EFFECTS OF CHELATE FORMATION
ON THE PROPERTIES OF
PROTEIN FIBERS

by

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From the thermodynamical study of the chelate reactions in synthetic chelate resins by author,[1~4] some new applications of these reactions were considered. As protein fibers, silk and wool were cnosen.

The cyclic compound, in which a metal is joined to two or more donor groups of a single molecule or ion, are particularly important since they have exceptionally high stability and, in many cases, have remarkable and valuable properties, In this paper chelate reactions were used for the purpose of improving of chemical and properties of silk and wool

I METHOD OF DETERMINING THE FORMATION OF
CHELATE IN PROTEIN TIBER.

All metal chelates may be considerered as formed by the displacement of one or more usually weak acidic protons of the chelating agent by a metal ion. Thus, the addition of glycine to a solution of cupric salt causes a drop in the pH. The reaction may be also considered as the combination of the metal ion with the anion of the chelating agent, accompanied by a shift in the ionization equilibrium of the amino acid. In a qualitative sense, therefore, it may be concluded that chelate formation of metal ions with amino acids and with the acid form of other chelating compounds results in a pH drop, and the greater the tendency for metals to combine with a given chelating compound, the greater drop in pH.

1) **pH Titration Curves of Silk[5]**

In Fig. 1, the pH titration curves for silk in the presence of metal ions are shown. The results show the addition of metal ions lowers the pH titration curves of Fibroin in the presence of KCI. In order to compare these results with free amino acid, o-amino benzoic acid was used. The addition of metal ion to this acid which has free amino group and carboxyl group lowers the pH of the solution greatly and appreciable chelation occures even in the strong acid region.

There are many peptide linkags in protein, therefore, the effect of these peptide linkages on the results of pH titration tests were considered. From the pH titration curves in Fig. 2, it is easily understood that the effect of peptide linkage on the pH titration curves of silk in the presence of metal ions is samll.
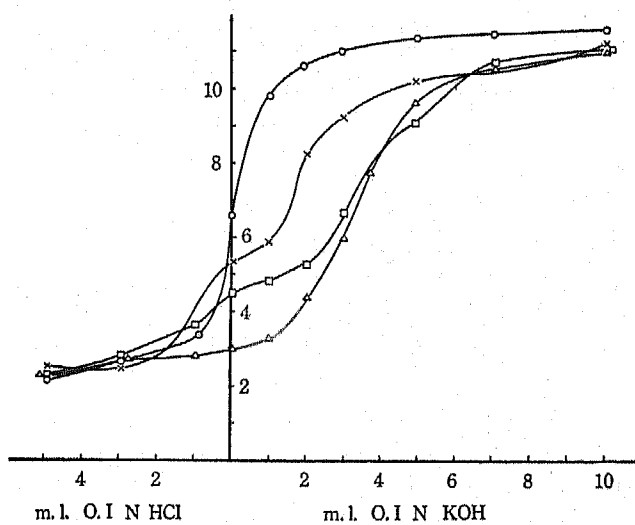
Fig. 1 pH titration Curves of Silk Fibroin in the
presence of KCl

    ○ Silk Fibroin   × Silk Fibroin+Cu
    □ Silk Fibroin+Al   △ Silk Fibroin+Fe
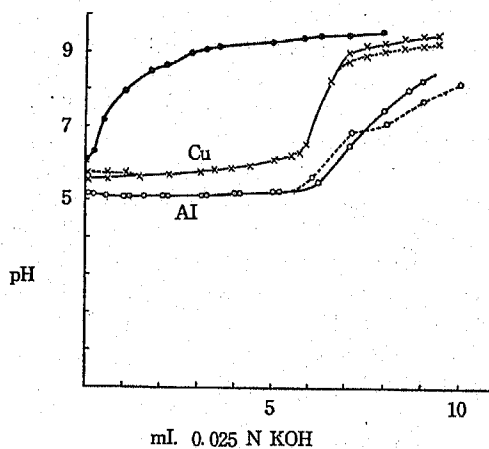

Fig. 2 pH Titration curves of Glycine anhydride in the
presence of KCl dotted line Cu, Al
        ● Glycine anhydride
        × Glycine anhydride+Cu
        ○ Glycine anhydride+Al

Interaction of o–amino benzoic acid with Cu and Al is greater than that of Fibroin with the same metals.

    2) **pH Titration Curves of Wool.** [6]

    pH titration curves of wool protein were obtained in the absence and in presence of heavy metallic ions such as Hg, Cu and Al. In order to get the accurate results, pH titrations with KOH and HCl were made of these metallic ions under the same condition as in the case of wool together with metallic ions. 0.4g of air dried wool was put into the bottle containing 40 ml of the following solution. Each bottle contained 10 ml of 2M KNO₃ solution, the definite quantity of 0.02N KOH or HCl and was filled to 40 ml with water. In Fig. 3 the addition of the metal ions to wool causes a drop in pH in the cases of Hg and Cu. Cystine, an important constituent of wool, was used for the determination of pH titration. From these tests, cystine
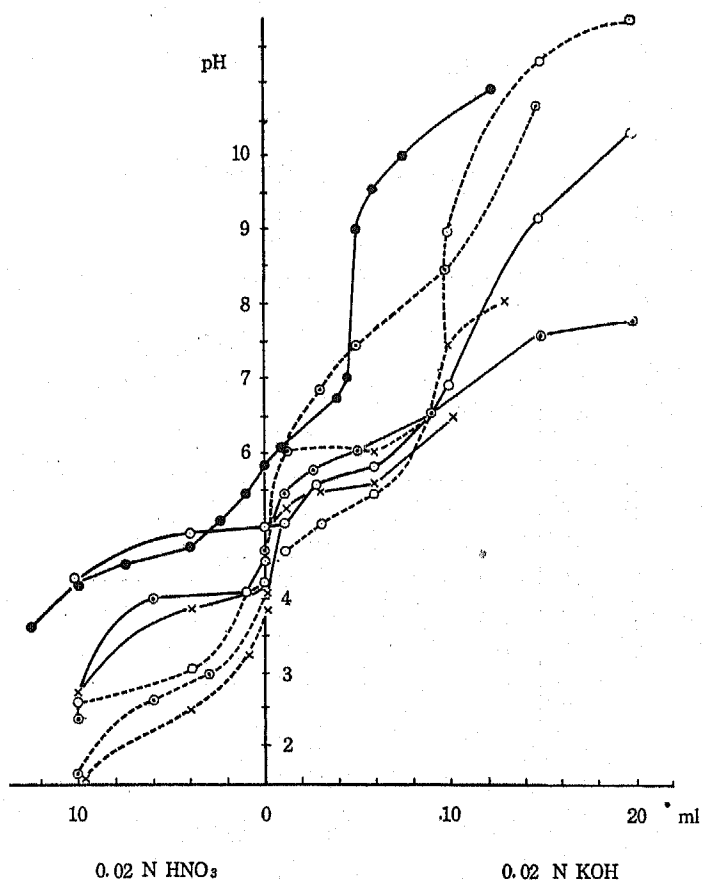

Fig. 3   pH Titration Curves of wool
       ● WOOL, ······Cu, ——× WOOL+Cu, ······○ Al
       ······◉ Hg,   ——○ WOOL+Al, ——◉ WOOL+Hg

seems to form chelate with Hg, Cu and Al.

II   EFFECTS OF CHELATE FORMATION ON THE MECHANICAL PROPERTIES OF PROTEIN FIBERS.

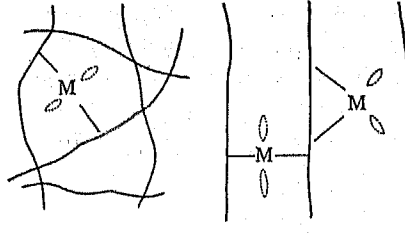### 1) The change in mechanical properties of Silk by chelate Formation.[7]

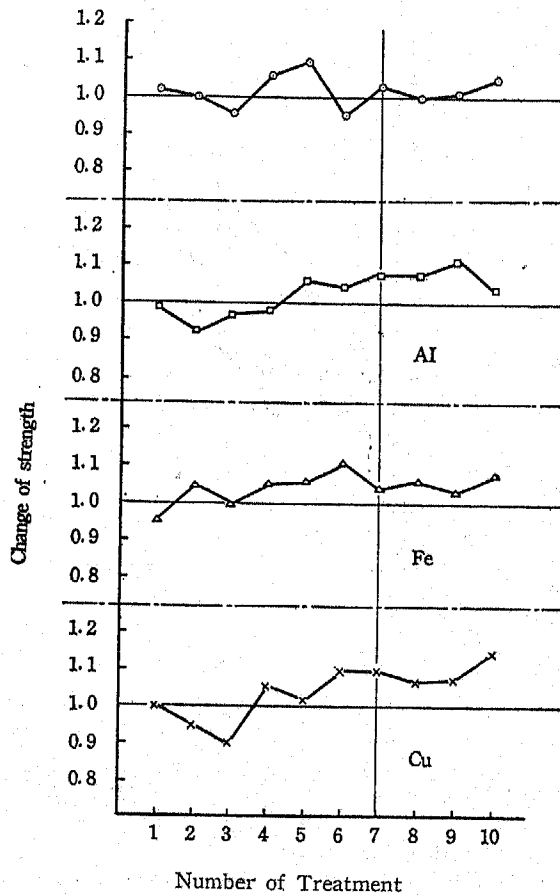Fig. 4 Model of chelation in fiber

Number of Treatment
Fig. 5 Change of Strength by Chelate Formation

If the chelate forms as is imagined in Fig. 4, the strength of fiber will change. The strength and elongation of the fibroin treated with heavy metal ions in various pH solutions were compared with those of the fibroin treated in the corresponding pH solutions without metal ions. The results were shown in Fig. 5. The strength of the treated fibroin increases in acidic region in all cases in comparison with those of the fibroin untreated and treated in the absence of heavy metal ions. The elongation has a tendency to decrease. The increase of strength of the treated silk is seen in the pH region where the chelate formation is expected. Cu, Al and Fe were used as heavy metal ion throughout those experiments.

    2) **The Relationship between the amount of Cu absorbed by silk and the strength of silk.** [8]

    From above results, it is easily understood that the more chelates in fiber is the more it becomes stable. The effects of the time of treatment and pH of the solution on the absorption of Cu by silk were considered. Then, Absorption of Cu
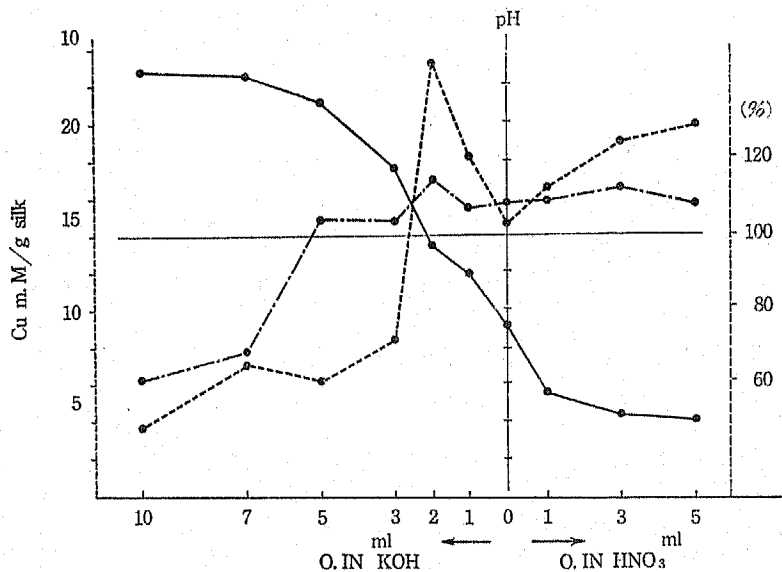

Fig. 6 Absorption of Cu and Change of strength of the silk treated with Cu in various pH solutions

and change of strength of the silk treated with Cu ion in various pH solutions were determined as mentioned before. (Fig. 6.). The strength of silk greatly increased at the point of the greatest absorption of Cu in Fig. 6. This tells us that chelation actually favours the strength of silk. The other factors which affect the strength of silk were taken into account. Acid treatment of silk is known to change the strength under certain conditions, therefore, silk was treated with acetic acid, hydrochloric acid and nitric acid respectively. The results were shown in Tables 1 and 2.

Table 1   Change of strength (%) of acetic acid treated silk

Time of Treatment (hr)	1.5	24	48
Change of Strength	99.5	101.5	106.1

0.5% of acetic acid was use for this experiment.

Table 2   Change of strength of acid treated silk after 24 hr dipping

ml. of 0.1 N Acids in 40 ml.	2	5	10
HNO_3	102.8	91.9	103.7
HCl	105.1	99.5	98.7
CH_3COOH	93.2	98.5	108.1

To neglect the effect of acetic acid treatment on the strength of Cu treated silk and that of Al treated one, washing of these treated silk with acetic acid was omitted, and the strength of those silk was measured as before, the results were shown in Table 3.

Table 3   Change of Strength (%)

Number of treatment	1	2	3	4	5	6	7	8	9	10
Al treated silk	100.6	106.1	103.7	104.2	98.2	103.1	104.6	105.3	102.6	102.9
Cu treated silk	71.2	74.4	102.9	103.7	108.6	107.6	107.5	110.7	105.9	110.1

Each sample was treated in the following solution

	0.1N			KOH			0.1N			HNO_3	
Number of Treatment	1	2	3	4	5	6	7	8	9	10	
ml. of KOH or HNO_3	10	7	5	3	2	1	0	1	3	5	

From Table 3, it is clear that chelation in silk fiber is favourable for the strength of it.

   3) **The Change in Absorption of Cu by wool with Streching.** [9]
   It is widely known that the chelate formation is greatly affected by the structure of a compound. If the compound which is able to form chelate in a normal condition is deformed by streching, then it will become impossible for it to form chelate with the same metal ions as in the normal condition. Ordinary low molecular weight compounds are not suitable for this study, so high molecular weight compounds were chosen. It being difficult to choose a synthetic chelate resin for above object, wool

was used. The absorption of Cu by the unstreched wool and the 150% streched wool was determined in 0.01M Cu solution and the following results were obtained.

        0.26 m.M/g. unstreched wool

        0.16 m.M/g. streched wool

Not only the deformation of chelating structure but also rearrangement of molecules in wool has a relation to the difference in absorbability of Cu by wool.

III   EFFECTS OF CHELATE FORMATION ON THE CHEMICAL PROPERTIES OF PROTEIN FIBERS.

1) The change in durability of wool toward alkali solution by chelate formation[10]

Having lone pair electrons, active groups and easily decomposable structures, un-stable compounds are reactive toward various reagents. Chelate will form selectively at the unstable position in a molecule, therefore, small quantity of metal ions will be enough to change the chemical properties of those compounds.

If this idea is correct, the chelate reaction will be able to be applied to the improve-ment of fiber. One of the most characteristic chemical properties of wool is the ease with which it is degraded in alkali solutions. A 5% solution of caustic soda at boiling temperature completely dissolves wool in a few minutes. Such degradation is closely associated with the lability toward alkalis of the disulfide groups in the cystine of the wool.

The injurious effect of alkali solution is of wide practical importance in view of the numerous alkali treatments which wool undergoes in being converted from raw stock to the finished fabric, in addition to the alkaline laundering which wool fabrics may receive in use.

A) The solubility of wool in alkali solution

Wool, Lincoln, was degreased with trichlorethylene for 8 hours, then washed with water and dried. The absorption equilibrium of metal ion of wool was deter-mined. In case of Cu ion, equilibrium attained in 30 hours at $26\cdots28°$ C, while the absorption of Hg by wool increased with time.

10g. of the purified wool were immersed in the following solution.

        H_2O 200ml (contained non ionic surface active agent)

        2M KNO_3 50ml

        0.05M $Cu(NO_3)_2$ or $Hg(NO_3)_2$ 50ml

The solubility of wool in alkali solution was determined in 0.1N NaOH at 65°C. The alkali solubility of non treated wool was compared with those of Hg treated wool and Cu treated one, and the results were shown in Fig. 7. A steep slope of solubility curve of wool means that the wool used is not stable for alkali, therefore, Hg treated wool and Cu treated one are more stable than non-treated wool. And the difference of the solubility between non-treated and treated wool became greater as the time of treatment increased.

B) Effect of chelate formation on change of Sulfur content in alkali treatment.

It was found that, although the atomic ratio of nitrogen to sulfur in wool is about 10 to 1, these elements are removed from wool in the ratio of about 1 to 1 by the action of an alkali during the first stages of its attack. If wool protein were simply being dissolved, there should be 10 atoms of nitrogen in the solution for every
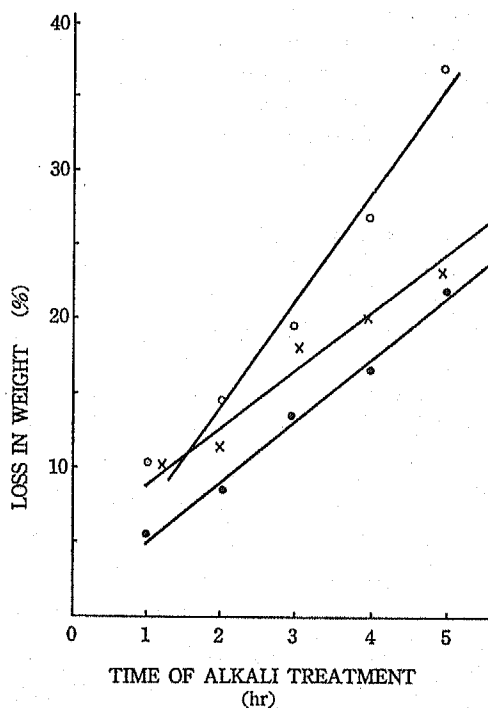
Fig. 7 Solubility of wool

◯ nontreated wool,   × Cu treated wool,   ● Hg treated wool,

atom of sulfur but the analysis showed that the sulfur was present in a much greater proportion. About 40% of the sulfur content of the wool is lost during alkali treatment which dissolves only about 9% of the wool, but thereafter is removed in proportion to the wool dissolved.

The determination of sulfur content was done with non treated wool and heavy metal ion treated wool, before and after the alkali treatment. A significant amount of sulfur lose was seen in the case of nontreated wool, while the less amount of sulfur lose was obtained in the case of metal treated wool.

These results show that the introduction of chelate bond made the wool more stable for alkali degradation.

Table 4 Change of Sulfur content in wool (%)

	Before Alkali treatment (A)	After Alkali treatment (B)	B/A
Non treated wool	4.42	2.96	0.67
Hg treated wool	4.35	4.02	0.93
Cu treated wool	4.20	3.86	0.92

Chelation occurred selectively at the unstable part of fiber, disulfide groups, as it was expected
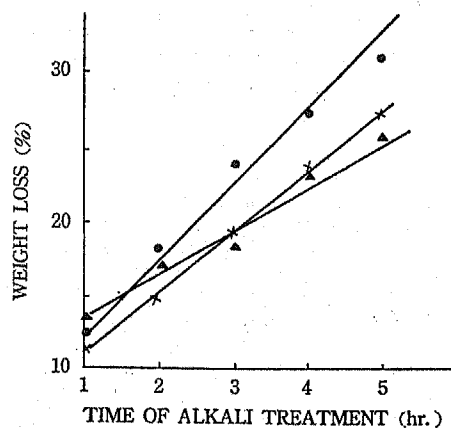

Fig. 8 Solubility of wool
   ● Nontreated wool.   × Cu treated
      wool.
   ▲ Recombined wool.

C)   The influence of the chelate forma-
tion at cystine bridge on the prope-
rties of wool[11]

   In order to study further, the effect of chelate formation at disulfide groups in wool on its properties, cystine bridge was cut and then treated with Cu solution to from metallic salt with sulfur and thus recombined wool was comared with the original wool and the chelated wool in alkali durability and in the change of sulfur in alkali treatment. Thioglycolic acid which is known to cut only cystine bridge in wool without damaging other part of it was used. Degreased Merino wool was used. Wool was immersed in 0.5M of thioglycolic acid in the ratio of 1:100 for 4 hours at 20°C and washed with water. The mixture of 50ml of 0.05M $Cu(No_3)_2$, 20ml of 0.5N HNO_3, 50ml of 3% aqueous solution of nonionic surface active agent and 200ml of water was used for Cu treatment. About 15g. of wool were immersed in the mixture at 30°C for 67 hours and washed and then dried. The results of alkali solubility tests were shown in Fig. 8.

   The recombined wool showed to have nearly the same durability toward alkali as the chelated wool. They have been much improved in alkali resistance in compar-ison with the original wool. The change of sulfur content during alkali treatment was also shown in Table 5.

Table 5   The change of Sulfur content in wool in alkali Treatment (%)

	Before treatment(A)	After treatment (B)	B/A
Nontreated wool	4.26	3.55	0.85
Chelated wool	4.34	4.11	0.94
Recombined wool	5.12	4.88	0.95

This also shows that the recombined wool has the same tendency as the chelated wool. From these results, the combination of Cu ion with sulfur in wool irrespective of Sulfur in cystine bridge or Sulfur in ionized state, has a relation to alkali dura-bility, therefore chelate formation in wool is preferable.

IV   THE CHANGE IN ABSORBABILITY OF Hg, Cu AND Ag BY WOOL IN ALKALI TREATMENT. [12)

   When wool is treated with alkali, the unstable part of wool will be decomposed

and some active groups or reactive parts will be produced remaining the form of fiber. This method was applied for the purpose of preparing a new chelating polymeric substances which can not easily be got by usual synthesis. It is expected that absorbability of metal by wool will change when wool is degraded with alkali solution. The degreased wool, Lincoln, was treated in 0.1N NaOH at 65°C. From the standpoints of chelate resins, the various chemical properties were considered, and the results were shown in Table 6.

Table 6 Effects of the Time of Alkali Treatment on wool

Time of Treatment (min)	S Content (%)	Absorption of Cu (m. M/g)	Absorption of Hg (m. M/g)	Absorption of Ag (m. M/g)	Cation Exchange Capacity (m. eq/g)	Anion Exchange Capacity (m. eq/g)	Moisture (%)
0	4.47	0.38	0.62	1.08	0.24	0.96	9.77
5	3.98	0.40	1.00	1.05	0.43	0.88	8.67
10	3.54	0.38	1.08	1.03	0.54	0.87	8.53
20	3.07	0.42	1.07	0.97	0.53	0.90	8.52
40	2.94	0.38	0.96	0.90	0.53	0.94	9.36
60	2.43	0.46	0.99	0.88	0.59	0.94	10.77

The absorption of Hg by wool was greatly increased when the wool was treated with alkali, while the absorption of Ag by wool decreased, and that of Cu was kept constant, in the earlier period of treatment. Hg absorption curve had a peak in it. Cation exchange capacity of wool became greater as the time of treatment became longer, while anion exchange capacity was kept constant or less than that of nontreated. When wool is treated with alkali, cystine linkage ···S···S··· is cut, and ···S⁻ which is thought to have an affinity to Hg is increased. This is the reason why the absorption of Hg by wool increased in the earlier period of treatment, and it decreaes with time as ···S⁻ is dissolved. If Ag is combined with cystine in the from of ···S······S···, then the absorption of Ag by wool will decrease with the time
$\diagdown$Ag$\diagup$
of treatment. If Cu is combined with polypeptide chains or both polypeptide chains and bridge which are not affected by alkali treatment, then it is easy to understand that the absorption of Cu by wool is kept constant with the time of treatment. The metal ions absorbed by wool could be easily displaced by washing with acid.

V THE ENERGY OF ACTIVATION IN ABSORPTION
OF Hg WITH WOOl[3]

In order to know how easily chelate reaction in fiber takes place, the energy of activation in the reaction of wool with Hg was determined. The reaction in wool is nct essentially the same as that in resin, but the degree of chelate formation in wool may be expected to be proportional to the absorption of heavy metallic ions, therefore, the velocity of the chelate reaction of wool with Hg was determined by

the absorption velocity of Hg by wool. As in the case of chelate resins, diffusion of metal ion in solid phase of wool is considered to be a serious factor for the determination of reaction velocity, so the following equation was applied,

$$\frac{dx}{dt}=\frac{K}{x^m} \qquad (1)$$

x; reaction rate (the quantity of Hg absorbed by wool in a state is divided by the quantity of Hg at equilibrium)
t; time
This equation may finally be transformed to ;

$$\log x=\frac{1}{n+1}\ \log K+\frac{1}{n+1}\ \log t+C \qquad (2)$$

Equation (1) is similar to Fick's law of diffusion.
According to the theory of activated diffusion, the energy of activation will be calculated from equation (3)
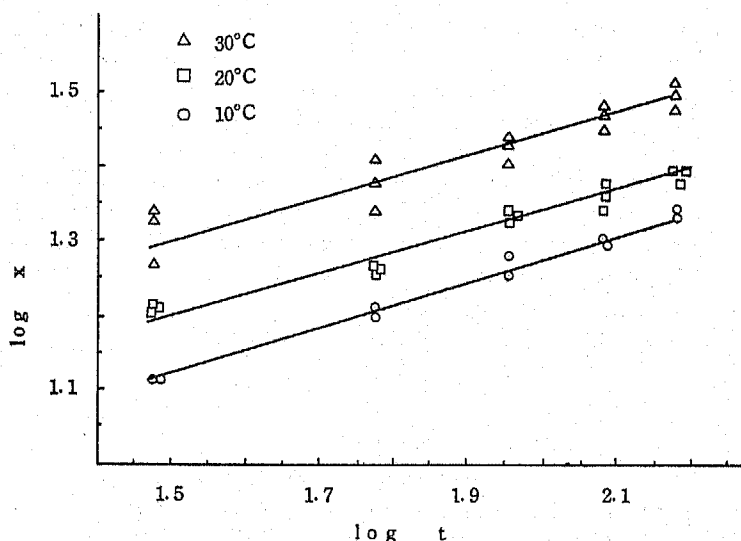

Fig. 9   log x—log t curves
x: Reaction rate % t: Time min.

$$E=\frac{R T_2 T_1}{T_2-T_1}\ \ln K_2/K_1 \qquad (3)$$

Equation (2) requires the plot of $\log x$ against $\log t$ to be a straight line, as long as $\log K$ is constant.
From the points in Fig. 9, the linear relationship between $\log x$ and $\log t$ is evident.
These experiments were done at three different temperatures. From the inclination of these straight lines and from the difference between $\log X_{t20}$ and $\log X_{t30}$ or or between $\log X_{t10}$ and $\log X_{t20}$, the energy of activation is computed and the following results were obtained.

6800 cal/mol 10—20°C
9600 cal/mol 20—30°C

Summary

The results of physico-chemical study on chelate reaction of synthetic chelate resins were apllied to the improvement of protein fibers. Formation of chelate in protein fibers was conveniently determined by pH titration curves as in the case of chelate resins. The effects of chelate formation in fibers on their properties were considered.

The increase of the strength of the metal treated silk was seen in the pH region where the chelate formation is expected. The strength of silk was greatest at the point of the greatest absorption of Cu. It is clear that chelation in silk fiber is favourable for the strength of it. By streching, absorption of Cu by wool decreases. Not only the deformation of chelating structure but also rearrengement of molecules in wool has a relation to this phenomena. Chelate will form selectively at unstable and reactive positions in a molecules, therefore, chelate reaction will be able to be applied to the improvement of fibers. From alkali solubility tests and change of Sulfur content in alkali treatment, Hg treated wool and Cu treated wool become more stable toward alkali than non-treated wool. Chelation occurred at disulfide groups and made it stable. The absorption of metal ions by wool changes when it is degraded with alkali solution. The absorption of Hg by wool increases and that of Ag decreases, while that of Cu is kept constant in the earlier period of alkali treatment. Diffusion of metal ions in solide phase of wool is considered to be a rate determining step of absorption of Hg by wool and the energy of activation in absorption of Hg by wool was determined. The results were shown as below;

$$6800 \text{ cal/mol} \ (10-20_oC)$$
$$9600 \text{ cal/mol} \ (20-30^\circ C)$$

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