

Reversible Crosslinks in Cotton Modified with *N*-Hydroxymethylacetylthioacetamide*

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Synopsis

The reaction of cotton fabric with *N*-hydroxymethylacetylthioacetamide ($\text{HOCH}_2\text{-NHCOCH}_2\text{SCOCH}_3$) yields acetylthioacetamidomethylcellulose, which can be saponified under mild conditions to the corresponding thiol derivative, mercaptoacetamidomethylcellulose. Some reactions of the thiol group are discussed, including oxidation to disulfide, repeated reduction-oxidation cycles, and blocking with alkyl halides. Chemical methods were used in establishing the occurrence and extent of the predicted reactions and their dependence on specific variables in the system. Changes in the crease recovery and tensile strength of the cotton derivative obtained in the reactions were found to be qualitatively consistent with the expected structures. The results of this work show that crosslinks can be obtained reversibly in cotton by this sequence of reactions. Intervening side reactions produce a gradual decrease in the yield of the desired products, however, as the number of cycles increases. Mercaptoacetamidomethylcellulose provides a limited but useful model for the investigation of reversible crosslinks in cellulose.

INTRODUCTION

The preparation of cellulose derivatives containing disulfide crosslinks has been of interest for some time. An analogy with reactions of wool fibers suggests that the preparation of fibrous cellulose derivatives in which crosslinks can be ruptured and rebuilt by reduction and oxidation reactions would provide new insight into the relationship of fiber properties to crosslink content and perhaps new methods for the control of those properties, which are primarily governed by the extent of crosslinking. The first extensive investigation of disulfide crosslinks in cotton cellulose was carried out by Schwenker and his co-workers.^{1,2} They reported the preparation of 6-thiocellulose from tosylated cotton and its oxidation to a 6,6' disulfide cotton derivative. The results obtained indicated that reduction and re-oxidation of disulfide-modified cotton fabric could be carried out and that changes in crease recovery were generally consistent with the postulated cleavage and re-formation of crosslinks.

Mack et al. and Hobart et al.^{3,4} studied the reaction of periodate-oxidized (dialdehyde) cotton with acetylthioacetamide disulfide ($\text{H}_2\text{NNHCOCH}_2\text{SSCH}_2\text{-CONHNH}_2$) and also reported qualitative evidence that disulfide bonds

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could be cleaved by reduction and re-formed by oxidation in this complex cotton derivative.

Attempts at introducing disulfide groups directly into unmodified cotton were reported while our work was in progress. A brief communication by Mueller and Swidler⁵ and a publication by Rath and his co-workers⁶ describe work on the modification of cotton with bis-*N*-methylol reagents containing disulfide groups, and Saur et al.⁷ have examined the reduction of cotton modified with 4,4'-bis(β -sulfatoethylsulfonyl) diphenyl disulfide.

The feasibility of introducing disulfide crosslinks into cotton, including unmodified cotton fabric, has thus been qualitatively demonstrated. Reduction and reoxidation experiments have been carried out, generally without attempts at determining the extent of reaction attained by chemical analysis. Throughout the work reported up to now the assumption has been made that changes in crease recovery resulting from reductive and oxidative treatments are solely dependent on, and directly proportional to, the cleavage and re-formation of disulfide bonds. The purpose of the work reported in the present paper has been the preparation of modified cotton fabric containing disulfide crosslinks, the investigation of its reactions by chemical analysis of functional groups formed, and the study of the relationships between the chemical changes occurring and the changes in the physical properties of the cotton derivatives.

EXPERIMENTAL

The reagent selected for this investigation of disulfide-modified cotton was *N*-hydroxymethylacetylthioacetamide (TAM),



This compound can be prepared in crystalline form from *N*-methylol-chloroacetamide and potassium thioacetate, and it reacts with cotton fabric in good yield under relatively mild conditions. The chemistry of the reactions covered by this investigation is summarized in the accompanying scheme (1).

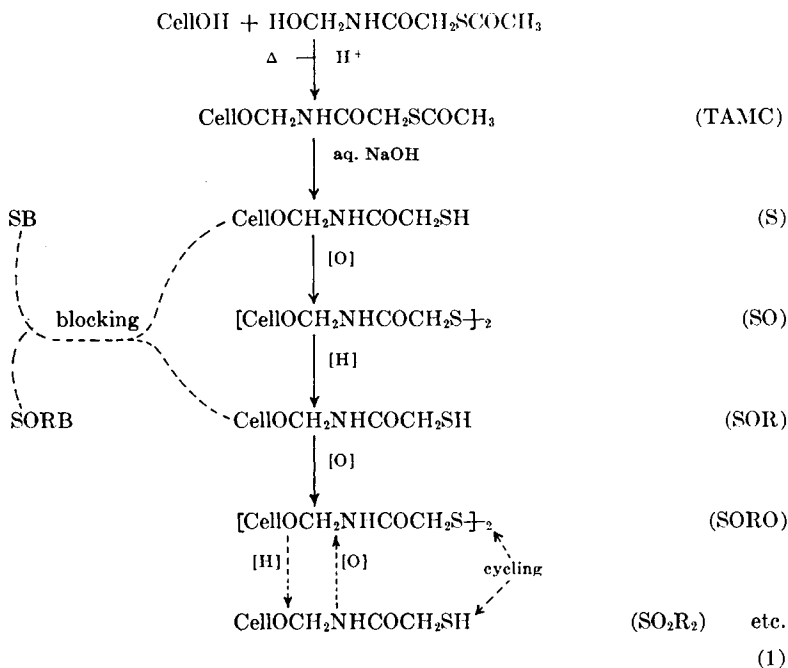
The chemical methods used for the preparation and analyses of the samples were as follows.

N-Hydroxymethylacetylthioacetamide

This substance was prepared in our laboratory. The material used had the following properties: m.p., 93–96°C. (m.p. 104–105°C. after recrystallization from benzene); S, 19.9% (calcd., 19.6%); bound formaldehyde, 17.7% (calcd., 18.4%).

Reaction of Cotton with TAM (TAMC)

Samples of cotton fabric (80 × 80 printcloth, desized and bleached, not mercerized) were padded with aqueous solutions containing TAM and magnesium chloride catalyst (12–15% MgCl₂, based on the weight of



reagent), dried (2 min. at 65°C.), cured (5 min. at 150°C.), washed thoroughly, and dried.

Saponification (S)

TAMC samples were treated under nitrogen with 0.2N NaOH (liquor ratio, 1:30) for 30 min. at room temperature, washed thoroughly with water, then with 2% acetic acid, and again with water, and dried.

Oxidation (SO)

The iodine oxidation method reported by Schwenker et al.^{1,2} was found to be unsatisfactory. Consumption of iodine can exceed the theoretical value and, on the other hand, iodine-oxidized samples exhibit residual reactivity with alkylating agents. The following alternate methods of oxidation were used.

Method 1: H₂O₂. Samples were treated with a 3% neutral aqueous solution of hydrogen peroxide (liquor ratio, 1:30) for 30 min. at room temperature, washed with water, and dried.

Method 2: DMSO-Fe. Samples were extracted repeatedly with dimethyl sulfoxide (DMSO) to replace water and then treated at room temperature for 30 min. in a 0.1% solution of Fe₂(SO₄)₃ in DMSO (liquor ratio, 1:30), while a vigorous stream of oxygen was passed through the solution. The samples were then washed with water and dried.

Method 3: Air. Samples were conditioned at 65% R.H. and 21°C. for the period indicated.

Reduction (SOR)

Reduction under neutral conditions was found to be desirable because it avoided side reactions resulting from alkaline hydrolysis of disulfide bonds. In the method used oxidized samples were treated under nitrogen with a 20% aqueous solution of tetrakis(hydroxymethyl) phosphonium chloride (THPC), adjusted to pH 7.0 with NaOH (liquor ratio, 1:30) for 2 hr. at 40°C., washed with water, and dried.⁹

Blocking (SB and SORB)

Samples were padded with a 5% aqueous solution of NaOH and then treated under nitrogen with a 5% solution of alkyl iodide in dimethylformamide (liquor ratio, 1:30) for 6 hr. at 50°C. They were then neutralized, washed with water, and dried.

Analysis of SH

The sample (ca. 0.08 g.) was treated in 100 ml. of pH 3.5 buffer solution containing 0.0125 g. of *N*-ethyl maleimide (NEMI) for 4 to 6 days at room temperature. The NEMI consumed by the SH groups was determined quantitatively from the change in ultraviolet absorbance at 300 m μ .¹⁰

Analysis of SS

The sample (ca. 0.24 g.) was treated with 25 ml. of nitrogen-flushed water containing 0.020 g. of dithiothreitol (DTT) for 24 hr. at room temperature, oxygen being excluded. The amount of cyclic disulfide formed from the DTT by the reduction of SS groups in the cellulose derivative was determined quantitatively from the ultraviolet absorbance at 284 m μ read against a reagent blank used for treating unmodified cotton.^{11,12}

Physical Test Methods

The crease recovery of dry (2 hr. conditioning), conditioned (24 hr. conditioning), and wet samples was measured by the Monsanto method (ASTM-D1295-60T), as specified. The tensile strength was measured by the strip method (ASTM-D1682-64).

RESULTS AND DISCUSSION

Analytical data obtained from TAMC (Table I) suggested that the postulated structure (acetylthioacetamidomethylcellulose) was the main reaction product. Unfortunately, the analytical values for the *S*-acetyl content could not be obtained with a satisfactory enough reproducibility to provide confirmation of the structure. The decrease in moisture regain observed in the TAMC samples compared with that of an untreated control was a disturbing indication that a side reaction involving crosslinking also had occurred, and additional evidence of crosslinking was found in the high crease recovery [240–250°C. (W + F) at ca. 2% sulfur] exhibited

TABLE I
Analytical Data on Acetylthioacetamidomethylcellulose

TAM, % on wt. of fabric	Weight gain, ^a %	S, %		N, %		M.R., %
		Calcd. ^b	Found	Calcd. ^b	Found	
22.1	12.8	2.52	2.46	1.12	1.05	4.4
13.4	10.4	2.08	2.00	0.92	0.97	4.6
8.6	7.5	1.55	1.49	0.68	0.74	4.9
6.6	6.7	1.41	1.20	0.62	0.60	5.0
4.3	4.1	0.86	0.85	0.38	0.40	5.6
2.6	2.3	0.50	0.48	0.22	0.26	6.0

^a Corrected for change in moisture regain.

^b Calculated from the corrected weight gain for $\text{CelloCH}_2\text{NHCOCH}_2\text{SCOCH}_3$.

by conditioned TAMC samples. Under the conditions of TAMC preparation two side reactions could lead to the formation of crosslinks: reaction of the cotton with formaldehyde, formed in a partial decomposition of the *N*-methylol reagent, and formation of disulfide crosslinks by oxidation of thiol groups produced in a hydrolytic cleavage of the *S*-acetyl groups.

Saponification of the TAMC samples under mild conditions resulted in a considerable lessening of crease recovery, suggesting that the high initial crease recovery of TAMC could be attributed to the presence of alkali labile crosslinks, possibly disulfide groups.

Figures 1 and 2 illustrate the changes in crease recovery observed as a result of reactions shown in scheme (1) and represented schematically as

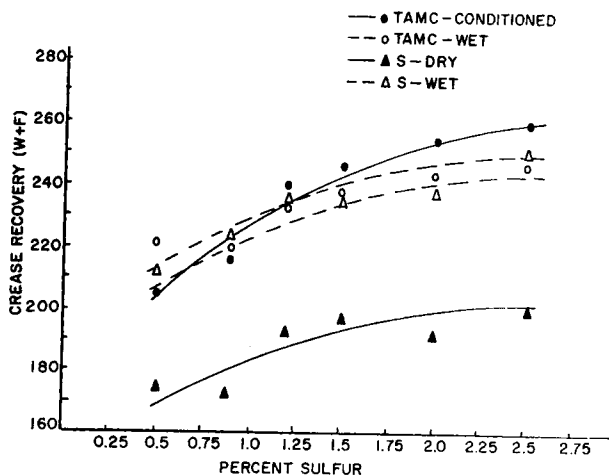
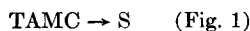


Fig. 1. Crease recovery changes as a function of sulfur content for TAMC and the saponified derivatives.

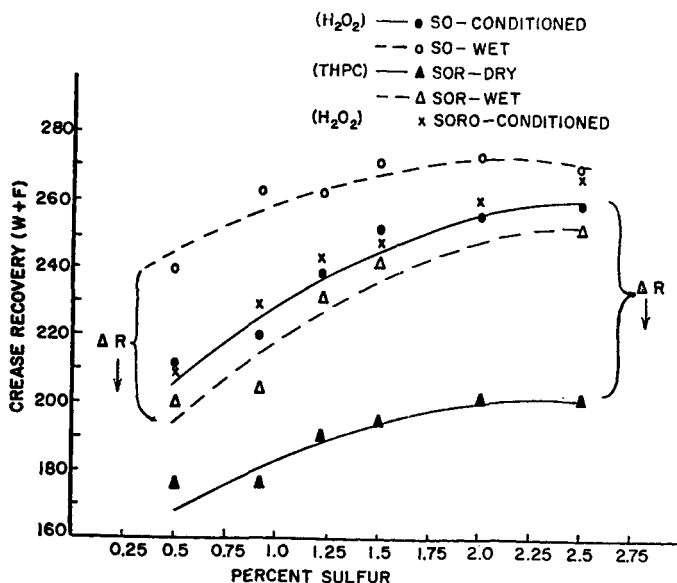


Fig. 2. Crease recovery changes as a function of sulfur content for oxidized and reduced derivatives.

Conditioned (24 hr.) crease recoveries are shown for TAMC and oxidized samples, assumed to be stable to air oxidation, while dry or immediate (2 hr. conditioning) crease recoveries are shown for samples expected to contain significant amounts of SH. These were assumed to be susceptible to oxidation and crosslinking in air during prolonged conditioning.^{2,3} At all sulfur contents examined the reactions did not affect the wet crease recovery appreciably. "Dry" crease recovery decreases as a result of saponification, is restored by oxidation, and returns to a low value after reduction. High crease recovery values are again reached after reoxidation of the reduced samples.

The results of these experiments were consistent with the chemical reactions postulated scheme (1), and the system appeared to be suitable for the proposed investigation of reversible disulfide crosslinks in cotton. A semiquantitative treatment of chemical data obtained from analysis of the functional groups present in the samples after each reaction revealed the complexity of the system and the gross approximations in the postulation of chemical facts on the basis of physical data such as crease recovery.

Figure 3 summarizes the analytical data obtained from samples of varying sulfur content: initially (TAMC), after saponification (S), after oxidation (SO), after reduction (SOR), and after reoxidation (SORO).

The total sulfur content did not change appreciably.

All TAMC samples have very low SH and SS content. Saponification of TAMC yields appreciable amounts of SH, but a significant amount of the sulfur present remains unaccounted for. After oxidation of the saponified sample the SS content is comparable to the SH content determined

prior to oxidation. Reduction re-forms SH groups in less than quantitative yield, and reoxidation yields samples in which both SS and SH are present in substantial amounts. In all samples (with the exception of TAMC), the percentage of sulfur present as (SH + SS) accounts for about 55-80% of the total sulfur present. Although the values obtained for SH and

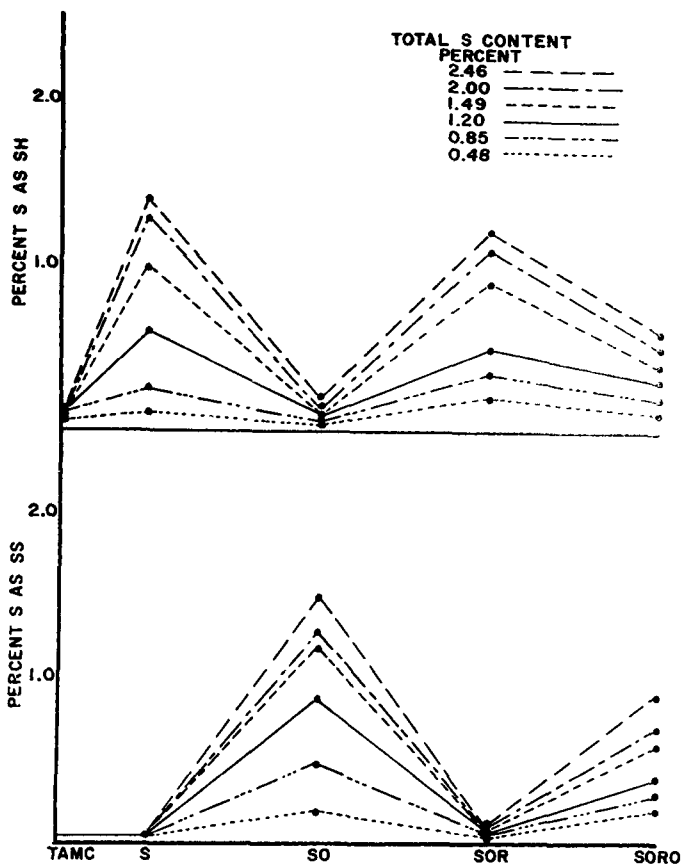


Fig. 3. Disulfide and sulfhydryl analytical data found in TAMC and in the saponified (S), oxidized (SO), reduced (SOR), and reoxidized (SORO) derivatives.

SS content were found to be somewhat dependent on the reagents and conditions used for the saponification, oxidation, and reduction reactions, the trends shown in Figure 3 remain generally valid and may be summarized as follows:

Reaction	Approximate yield
Saponification: TAMC→S	60% of available Sulfur to SH
Oxidation: S→SO	100% of available SH to SS
Reduction: SO→SOR	80% of available SS to SH
Reoxidation: SOR→SORO	60% of available SH to SS

TABLE II
 Dry Crease Recovery, Disulfide and Thiol Group Analysis During Extended Conditioning
 of Saponified (S), Oxidized (SO), and Reduced (SOR) Samples from TAMC
 of 2.4% Total Sulfur

Condit. time	Dry crease recovery (W + F)			S as SS, %			S as SH, %		
	S	SO	SOR	S	SO	SOR	S	SO	SOR
0	198	206	206	0.47	1.07	0.21	1.29	0.18	1.11
4 days	237	254	252	0.54	1.07	0.15	1.26	0.27	1.17
2 weeks	250	282	267	0.51	0.82	0.16	0.94	0.37	1.10
3 weeks	266	277	268	0.55	0.83	0.13	1.12	0.40	0.94
5 weeks	273	283	287	0.51	0.76	0.14	0.83	0.27	0.90
8 weeks	289	287	288	0.77	0.89	0.43	0.79	0.12	0.78
10 weeks	289	286	286	0.72	1.08	0.68	0.68	0.12	0.60

No explanation is available for the difference observed in the oxidation behavior of saponified (S) and reduced (SOR) samples.

In the modified cotton samples the analytical determination of the functional groups (SH and SS) reveals, in fact, only those groups which are accessible to the analytical reagent. It is possible that the true SH and SS contents might be higher than indicated by the analytical results, including, perhaps, substituent groups that are not reached by the reagents used for reactions or analyses. The fact remains that a significant portion of each cellulose derivative escapes analysis, characterization, and definition by the methods employed in this study.

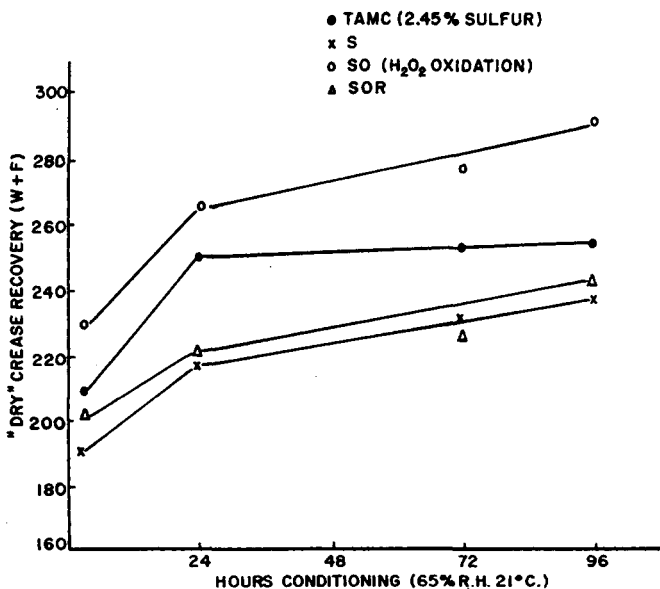


Fig. 4. Effect of conditioning on dry crease recovery of TAMC and its derivatives.

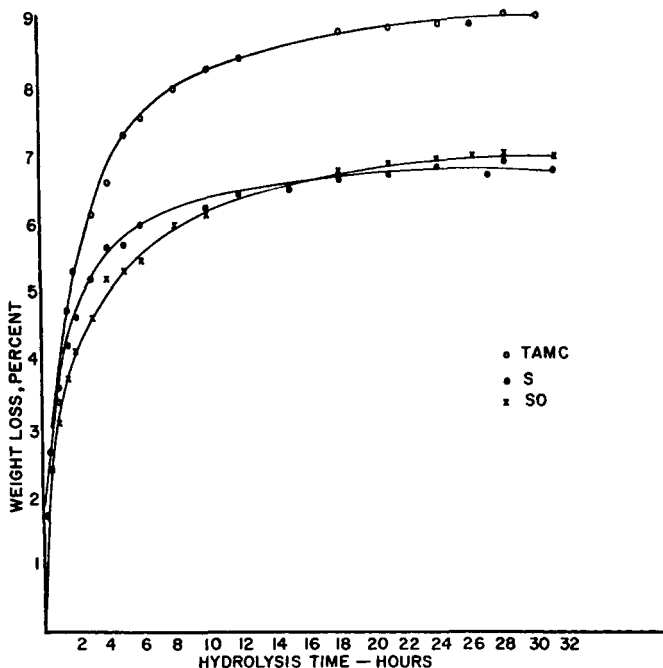


Fig. 5. Acid hydrolytic stability of TAMC and its derivatives in 0.1N HCl at 60°C.

Some attempts were made to alter the swelling behavior and accessibility of the cotton derivatives by carrying out the preparation of TAMC in the presence of nonvolatile swelling agents and also by carrying out reaction cycles on never-dried samples. No significant change in the general trends shown in Figure 3 was observed in the results of these experiments.

The effect of air oxidation on the crease recovery and SH and SS contents of the cotton derivatives (2.45% S) was investigated during extended conditioning at 65% R.H. and 21°C. In the course of this work immediate crease recovery values were determined also from the TAMC and oxidized (SO) samples that had previously been tested (Figs. 1 and 2) after 24 hr. of conditioning. Surprisingly, the immediate crease recovery of these samples was much lower than that obtained after conditioning. The magnitude of the effect of conditioning during 4 days is shown in Figure 4. It is evident that after 24 hr. dry crease recovery values increase even more for the "stable" TAMC and SO samples than for the "unstable" S and SOR samples.

Additional data shown in Table II illustrate the continuing rise in dry crease recovery with time of conditioning. The dry crease recovery of all three samples approached 290°C. after about 5 weeks of conditioning. An initial, high, wet crease recovery (250°C.) remained unchanged in the case of samples in the reduced state (S and SOR), while that of the SO sample increased from 256 to 280°C. (W + F) between 2 and 10 weeks of conditioning.

The dry crease recovery of the unmodified-cotton control increased

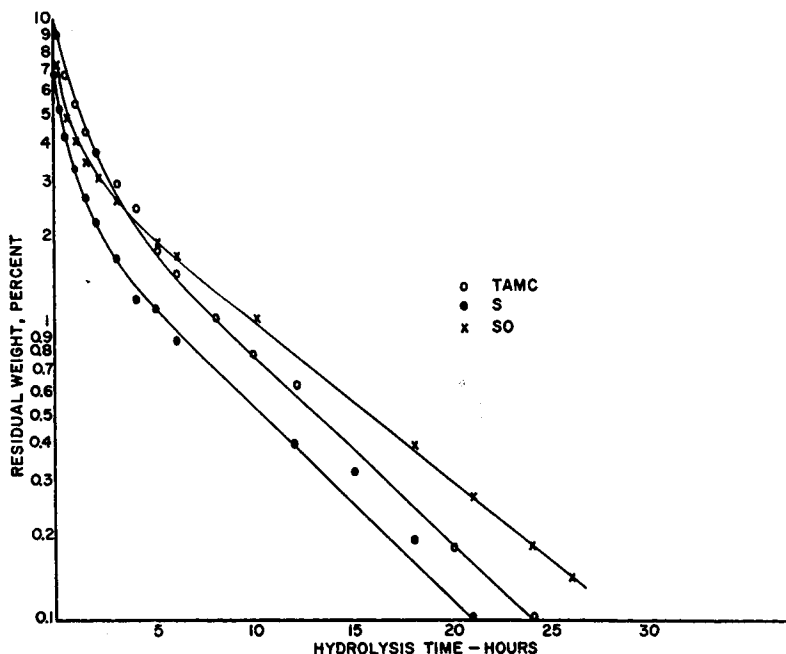


Fig. 6. Relation between the logarithm of residual substituent weight and the hydrolysis time for TAMC and its derivatives.

(148–169°C.) during 24 hr. of conditioning but remained unchanged (average value, 176°C.) during 13 additional days.

The SS and SH contents of the SO derivative did not change significantly during the 10 weeks. The SH contents of the S and SOR samples showed a tendency to decrease only during the last 5 weeks of conditioning, while the SS contents showed corresponding increases. Consideration of the constancy of the analyzable thiol and disulfide contents during the first 5 weeks provides evidence that simple air oxidation of thiol groups accessible to analysis cannot independently explain the observed increases in dry crease recovery. The formation of new disulfide crosslinks during conditioning, if it occurs, could be attributed either to a thiol-catalyzed disulfide interchange mechanism¹³ or to diffusion of atmospheric oxygen and oxidation of thiol groups in a region of the fiber that cannot be reached by analytical reagents. The fact that dry (2 hr. conditioning) crease recovery of samples stored under nitrogen for 2 months did not show an appreciable increase tends to support the second alternative.

Throughout this study evidence has been obtained that a significant portion of the substituents in the cotton derivatives is not accessible to reagents. Hydrolysis rate experiments were carried out, to explore the accessibility of the substituents to aqueous acid solutions. Dried samples (ca. 2.5 g.) of the TAMC, saponified (S) derivatives, or saponified and oxidized (with 3% H_2O_2) derivatives (SO) were treated with 100 ml. por-

tions of 0.10*N* HCl at 60°C., washed thoroughly with water, and dried (50° *in vacuo*). Weight-loss data resulting from specified hydrolysis times are shown graphically in Figure 5.

The weight loss leveled off after 30 hr. of hydrolysis time for the TAMC and S and SO derivatives, and sulfur-free and nitrogen-free products were obtained. Semilogarithmic plots of the data (Figure 6) show that in each case the weight loss occurs at two, distinct, pseudo first-order reaction rates. From all three derivatives the faster-hydrolyzing fraction was removed after 3-5 hr. of hydrolysis time.

TABLE III
Hydrolysis (60°C., 0.10*N* HCl) of TAMC and Saponified (S) and Oxidized (SO)
Derivatives from 2.4% Total Sulfur

Derivative	Hydrolysis rate constant ($\times 10^5$)		Total weight loss, %	
	k_1	k_2	At k_1	At k_2
TAMC	21	4.0	60	40
S	31	3.9	63	37
SO	35	3.4	52	48

Hydrolysis rate constants k_1 and k_2 for the substituents hydrolyzed at the faster and slower rates are shown in Table III, together with the proportions of the derivatives hydrolyzed at the two rates.

The hydrolysis rate data, supported by S and N elemental analyses, suggest a straightforward loss of substituent groups in the acid hydrolysis

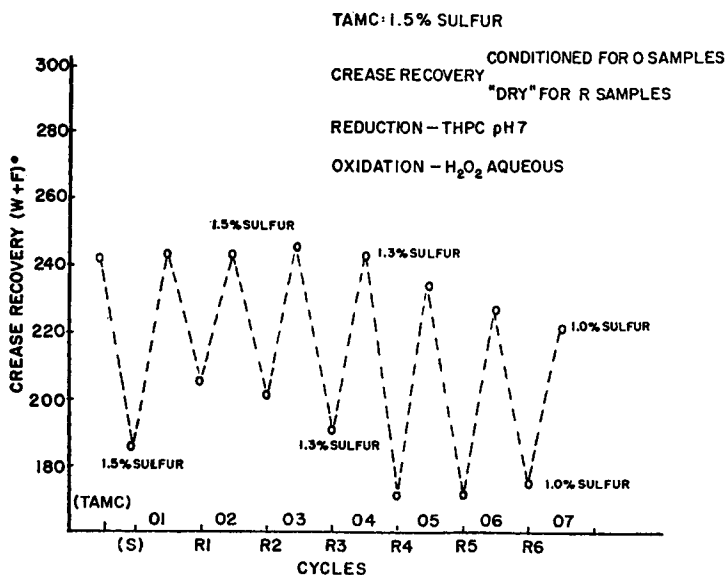


Fig. 7. Dry crease recovery changes of TAMC during oxidation-reduction cycles.

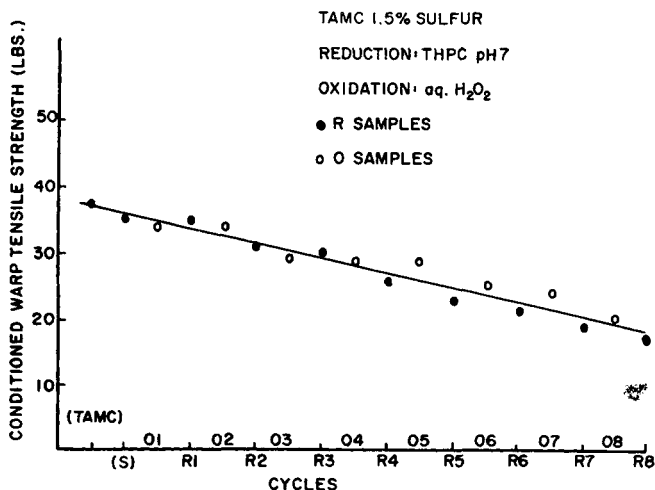


Fig. 8. Changes in tensile strength of TAMC during oxidation-reduction cycles.

by cleavage of $-\text{OCH}_2\text{N} \langle$. The percentage values obtained from the more readily hydrolyzed components correspond closely to those obtained for SH + SS in analytical determinations (e.g., see Table II), suggesting that differences in accessibility give rise to the two hydrolysis rates. Studies of hydrolysis rates of resinous materials on cellulose substrates have consistently shown¹⁴ that the resin is present as strongly and loosely bound fractions. The differences have been attributed to the cleavage of different linkages in the resinous component, but this does not appear to be a plausible explanation in the case of the TAMC derivative.

Even with the limitations discussed above a study of repeated oxidation-reduction cycles in this system was of interest. Figure 7 shows crease recovery changes for a sample of TAMC (1.5% sulfur), saponified and then exposed to multiple cycles of oxidation (H₂O₂) and reduction. A gradual decrease in the conditioned crease recovery of oxidized samples begins after the third or fourth cycle. The tensile strength of the samples (Figure 8) decreases gradually throughout, probably owing to oxidative degradation. As cycling continues, the tensile strength tends to be higher for the oxidized (crosslinked) samples than for the reduced. The sulfur content and the SH content of reduced samples after multiple cycles are shown in Figure 9. Loss of sulfur becomes significant after about three cycles and appears to parallel a decrease in the SH content of reduced samples besides the observed decrease in the conditioned crease recovery of oxidized samples shown in Figure 7. When a similar cycling experiment was carried out on a TAMC sample containing 2.45% sulfur, with the use of DMSO-Fe oxidations, sulfur loss was avoided, as shown by the upper curve for total sulfur in Figure 9, but the extent of oxidation determined by analysis of SH and SS content was very low after the first cycle.

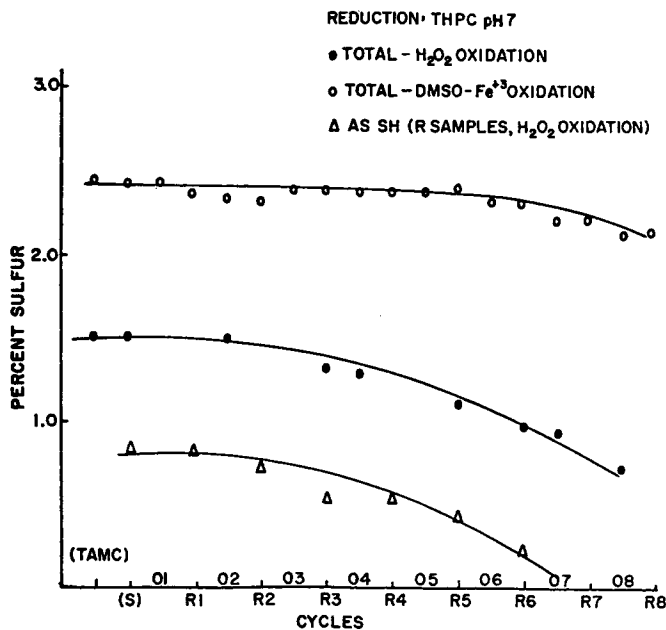


Fig. 9. Changes in sulfur content of TAMC during oxidation-reduction cycles.

Blocking of samples of high SH content (S and SOR) was studied with NEMI, iodoacetamide, and alkyl iodides. Some results of blocking experiments are shown in Table IV for a saponified TAMC sample (S) of 2.45% sulfur content. Comparable results were obtained when blocking reactions were carried out with saponified TAMC of lower sulfur content and also with TAMC samples that had been saponified, oxidized, and reduced

TABLE IV
Data on Thioacetamidomethylcellulose
Blocked with Alkyl Iodides and with NEMI^a

Blocking reagent	SH, %	Sulfur as —SS—, %	Conditioned crease recovery (W + F)		Tensile strength (warp), lb.	
	SB ^b	SBO ^b	SB	SBO	SB	SBO
None	1.21(S)	1.20(SO)	189 (S) ^c	257 (SO)	39 (S)	32 (SO)
C ₂ H ₅ I	0	0.12	206	203	36	35
C ₄ H ₉ I	0	0.17	211	213	36	36
C ₅ H ₁₁ I	0	0.10	218	224	35	35
C ₁₀ H ₂₁ I	0	0.12	230	229	35	37
C ₁₈ H ₃₇ I	0	0.18	213	226	36	30
ICH ₂ CONH ₂	0	—	208	230	39	35
NEMI	0	0	186	245	41	43

^a TAMC: 2.45% sulfur.

^b SB, saponified and blocked; SBO, saponified, blocked and oxidized (H₂O₂).

^c Conditioned 2 hr. only.

(SOR). Analytical determinations with blocked samples indicate that the alkylation reactions had reached essential completion. In samples blocked with alkyl iodides the conditioned crease recovery and tensile strength remain essentially unchanged when the samples are oxidized with 3% H_2O_2 . The conditioned crease recovery of the sample blocked with NEMI, on the other hand, increases after oxidation. These observations again suggest the presence of thiol groups inaccessible to the NEMI reagent under the conditions used.

SUMMARY AND CONCLUSIONS

In the light of the results reported in this paper the following conclusions are warranted.

(1) The reversibility of crosslinks in disulfide-modified cotton has been confirmed for the system investigated, namely, the product obtained from the reaction of unmodified cotton with *N*-hydroxymethylacetylthioacetamide, saponification, and oxidation.

(2) In this system, as in others investigated previously, the dry crease recovery changes in the expected manner, as crosslinks are formed and ruptured in oxidation and reduction reactions. However, chemical analysis of functional groups reveals that the reaction yields are far from quantitative and that a significant portion of the functional groups present remains inaccessible to reagents during reactions or analyses or both.

(3) The dry crease recovery of acetylthioacetamidomethylcellulose (TAMC) and of its saponified (S), oxidized (SO), and reduced (SOR) derivatives increases with increasing time of conditioning, while the apparent thiol and disulfide contents change only slowly and after prolonged conditioning.

(4) Available experimental evidence suggests that in the system studied a fraction of the substituents are readily accessible to reagents, while the remainder react at a much slower rate.

Hydrolysis rate studies revealed thioacetamidomethyl substituents in TAMC and in the saponified (S) and oxidized (SO) derivatives having two distinctly different hydrolytic stabilities differing by approximately one order of magnitude.

(5) Multiple oxidation-reduction cycles can be carried out on the cotton derivatives, but oxidative degradation in the case of H_2O_2 oxidation and decreasing reaction efficiency in the case of DMSO-Fe oxidation limit the number of effective cycles in this sequence.

(6) Alkylation of mercaptoacetamidomethylcellulose with alkyl iodides forms stable thioether derivatives, seemingly free from crosslinks, and offers an interesting route to the preparation of new cotton derivatives.

References

1. R. F. Schwenker, Jr., L. Lifland, and E. Pacsu, *Textile Res. J.*, **32**, 797 (1962).
2. R. F. Schwenker, Jr. and L. Lifland, *Textile Res. J.*, **33**, 107 (1963).
3. C. H. Mack, H. H. McGregor, Jr., and S. R. Hobart, *Textile Res. J.*, **35**, 353 (1965).

4. S. R. Hobart, C. H. Mack, and C. P. Wade, *Textile Res. J.*, **36**, 30 (1966).
5. W. A. Mueller and R. Swidler, *Textile Res. J.*, **34**, 656 (1964).
6. H. Rath, K. Brederick, W. Möck, and M. Diamantoglou, *Melliand Textilchem.*, **1**, 97 (1965).
7. W. Sauer, O. A. Stamm, and H. Zollinger, *Textilveredlung*, **1**, 4 (1966).
8. C. G. Overberger, K. H. Burg, and W. H. Daly, *J. Am. Chem. Soc.*, **87**, 4125 (1965).
9. A. D. Jenkins and L. J. Wolfram, *J. Soc. Dyers Colourists*, **79**, 55 (1963).
10. R. W. Burley and F. W. A. Horden, *Textile Res. J.*, **27**, 615 (1957).
11. W. W. Cleland, *Biochemistry*, **3**, 480 (1964).
12. H. D. Weigmann and L. Rebenfeld, *Textile Res. J.*, **36**, 202 (1966).
13. H. D. Weigmann, L. Rebenfeld, and C. Dansizer, *Textile Res. J.*, **35**, 604 (1965).
14. W. B. Achwal and B. B. Pitale, *Textile Res. J.*, **36**, 844 (1966).

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