THE COLLOIDAL BEHAVIOUR OF SERICIN. IV.*

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Sec. XII. The Colour Reactions of the Sericin Solution.

For the aqueous solution of sericin the biuret, xanthoproteic, and Millon's reactions have been long known.

(1) **The Biuret Reation.** This reaction of sericin was completely investigated by R. Inoue⁽⁴¹⁾. He applied it for the determination of "kaijo" of cocoons, because the tinge of colour produced in the biuret reaction varied with the "kaijo" of cocoons.

(2) **The Yellow Colouration.** An almost colourless or a slightly yellowish solution is obtained when the white cocoon layer is boiled with water. M. Oku⁽⁴²⁾ investigated the colouring matters of the cocoon layer and showed the existence of a flavone-like glucoside in its alcoholic extract. As the colourless glucoside gives yellow colour by hydrolysis, the colour of the aqueous solution of the cocoon layer may be produced partly by the hydrolysis of this substance during the boiling of cocoons in water. And this yellow colouration was also produced by the addition of alkaline substances (NaOH, ammonia, etc.) to a cold aqueous solution of sericin. But we can easily get the yellow colouration by adding a small solid mass of quick lime to a sericin

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* Continued from p. 302 of this volume.

(41) R. Inoue, *Bull. Seric. and Silk Ind. Japan*, **3** (1931), 171.

(42) M. Oku, *J. Agri. Chem. Soc. Japan*, **6** (1930), 1103.

solution. The aqueous or alcoholic solution obtained from the cocoon having good "kaijo" showed generally lighter colouration than the one having bad "kaijo". By adding quick lime to an alcoholic solution of glycocoll a similar beautiful colouration resulted, but its colour turned to violettish gray in about ten hours at room temperature.

(3) **Engel's Colour Reaction.** E. Hiratsuka(43) showed that amino-acids and other nitrogen compounds existed in the silk gland, and also R. Inoue noticed that in the sericin solution there was a free or a loosely combined amino-acid giving a weak ninhydrine reaction.

The characteristic blue colour reaction of the glycocoll solution with phenol and sodium hypochlorite solutions was found by Engel and is called Engel's reaction. As shown by O. Früth(44) the blue colouration is given not only by glycocoll, but by many other substances like ammonium salts and biuret. Glycocoll, however, gives the deepest blue colour which is stable during a few days. The alcoholic solution of sericin also gave Engel's reaction, but the colour was rich in greenish tinge like that given by ammonium salts. The difference in depth of colour was hardly recognized with the difference in "kaijo", though bad "kaijo" was apt to show somewhat deeper colour. From these results it can be seen that the blue colouration given by the alcoholic solution of sericin is similar to those given by solutions of glycocoll, aspartic acid, and ammonium oxalate, which give all violet fluorescence under ultra-violet rays. As it was mentioned in the introduction, R. Inoue observed that glycocoll and aspartic acid were contained in a large amount in the sericin from the inner layer of cocoons.

(4) **Markris' Colour Reaction.** Markris (1931)(45) detected a minute quantity of ammonia by using a 5 per cent. solution of tannin and a 20 per cent. solution of silver nitrate. When this reaction was carried out with an alcoholic solution of cocoon layer, a very slightly whitish turbidity took place first, and then slightly brownish dark yellow colour followed in five minutes. But the development of the colour was not so quick as in the case of free ammonia.

(5) **Colour Reaction with Concentrated Sulphuric Acid.** When 0.5 c.c. of an alcoholic solution of sericin, after adding three drops of a 3 per cent. alcoholic solution of α-naphthol, was thoroughly mixed with 5 c.c. of cold 75 per cent. sulphuric acid, a yellowish green colouration took place. If a

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(43) E. Hiratsuka, loc. cit.

(44) O. Früth, *Biochem. Z.*, **240** (1931), 56-61.

(45) Markris, *Z. anal. Chem.*, **84** (1931), 241-242.

carbohydrate were present in solution, violettish red or red colouration would develop as modified Molisch reaction. Glycocoll and ammonium oxalate gave a colouration similar to that of sericin.

(6) **Arginine Colour Reaction.** When two drops of a 0.1 per cent. alcoholic solution of α-naphthol and a few drops of a 5 per cent. sodium hypochlorite were added to 3 c.c. of a sericin solution, a deep brownish red colouration took place. The sericin solution obtained from cocoons of good "kaijo" gave more intense colour than that from cocoons of bad "kaijo". From these results it is probable that flavone-like compounds, free amino-acids like glycocoll and aspartic acid, and ammonium salts like ammonium carbonate and oxalate, are contained in a very minute quantities in the aqueous solution of sericin obtained by boiling cocoon layers in water for 30 minutes, and that the solubility of sericin in water depends partly on the basic groups like arginine-group which have intimate relation with water molecules.

Sec. XIII. The Gold Number of the Sericin Solution.

The protection of less stable sols by organic sols was first definitely recognized by E. von Mayer and Lottermoser in 1897. Lottermoser reached the conclusion that on addition of a very stable colloid, such as albumin, gelatin, and gum arabic, to a silver sol, no precipitation is caused by electrolytes before the stable colloid is gelatinized. The degree of protection is expressed by the "gold number", that is the weight in mg. of a colloid which just fails to prevent the change from red to violet when 1 c.c. of a 10 per cent. solution of sodium chloride is added to 10 c.c. of a gold sol (0.0053–0.0058 per cent.). Some examples are quoted from Zsigmondy's table in Table 66.

Table 66.

Colloid	Gold number
Gelatin	0.005–0.01
Casein (in ammonia)	0.01
Egg-albumin	0.15–0.25
Gum arabic	0.15–0.25, 0.5–4.0

K. Yamamoto (1929)(46) investigated the protective action of sericin precipitated by alcohol and observed that the gold number of sericin was approximately equal to that of gelatin. He also noticed that the protective action of sericin obtained from inner layer of cocoons was less than that of sericin obtained from outer layer.

From many investigations we can reach the conclusion that the stability of colloid particles depends mainly on the chemical factor conditioned by the

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(46) K. Yamamoto, *Reports Gunze Phys. Chem. Lab. Japan*, **4** (1929), 23-27.

constitution of colloid particles and on the electrostatical factor conditioned by adsorption and hydration.

S. Liepatoff (1930) considered that the stability of colloidal particles depends on their charges and the degree of saturation of their surfaces. To the protective action of colloids, C. Marie and N. Marinesco (1931) gave an explanation that the colloid particle is covered with the polarized protecting body and becomes similar to the latter in its behaviour.⁽⁴⁷⁾

The data obtained are given in Table 67. From my results it can be seen that the gold number of sericin is nearly equal to that of egg-globulin (0.02–0.05), gelatin (0.007–0.06), and amorphous albumin (0.03–0.06), and that it depends on the concentration of sericin and also on the "kaijo" of cocoons at the constant *p*H value and temperature.

Table 67. Effect of concentration on the gold number of sericin (at 30°C.).

Good "kaijo"		Mid. "kaijo"		Bad "kaijo"	
Conc. (%)	Gold numb.	Conc. (%)	Gold numb.	Conc. (%)	Gold numb.
0.075	0.03	0.095	0.0285	0.07	0.0385
0.10	0.03	0.10	0.03	0.125	0.0312
0.12	0.027	0.15	0.03	0.15	0.030
0.20	0.020	0.21	0.021	0.23	0.023
0.21	0.021	—	—	—	—

(1) **Effect of *p*H.** The gold number of sericin is maximum at the isoelectric point as shown by Fig. 27.

Table 68.

"Kaijo"	*p*H giving maximum gold number
Good	4.20
Middle	4.25
Bad	4.30

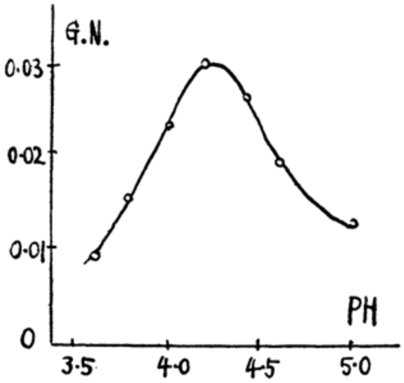

Fig. 27.

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(47) C. Marie and N. Marinesco, *Compt. rend.*, **192** (1931), 92–94.

(2) **Effect of Temperature.** As already stated, the aggregation of sericin micellæ varies with temperature, so its protective action must be raised with it. Above 60°C. sericin particles exist in sol-form, and the protective power increases remarkably as shown in Table 69 and in Fig. 28.

Table 69. Effect of temperature on gold number.

Temp. (C.)	Good	Mid.	Bad
	0.1%	0.11%	0.085%
0	0.030	0.033	0.0375
20	0.03	0.0325	0.0375
40	0.025	0.028	0.030
60	0.012	0.019	0.0243
80	0.01	0.016	0.0215

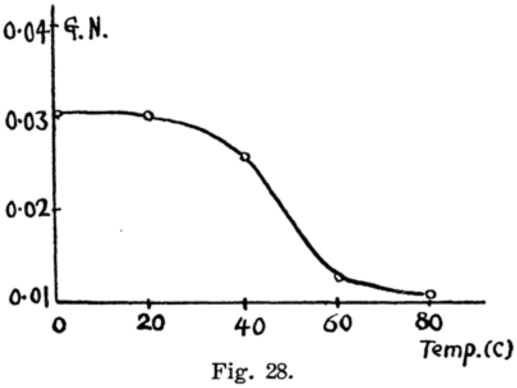

Fig. 28.

(3) **Effect of Aging.** The gold number of sericin increased on aging the solution, probably because the sericin particles formed larger aggregates of less protective power.

Sec. XIV. The Protective Actions of the Sericin Solution.

(1) **The Emulsifying Properties of the Sericin Systems.** When a liquid immiscible with water is shaken with an aqueous solution of sericin, a very stable emulsion results. On standing for a few days the emulsion creams in a separate layer. In the following experiments each emulsion was made with 5 c.c. of a sericin solution and 10 c.c. of an immiscible liquid in a test-tube with a diameter 2 cm.. Such test-tubes were stoppered and shaken by a shaking machine for half an hour, and then, after giving two hundred shakes by hand, they were set aside. The volume of stable emulsions was read from time to time.

(a) **Effect of Concentration of Sericin.** First I investigated how the emulsifying properties vary with the sericin content. Experiments were carried out by mixing the sericin solutions (0.08–0.8%) with various liquids in the manner described above. The results are given in Table 70, where the emulsifying power is expressed by the formula $\frac{V-v}{V}\times 100$, V being the initial volume of emulsion (15 c.c.), and v the volume of aqueous layer separated from the emulsion after a definite time.

Table 70. Benzene and good "kaijo."

Conc. of sericin (%)	Emulsifying power			
	10 min.	30 min.	60 min.	120 min.
0.802	98.87	97.73	97.16	96.59
0.65	100.0	99.14	97.99	97.65
0.484	100.0	99.95	99.92	99.89
0.32	100.0	100.0	100.0	100.0
0.26	100.0	100.0	100.0	100·0
0.084	100.0	98.13	96.25	96.25

At the low concentrations (from 0.25 to 0.35 per cent.) the emulsifying power of the sericin solution was strongest and it diminished gradually with time, following a monomolecular reaction. After two hours there was no remarkable change in it.

These results are expressed in Fig. 29. Sericin had a maximum emulsifying power for liquids examined and the position of the maximum seems to depend on their dielectric constants (Table 71).

Table 71. The emulsifying powers of various sericin systems.

Conc. (%)	CCl_4	$CHCl_3$	Poupa oil	Olive oil
0.802	92.14	95.51	74.11	85.12
0.65	90.74	94.12	84.78	94.12
0.48	89.79	93.34	87.22	100.0
0.32	86.52	92.14	92.53	100.0
0.26	85.39	90.76	93.01	96.25
0.08	73.52	87.94	97.50	93.34

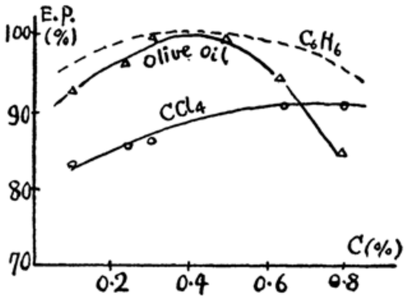

Fig. 29.

At the constant concentration, sericin obtained from cocoons having good "kaijo" had a greater emulsifying power than that obtained from cocoon having bad "kaijo". This may be due to the difference in adsorption of sericin particles on the interface of liquid particles. Pupa oil manifested a reverse relation.

(b) **The Adsorption of Sericin on the Interface.** In order to determine the extent of adsorption of sericin on the interface of the emulsion, analyses of the aqueous phase before emulsification were compared with those of the first few c.c. of the aqueous phase that separated from the emulsion. The micro-Kjeldahl-method for determination of nitrogen in proteins was used to determine the sericin content. The results are shown in Table 72.

Table 72.

Nitrogen content in mg. per c.c. (For good "kaijo").

Original solution	Benzene			Chloroform		
	Separated solution	Difference	Emuls. power	Separated solution	Difference	Emuls. power
1.290	1.213	0.077	96	1.135	0.155	95
1.071	0.976	0.095	97	0.937	0.134	94
0.785	0.626	0.159	99.9	0.665	0.120	93
0.528	—	—	—	0.455	0.073	92
0.428	—	—	—	0.357	0.071	90
0.143	0.075	0.068	96	0.107	0.036	87

It can be seen that sericin was taken from the aqueous phase and concentrated on the interface of the emulsion, and that oil particles were stabilized by the accumulation of sericin particles on their interfaces.

(c) **Effect of *p*H.** The emulsifying power of sericin depends further on the hydrogen-ion concentration of the medium, for the state of aggregation of sericin is remarkably influenced by the *p*H value.

Fig. 30, showing the influence of *p*H on emulsifying property of the sericin solution, shows that, if *p*H of the sericin solution is near the isoelectric point, the least stable emulsion is formed. The *p*H values corresponding to the minimum emulsifying power are given in Table 73.

Table 73.

"Kaijo"	C_6H_6-H_2O emuls.	Pupa oil-H_2O emuls.
Good	*p*H 4.0	*p*H 4.3
Middle	4.5	4 6
Bad	4.8	4.8

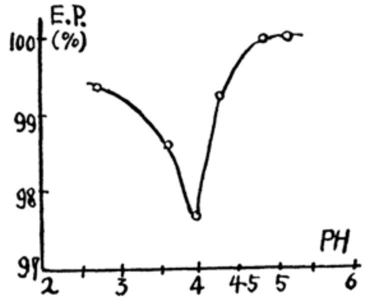

Fig. 30.

In the cases of CCl_4, $CHCl_3$, C_6H_6, and aniline the same results were obtained, but benzene did not produce an emulsion at the more acid side than pH 3.5. Change of pH of the sericin solution affects its emulsifying properties in a manner closely related to the effect of change of pH on the surface tension and viscosity of the sericin solution.

(2) **The Protective Action of Sericin against the Oxidation of Pyrogallol in the Presence of the Purpureo-salt.** With Prof. Y. Shibata I investigated the oxidase-like action of complex cobalt salts, and found that the aqueous solution of pyrogallol was catalyticcally oxidized in the presence of the purpureo-salt, pentammine cobalt chloride, even in a very small concentration and it became gradually brownish yellow.(48)

Taking the colour as expressing the degree of oxidation, the colour developed in 15 minutes in a number of solutions, prepared by adding 0.1 c.c. of a 10 mg. mol. solution of purpureo-salt to 10 c.c. of a 1 mg. mol. solution of pyrogallol with the addition of sericin in various concentrations, were compared by a colorimeter with that of a standard solution prepared similarly but without sericin. Sericin protected pyrogallol from oxidation catalysed by the purpureo-salt, the height (h in Fig. 31) of the solution at the colorimeter increasing with increasing concentration of sericin. The sericin solution obtained from cocoons of good "kaijo" had stronger protective power than that obtained from cocoons of bad "kaijo", as in the case of the gold number of sericin.

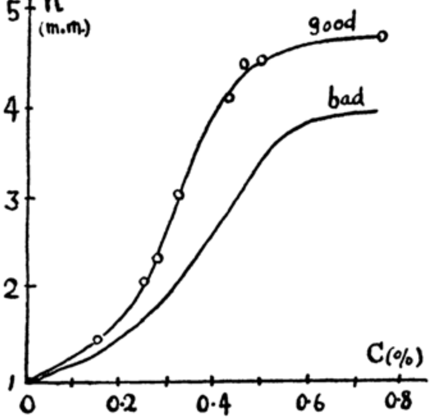

Fig. 31.

(3) **The Protective Action of Sericin against the Decomposition of Hydrogen Peroxide in the Presence of Platinum Sol.** It was early recognized by Groth that gelatin possesses a power to protect hydrogen peroxide from decomposition catalysed by platinum sol. Sericin showed a protective action against the decomposition of hydrogen peroxide (Fig. 32).

As it is well known, the decomposition of hydrogen peroxide in the presence of platinum sol is a mono-molecular reaction expressed by

$$K=\frac{2.303}{t}\cdot\log\frac{a}{a-x},$$

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(48) Y. Shibata and H. Kaneko, *J. Chem. Soc. Japan*, **44** (1922), 833–884.

Table 74. Reaction velocity constant, $K \times 10^3$.

t (min.)	Volume of sericin added (c.c.)			
	0	0.1	0.5	0.7
0	—	—	—	—
5	30.24	(24.40)	(15.85)	(12.42)
10	30.33	(26.43)	(17.48)	(17.61)
15	31.67	27.16	25.00	19.09
20	32.29	28.51	25.07	18.30
25	33.83	29.74	24.84	19.13
30	33.19	30.50	24.00	20.70
60	32.96	30.25	24.93	20.74
mean	32.07	29.23	24.77	19.59

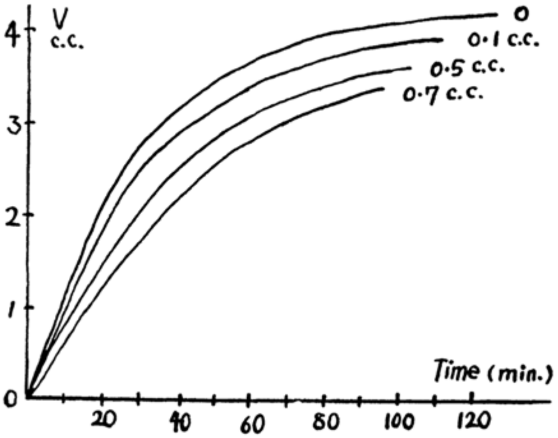

Fig. 32.

where K is the reaction velocity constant, x and a are the quantities of oxygen evolved at time t and at infinite time t_∞ respectively.

The reaction velocity constant was found to be reduced by the addition of sericin as shown in Table 74. The velocity constant K was inversely proportional to the concentration of sericin in low concentration.

Table 75. Effect of aging.
Pt-sol, 0.5 c.c.; sericin, 0.5 c.c.

Time of aging min.)	Good "kaijo"			Bad "kaijo."	
	0.44%	0.43%	0.32%	0.42%	0.35%
0	1.40	1.53	2.22	1.81	2.28
40	1.59	1.63	2.41	2.11	2.52
90	1.70	1.80	2.50	2.13	2.65
120	1.62	1.71	2.38	1.98	2.45
180	1.25	1.38	1.97	1.52	2.01
240	0.81	0.85	1.55	1.00	1.58
300	—	0.67	—	—	—

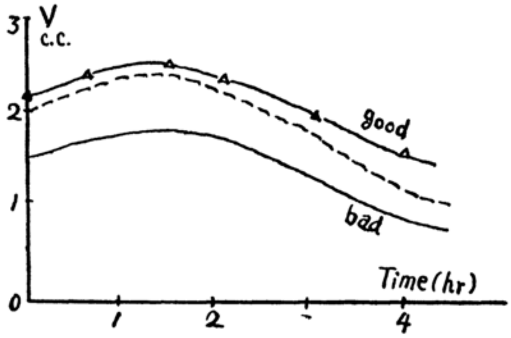

Fig. 33.

On aging the sericin sol at 13°C., its protective action against the decomposition reached a minimum value (Table 75 and Fig. 33). Near this point, many physico-chemical properties of the sericin solution showed also remarkable changes.

(To be continued.)