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The disulfide interchange reaction of hydrolyzed hair keratin and bis-dinitrophenyl cystine in concentrated hydrochloric acid

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Synopsis

The disulfide exchange reaction of cystine from hair keratin hydrolysates and bis-dinitrophenyl-cystine in concentrated hydrochloric acid was investigated. The reaction rate constants for the forward and the reverse reaction were found to be 12.8 and 5.0 hr^{-1}, respectively. The equilibrium constant was 2.56.

Using this reaction, the cystine contents in hydrolysates from intact and peroxide-bleached Caucasian hair were determined. The corresponding liquid retentions for the hair samples were also measured. A correlation between the decrease in cystine content due to the bleaching (from 647 μ mole/g for intact hair to 450 μ mole/g for 5 times bleached hair) and the increase in the liquid retention (from 38 to 53%) was demonstrated.

INTRODUCTION

The disulfide bonds of cystine in hair keratin contribute significantly to the physical and chemical properties of hair fibers. These bonds can be ruptured in a variety of ways including bleaching, waving, or exposure to UV radiation. Hair fibers undergoing these reactions will change their properties. An estimate of the number of disulfide bonds broken by these treatments would indicate the extent of these reactions with the hair.

A disulfide interchange reaction in a concentrated acidic medium was demonstrated by Sanger (1,2) using bis-dinitroprophenyl cystine (DNP-S-S-DNP) and cystine (Cy-S-S-Cy) as model compounds

$$\underset{(A)}{\text{Cy-S-S-Cy}} + \underset{(B)}{\text{DNP-S-S-DNP}} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} 2\ \underset{(C)}{\text{Cy-S-S-DNP}} \qquad \text{(eq. 1)}$$

The mechanism of this reaction was deduced by Benesch and Benesch (3) and can be represented by the following scheme:

$$\text{INITIATION: RSSR} + \text{H}^+ \rightleftharpoons \text{RS}^+ + \text{RSH}$$

$$\text{EXCHANGE: RS}^+ + \text{R'SSR'} \rightleftharpoons \text{RSSR'} + \text{R'S}^+$$

$$\text{INHIBITION: R'S}^+ + \text{RSH} \rightleftharpoons \text{RSSR'} + \text{H}^+$$

Glazer and Smith (4) employed this reaction to estimate the amount of half-cystine in proteins. However, the reaction rate constants (k_1, k_2) and the equilibrium constant ($K = k_1/k_2$) were not determined. Without these parameters, multiple experiments with several concentrations of a protein were required to determine the cystine content.

Herewith we are reporting a study on the exchange reaction of cystine in hair keratin hydrolysates and bis-DNP(dinitrophenyl) cystine to obtain the equilibrium and reaction rate constants. These constants in conjunction with the equilibrium or the kinetic data are used to determine the cystine contents in virgin and oxidized (bleached) hair fibers.

METHOD

MATERIALS

Analytical grade chemicals were used in the study without further purification. Bis-DNP-cystine was obtained commercially. Brown Caucasian hair was used as the source of hair keratin. The hair was cleaned by twice shampooing and repeated rinsing with distilled water. All glassware was acid washed and rinsed well with distilled water prior to use.

EXPERIMENTS

A known amount of hair, ranging from 0.05 to 0.5 g, was digested in 25 ml of 12 N HCl at 50°C for 48 hours. Then the hydrolysate was filtered using a Milipore glass filter and diluted to 250 ml with 9.6 N HCl. A 20-ml aliquot was subsequently transferred to a screw cap vial containing bis-DNP-cystine. The concentration of bis-DNP-cystine used ranged from 1.2 to 10 mg per ml of hair hydrolysate. The vial was wrapped in aluminum foil to protect it from exposure to light and placed in a 39 ± 0.5°C water bath. At certain time intervals, i.e., 1, 2, 3, 4, 8, 10, 15, 23, and 30 days, 1 ml of the reaction solution was withdrawn and diluted with 1.5 ml water. The unreacted bis-DNP-cystine in the solution was removed by extraction with 3 ml ethyl ether 6 times. The aqueous phase was quantitatively transferred to a 25-ml volumetric flask and diluted with 6N HCl. The concentration of mono-DNP-cystine in the solution was determined spectrophotometrically at 357 nm using a Cary 17D spectrophotometer. A sample of the hydrolysate without the reagent was taken through the complete procedure as the control. All experiments were run in duplicate.

OXIDATION OF HAIR

Hydrogen peroxide was used to bleach hair. Two solutions were prepared for the experiment. Solution A contained 10 g urea, 3 g glycerine, 7 g sodium chloride and 12 g ammonia hydroxide (30%) in 68 ml of distilled water. Solution B was a 6% (by weight) hydrogen peroxide solution. A mixture of 10 ml of each solution was heated to 32 ± 0.5°C in a glass beaker. Then a 2 g hair tress was immersed in the mixture for 30 minutes. The temperature of the solution was maintained by a water bath and the solution was agitated throughout this period. The oxidized hair tress was rinsed thoroughly with distilled water and dried.

DETERMINATION OF LIQUID RETENTION

To determine the percentage of liquid retained by hair, a 0.2 g hair tress was immersed in a pH 7.00 buffer with 2 drops of 1% Triton X 100 solution. The hair was left in

the solution for 30 minutes. Then it was transferred to a centrifuge tube and centrifuged for 10 minutes at 1000 G. The tube had a wire mesh platform in the middle section to prevent the centrifuged hair from mixing with the liquid. Immediately following the centrifugation, the weight of the hair sample was determined. Then the hair sample was dried in a 105°C oven for 2 hours. When the sample was cooled, it was weighed again. The percentage of liquid retained was calculated from the difference of wet and dry weights divided by the dry weight.

RESULTS AND DISCUSSION

DETERMINATION OF THE EQUILIBRIUM CONSTANT

The disulfide interchange reaction is very slow in hydrochloric acid having a concentration below 9N (2). In determining the concentration of Mono-DNP-cystine in the reacting solution, the acid concentration was diluted to about 2.4N during extraction. Therefore, because the reaction was stopped in the separation process, the concentration of mono-DNP-cystine should not be affected by removal of bis-DNP-cystine during the procedure.

The reaction reached equilibrium in about 20 days. At equilibrium, the concentration of each species in the reacting solution may be expressed by

$$\frac{[C]^2}{[A][B]} = \frac{k_1}{k_2} = K \qquad \text{(eq. 2)}$$

where k_1 and k_2 are the forward and reverse reaction rate constants and K is the equilibrium constant. The other parameters, A, B, and C, are species shown in eq. 1. Under the chosen conditions, the solution was saturated with bis-DNP-cystine (B) throughout the experiment; the concentration of B was constant at 1.3 m mole/l (4). From the law of mass conservation, the concentration of cystine ([A]) is

$$[A] = [A]_0 - 0.5[C] \qquad \text{(eq. 3)}$$

where $[A]_0$ is the concentration of A at $t = 0$, i.e., the amount of cystine in hair keratin. It may be rewritten as

$$[A]_0 = m[Cy] \qquad \text{(eq. 4)}$$

where [Cy] is the amount of cystine in 1 g of hair and m is the weight of hair per liter of hydrolysate. Eq. 2 can then be reduced to

$$[C]^2 = K'(m[Cy] - 0.5[C]) \qquad \text{(eq. 5)}$$

where

$$K' = K[B] \qquad \text{(eq. 6)}$$

The solution for eq. 5, i.e., the concentration of mono-DNP-cystine at equilibrium, would be

$$[C] = 0.5(-0.5K' + Q) \qquad \text{(eq. 7)}$$

where

$$Q = (0.25K'^2 + 4K'\, m[Cy])^{1/2} \qquad \text{(eq. 8)}$$

To determine the equilibrium constant, K, and the amount of cystine per gram of hair, [Cy], from the equilibrium concentration of mono-DNP-cystine, [C], eq. 5 may be rearranged to

$$m = \frac{[C]^2}{K'[Cy]} + \frac{0.5}{[Cy]}[C] \qquad \text{(eq. 9)}$$

Eq. 9 is a simple equation of m expressed in quadratic form of [C] with $1/K'[Cy]$ and $1/[Cy]$ as constant parameters. Therefore, [Cy] and K′ (or K) can be determined using a least squares regression method with several values of m and [C] determined experimentally. Figure 1 shows the relationship between the equilibrium concentration of mono-DNP-cystine, [C], obtained and the amount of hair per liter of hydrolysate (m). The regression curve of eq. 9 is shown as the solid line in Figure 1 with $[Cy] = 6.54 \times 10^{-4}$ mole/g dry hair and $K' = 3.33 \times 10^{-3}$. The half-cystine content in Caucasian virgin hair obtained here (1308 μ mole/g hair) is well within the range of the literature

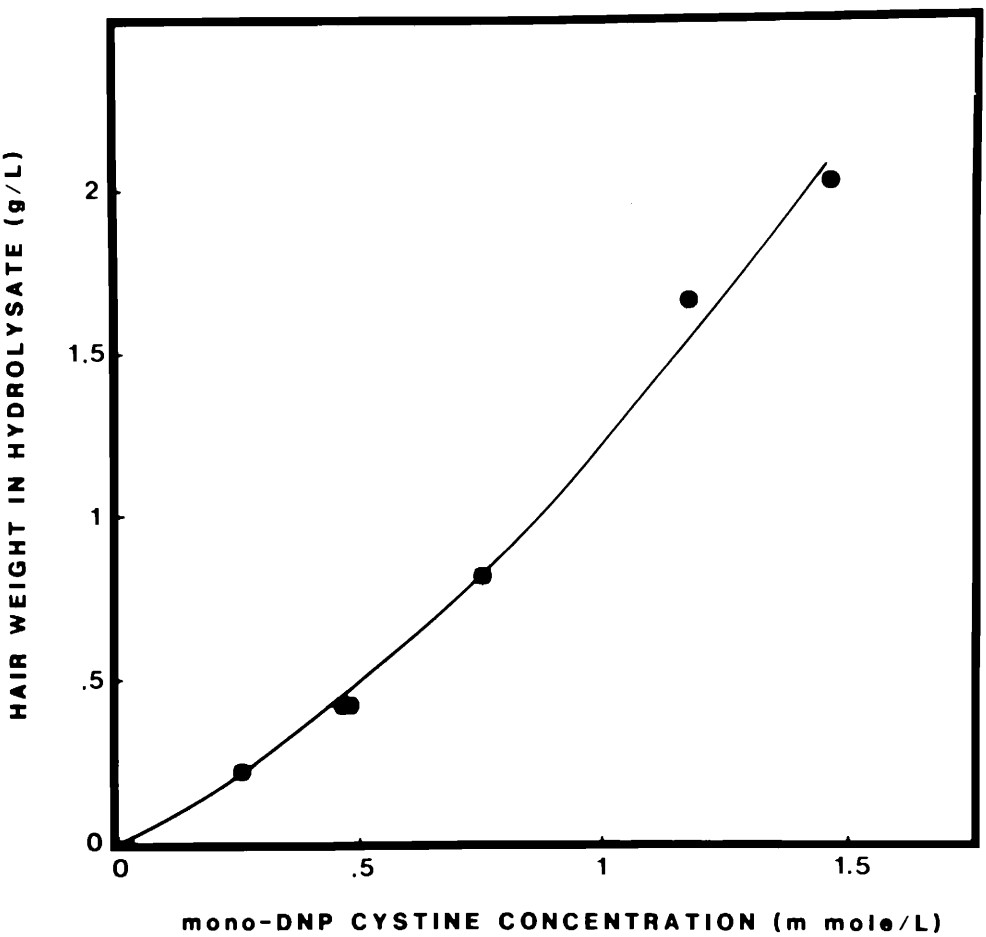

Figure 1. The relationship between yield of mono-DNP-cystine at equilibrium and concentration of hair in hydrolysate used in disulfide interchange reaction. The closed circles represent experimental data and the curve was calculated from eq. 5 with $K' = 3.33 \times 10^{-3}$ and $[Cy] = 6.54 \times 10^{-4}$ mole/g hair.

values (1269–1650 μ mole/g hair) (5). The equilibrium constant K for the interchange reaction in strong acidic medium (≥9.6N) was determined to be 2.56.

DETERMINATION OF THE REACTION RATE CONSTANTS

The rate equation for the interchange reaction is

$$\frac{d[C]}{dt} = k_1[A][B] - k_2[C]^2 \qquad \text{(eq. 10)}$$

or

$$\frac{1}{k_2}\frac{d[C]}{dt} = K'(m[Cy] - 0.5[C]) - [C]^2 \qquad \text{(eq. 11)}$$

The solution for eq. 11, i.e, the formation of mono-DNP-cystine (C) as a function of time (t) is

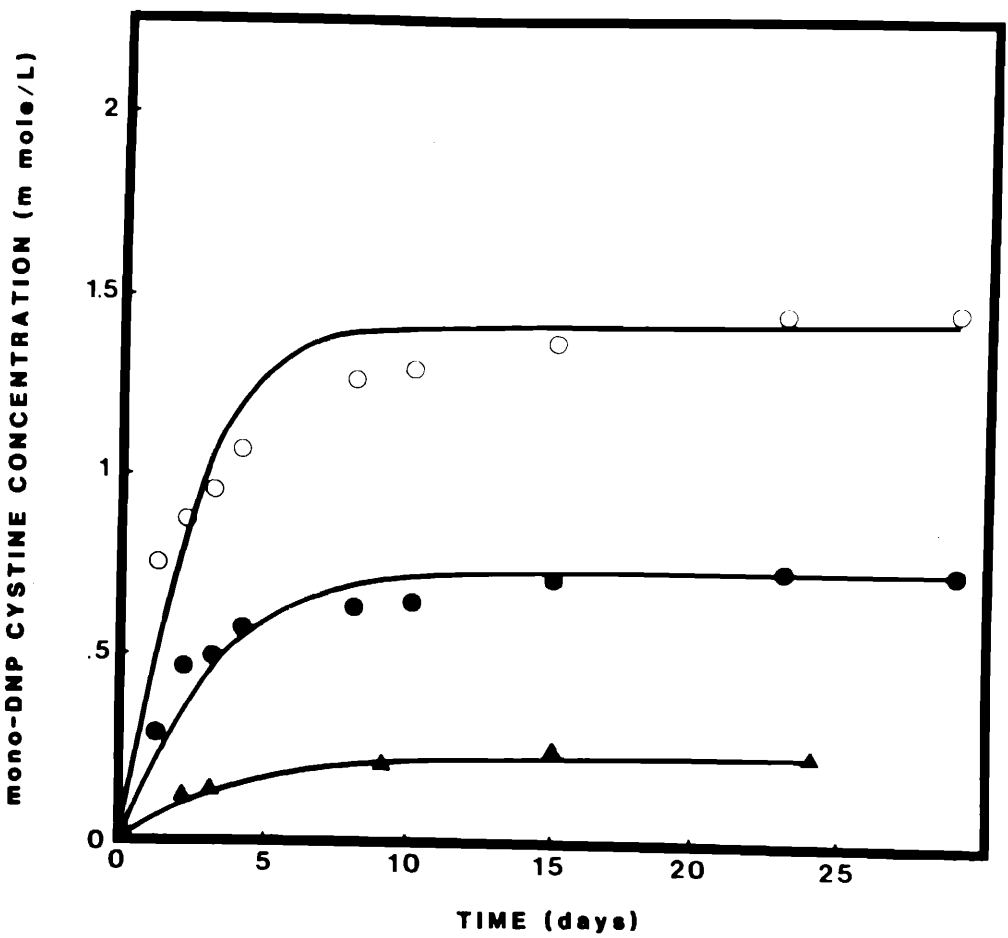

Figure 2. Formation of mono-DNP-cystine as a function of time for various concentrations of hair hydrolysate from intact hair. The curves were calculated from eq. 12 with $K' = 3.3 \times 10^{-3}$, $k_2 = 5.0$ hr^{-1}, and $[Cy] = 6.54 \times 10^{-4}$ mole/g hair. Key: ○ = 0.5 g; ● = 0.2 g; ▲ = 0.05 g of hair in 250 ml of 9.6 N HCl.

$$[C] = \frac{0.5(0.5K' - Q)(e^{-k_2Qt} - 1)}{1 - \frac{0.5K' - Q}{0.5K' + Q} \cdot e^{-k_2Qt}} \qquad \text{(eq. 12)}$$

Eq. 12 may be rewritten as

$$-k_2Qt = \ln\left(\frac{[C] + 0.5(0.5K' - Q)}{[C] + 0.5(0.5K' + Q)} \cdot \frac{0.5K' + Q}{0.5K' - Q}\right) \qquad \text{(eq. 13)}$$

Since K′ and Q (or [Cy]) are known, the right hand side of eq. 13 may be plotted as a function of time (t) from the kinetic data. The slope of this straight line is $-k_2Q$. Therefore, the reaction rate constants k_2 and k_1 can be determined. The values for k_1 and k_2 for the hair keratin obtained using the least square linear regression are 12.80 and 5.00 hr^{-1}, respectively. Figure 2 shows the formation of mono-DNP-cystine as a function of time for some hydrolysate concentrations. The curves in the figure were calculated from eq. 12.

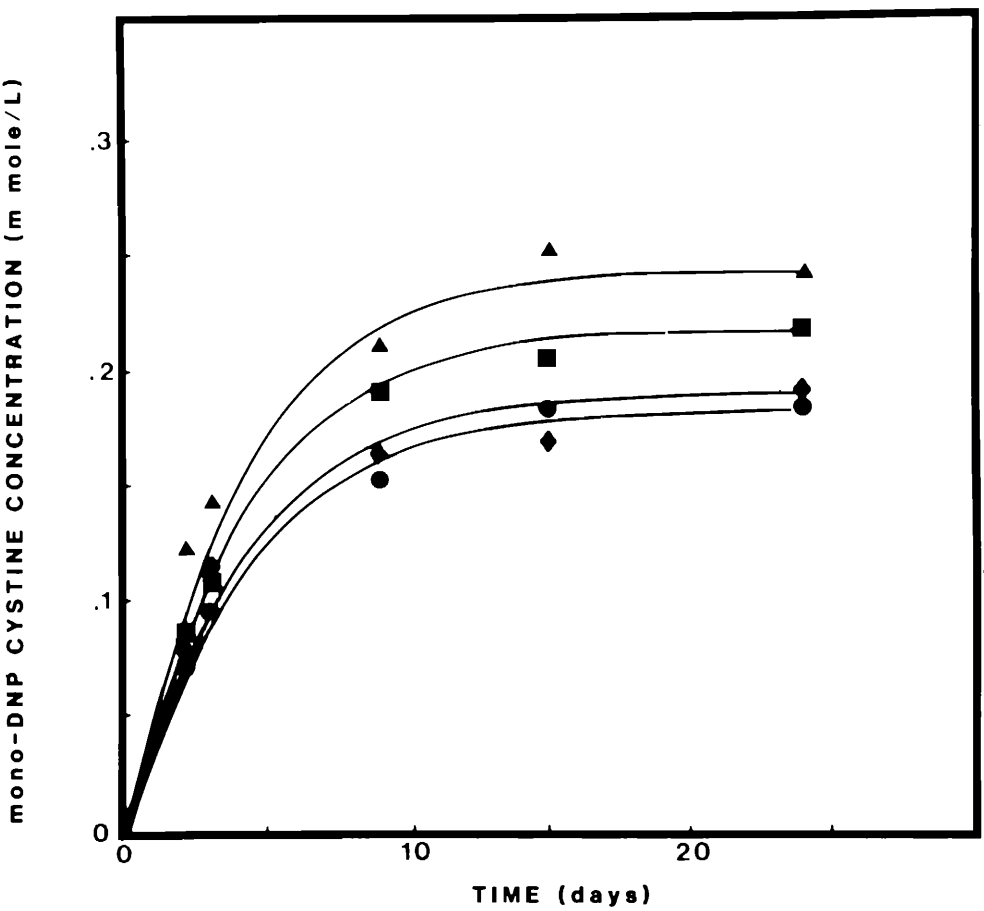

Figure 3. Formation of mono-DNP-cystine as a function of time using hydrolysates of hair which had undergone various degrees of bleaching in alkaline hydrogen peroxide solution. The curves were calculated from eq. 12 with $K' = 3.33 \times 10^{-3}$, $k_2 = 5.0$ hr^{-1}, and [Cy]s from Table I. Key: ▲ = untreated; ■ = 1× bleached; ◆ = 3× bleached; ● = 5× bleached.

CYSTINE CONTENT IN OXIDIZED HAIR

Figure 3 depicts rates of mono-DNP-cystine production for hair at different levels of peroxide treatment. The amount of mono-DNP-cystine formed is related proportionally to the level of the treatment. This indicates an increase in the disulfide bonds broken as the hair was subjected to more oxidation. The cystine concentration in the oxidized keratin fibers could be calculated from the concentration of mono-DNP-cystine at equilibrium using eq. 5 and the equilibrium constant obtained above. The amounts of hair used, the concentration of mono-DNP-cystine at equilibrium, and the calculated cystine contents are listed in Table I.

The rates of mono-DNP-cystine formation as a function of time for the bleached hair are shown in Figure 3. The curves in the figure are calculated from eq. 12 using the same reaction rate constants (k_1 and k_2) as for the virgin fibers. It can be seen that good agreement ($r = 0.988$, $t = 23.14$) exists between the experimental data for the oxidized hair and the rates predicted using the constants derived from untreated hair.

In the peroxide bleaching process, the perhydroxy anion (HO_2^-) is the reactive species, and its attack on hair keratin appears to be focused on the disulfide bond (6). As the cystine content decreases, there is a corresponding increase in cysteic acid which is the only established major end product of the oxidative cleavage (7,8). Any other reaction intermediates formed would be unstable under alkaline conditions and would revert to cystine and cysteic acid during hydrolysis. The conversion of the disulfide group to two more hydrophilic cysteic acid groups will increase the liquid retention of the oxidized hair fiber (9).

The disulfide concentrations and the corresponding liquid retentions for the peroxide bleached hair fibers are listed in Table II and shown in Figure 4. As expected, the disulfide concentration decreases and the liquid retention increases when the number of treatment increases.

CONCLUSION

Sanger's (1,2) experience in producing a mixed disulfide from the reaction of two symmetric disulfides was successfully applied to hair keratin disulfide analysis. Using eq. 5 with different amounts of hair in the hydrolysate and the corresponding equilibrium concentrations of the mixed disulfide formed, the cystine content in hair and the equilibrium constant for the exchange reaction were determined. The forward and the

Table I
Experimental Results and Calculated Cystine Content* for Bleached Hair

Number of Treatments	Amount of Hair in Hydrolysate m (g/l)	Con. of Mono-DNP Cystine at Equilibrium [C] (m mole/l)	Calculated Cystine Content in Hair [Cy] (μ mole/g hair)
0	0.20184	0.245	647
1	0.21216	0.211	553
3	0.2086	0.187	489
5	0.2204	0.181	450

* Using eq. 5 with $K' = 3.33 \times 10^{-3}$.

Table II
Cystine Content and Liquid Retention of Bleached* Hair

No. of Treatments	Cystine Content (μ mole/g hair)	Liquid Retention (%)
0	647	37.5
1	553	41.3
3	489	45.3
5	450	52.8

* Hair was bleached in alkaline hydrogen peroxide solution.

reverse reaction rate constants were also determined from the kinetic data using eq. 12.

Hair fiber bleaching by peroxide oxidation was accompanied by a decrease in disulfide bonds (from 647 to 450 μ mole/g of hair) and an increase in liquid retention (from 38 to 53%). The data indicate that the disulfide analysis prior to and after oxidative treatment can be used to determine the decrease in cystine content caused by the treatment.

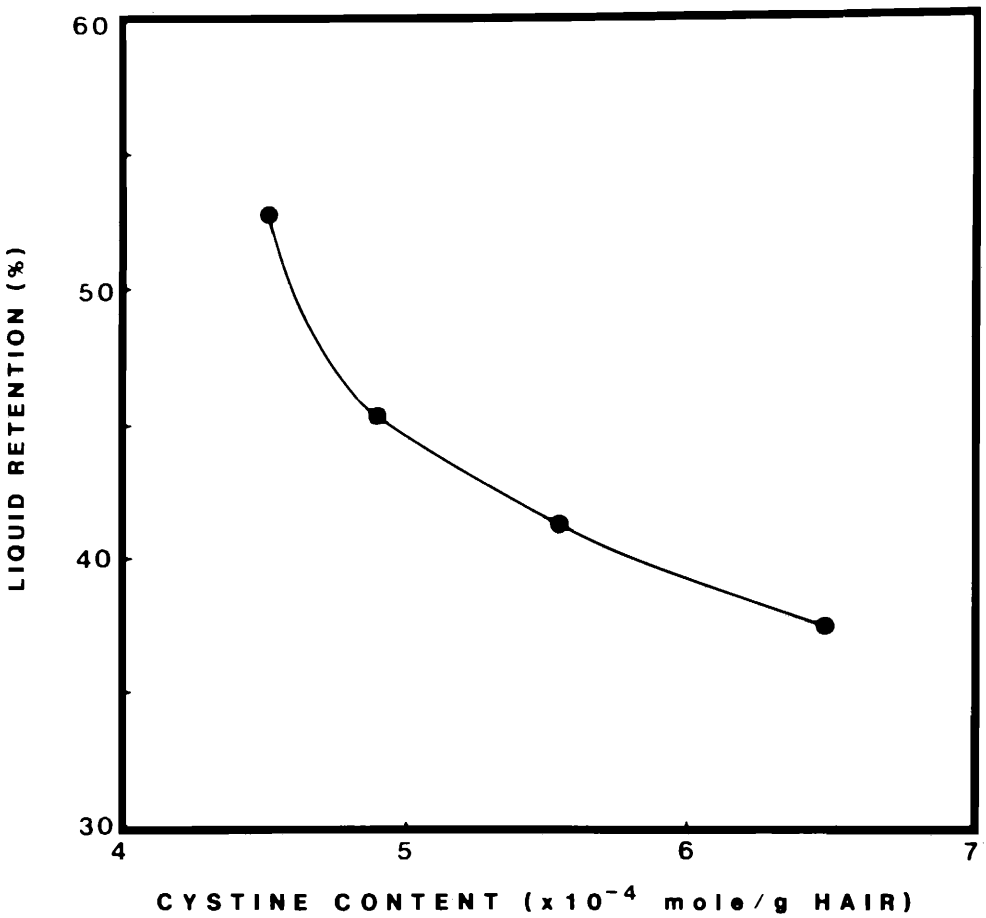

Figure 4. The relationship between liquid retention and the cystine content of bleached hair.

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