A strong support for this view was obtained from the liquid retention measurements of reduced and reduced-alkylated hair (Table V). The reduction treatment alone causes a large increase in swelling in both water and aqueous alcohol. Alkylation of reduced hair with methyl iodide or N-ethyl maleimide slightly intensifies the swelling. This increment in hydration is probably caused by the elimination of weak hydrogen bonding involving the sulfur hydrogen and the apparent inability of the methyl or N-ethyl derivatives to establish any specific interactions with the environment. On the other hand, alkylation with either N-hexyl or N-heptyl maleimides brings about significant decrease in hydration. Obviously, the introduction of hydrophobic residues can impart

TABLE V
Effect of Apolar Residues on the Swelling Properties of Hair

Per Cent Reduction	Alkylating Agent	Per Cent Swelling ^a in:				
		Water	50 Per Cent Methanol	50 Per Cent Ethanol	20 Per Cent Propanol	50 Per Cent Propanol
0	None	31.4	n.d. ^b	n.d. ^b	31.6	32.2
45	None	39.9	35.8	37.4	42.6	45.2
45	Methyl iodide	47.2	42.2	46.2	47.8	49.2
45	N-ethyl maleimide	45.3	41.0	45.5	48.6	51.1
45	N-hexyl maleimide	33.9	33.7	37.8	39.5	46.3
45	N-heptyl maleimide	34.8	33.6	37.7	40.9	46.4
82	N-hexyl maleimide	31.0	n.d. ^b	n.d. ^b	44.9	56.5
82	N-dodecyl maleimide	28.2	n.d. ^b	31.9	36.4	47.5
82	N-benzyl maleimide	31.5	n.d. ^b	34.1	35.4	46.1

^aCalculated on the treated weight of the fiber.

^bNot determined.

substantial conformational stability to the reduced keratin structure and successfully resist the swelling pressure.

This conformational stability is, of course, lost in aqueous alcohols, where the hydrophobic interactions between the apolar residues present in hair are prevented. Such a differential response to aqueous solvents offers a unique way of hair manipulation, particularly with regard to setting.

7. Mechanical properties: Swelling data have shown that the imbibition of water by keratin can be restricted by hydrophobic modification of the fiber. Although, the precise nature of the intermolecular arrangement remains a subject of controversy (10, 11), the stabilization of hydrophobic bonds by water is not disputed. The strength of the hydrophobic bond is represented by the tendency of nonpolar groups to adhere to one another. The free energy of this process has been assessed (12), and in the case of the interaction of 2 methyl groups, was found to be -0.73 kcal/mole for alkyl sidechains. The increment in the free energy of binding was in the order of -0.37 kcal/mole per CH_2 group. The overall contribution of these hydrophobic crosslinks to the stabilization of the keratin structure will depend on the size, shape, and number of the introduced apolar residues.

Some further insight on these hydrophobic interactions was obtained from a study of the mechanical properties. It is well known that the wet strength of intact hair bears a linear relationship to the cystine content over a wide range of reduction levels (13). Using this linear relationship as a guide, a preliminary assessment of the stabilization effect arising from hydrophobic interactions was obtained from the mechanical behavior

Table VI
Yield Stress of Hair Alkylated with N-Hexyl Maleimide

Reduction Level, Per Cent	Yield Stress, g/denier in:					
	Water		20 Per Cent Propanol		50 Per Cent Propanol	
	Calculated ^a	Observation	Calculated ^a	Observation	Calculated ^a	Observation
0	0.42	0.42	0.36	0.36	0.36	0.36
31	0.28	0.40	0.25	0.30	0.25	0.27
42	0.22	0.32	0.21	0.17	0.21	0.14
82	0.08	0.27	0.06	0.11	0.06	0.10

^aCalculated yield stress = (intact hair yield stress) (100-per cent reduction/100).

of alkylated fibers (Table VI). The alkylation was performed at 3 reduction levels using N-hexyl maleimide as the alkylating agent.

If one relies on the fact that the percent reduction in work to stretch a reduced fiber is directly proportional to the extent of reduction, then it is evident that the alkylated fibers do not exhibit such a loss of strength. In water, the formation of hydrophobic bonds, via interaction of the hexyl residues, results in significant stabilization of the keratin structure. Although, some repairing effect was anticipated, the extent of the stabilization and particularly the resistance of the treated fibers to the external stresses was unexpected. Even with the maximum overlap of the apolar sidechains introduced in the alkylation step, the average strength of the newly formed bonds would not exceed 5 kcal. This is only a fraction of the energy loss which accompanies the breakdown of cystine crosslinks (~50 kcal/mol). It is apparent that the hydrophobic interactions which accompany the blocking of cysteine residues are very intensive, although, a possibility of cooperative multichain hydrogen bonding in a hydrophobic environment cannot be excluded.

8. *Setting properties of alkylated hair:* The alkylation of reduced hair limits the swelling of hair in water. On the other hand, such hair can be readily deformed in alcoholic media. An attempt was made to utilize this change in swelling characteristic for the setting purposes. Thus, the hair was swollen in 50 per cent propanol and set on rollers. In the presence of alcohol, the alkylated hair is very pliable and moldable and conforms readily to the desired configuration. After setting the hair, the alcohol was removed by rinsing with water. Removal of the alcohol leads to the formation of hydrophobic crosslinks, which stabilize the new (set) configuration. Hair alkylated with N-heptyl maleimide retained the set in liquid H₂O, while a tress alkylated with N-ethyl maleimide straightened within 1 to 2 min. This demonstrates that a water-resistant set can be attained by the introduction of apolar residues, providing that a sufficiently long sidechain is used. A 7-carbon alkyl chain appears to fulfill this requirement.

The set stability of tresses was also assessed by the conventional manner. This involves the exposure of set tresses to controlled conditions of humidity and temperature and measuring the extent of relaxation with respect to time. Following the reduction, the tresses were rinsed with water and then alkylated with N-heptyl maleimide for 2 h. The setting was performed with 50 per cent propanol; after thorough rinsing with water the

Table VII
Set Stability of Hair Following Alkylation with N-Heptyl Maleimide

Sample	Hanging Lengths (cm) ^a After Following Relaxation Time (min):						
	0	5	7.5	10	15	30	150
Intact (H ₂ O set)	2	7.5	11	12	13	13	13
Intact	2	5.0	7	8	10	11	12
Reduced/heptyl maleimide	2.5	3.5	4	4	4.5	5.0	6

^aMeasured at 84 per cent RH, 85°F.

Table VIII
Effect of Reduction Level on the Set Stability of Alkylated Hair

Treatment	Hanging Lengths (cm) at Relaxation Times (min):					
	3	6	9	12	20	45
Intact (H ₂ O)	2	3	5.5	8	10	11
Intact	2.5	3	3.5	5.5	8.5	10.5
15' Reduction/heptyl maleimide	2.5	5.0	6	7.5	9	10
30' Reduction/heptyl maleimide	2	3	3.5	4	5	6.5
60' Reduction/heptyl maleimide	2	2.5	3.0	3.5	4	5.0

tresses were air dried overnight on the rollers (alternatively, the set hair was dried with a hand held dryer). The set stability is shown in Table VII.

The alkylation results in excellent set stability during a prolonged exposure to conditions of high humidity. Additional experiments indicated that increasing the size of the alkyl substituent above C₇ offered no benefit.

Optimal interaction of the hydrophobic sidechains or, indeed, the mobility of such chains, may not be possible in the case of the dodecyl residues because of steric factors. Thus, the maximal stabilization of the keratin would not be attained. It is of interest to note that the best results have been obtained with hair alkylated either with heptyl or with benzyl maleimides.

The effect of alkylation on the set stability (measured at 84 per cent RH, 85°F) was determined at several reduction levels (Table VIII). It appears that alkylation of reduced hair having less than 50 per cent cleavage does not greatly improve the setting properties.

An important consideration in the reduction-alkylation process is the respective bond energies of the system. As discussed earlier, disulfide bonds represent approximately 50 kcal/mol of stabilization energy. The introduction of two apolar residues (heptyl sidechain) for the blocking of a reduced disulfide will contribute only about 3 kcal/mol of binding energy. At low reduction levels, the introduction of a few hydrophobic crosslinks cannot compete with the residual disulfides which significantly contribute to the overall setting characteristics of intact hair. At very high reduction levels, only a

few restraining disulfide crosslinks remain, thus, the stabilizing influence of the hydrophobic crosslinks becomes more prevalent in maintaining the native structure of the keratin.

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