THE COLLOIDAL BEHAVIOUR OF SERICIN. II.*

By **Hideo KANEKO.**

Received January 26th, 1934. Published June 28th, 1934.

Sec. III. The Surface Tension of Sericin Sol.

H. Iscovesco(11) (1910) and Zlobicki observed that solutions of many hydrophilic colloids (egg white, ovoglobulin, starch, gum arabic) have a lower surface tension than pure water. The surface tension of normal blood serum is lower than that of pure water, and it is reasonable to assume that the decrease in surface tension of water is mostly caused by the proteins.

The experimental materials and the method are the same as described in the previous section.

(1) **Variation of Surface Tension with Temperature.** Fano and Mayer(12) (1907) observed that the surface tension of the blood serum of any animal increases with fall in temperature, as is seen with water and with any other solution. I measured the surface tension of sericin sol with du Noüy's tensiometer at various temperatures. The results are shown in Table 15 and Fig. 9.

Table 15.

Surface tension (dyne/cm.) of sericin sol (good "kaijo").

Temp. (C.)	0.48%	0.304%	0.228%	0.075%
0	59.00	61.00	61.50	63.70
10	56.89	58.91	58.80	62.00
20	54.22	56.50	57.10	60.32
30	52.33	53.81	55.75	58.01
40	49.49	51.00	53 61	55.50
50	46.78	48.50	51.40	54.33
60	44.45	45.95	49.57	51.65
70	43.02	44.25	48.96	50.36

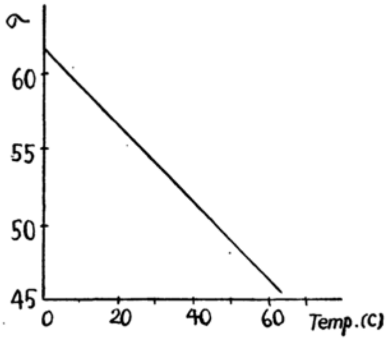

Fig. 9.

---

* Continued from p. 221 of this volume.

(11) H. Iscovesco, *Compt. rend. soc. biol.*, **69** (1910), 491, 537, 566, 622.

(12) Fano and Mayer, *Arch. fisiol.*, **4** (1907), 164.

The surface tension of sericin sol diminishes linearly with rise in temperature until about 60°C.. The value of $\frac{\mathrm{d}\sigma}{\mathrm{d}T}$ for sericin sol obtained from the cocoon of good "kaijo" is greater than that for sericin sol obtained from the cocoon of bad "kaijo." Tangents of the angles of inclination of surface tension-temperature curves are shown in Table 16.

Table 16.

	"Kaijo"		
	Good	Middle	Bad
Tan α	1.0575	0.7813	0.7346
Conc. (%)	0.304	0.346	0.309

The relative lowering of surface tension is expressed by the term of $\frac{\sigma_0-\sigma}{\sigma}$, where σ_0 and σ are respectively the surface tension of water and that of sericin sol. Some of results obtained are given in Table 17 and Fig. 10.

Table 17.

The values of $\Delta\sigma$.

Temp. (C.)	Good (0.304%)	Mid. (0.347%)	Bad (0.309%)
0	0.2344	0.3005	0.3095
10	0.2553	0.3221	0.3271
20	0.2849	0.3417	0.3419
40	0.3715	0.3941	0.4032
60	0.4646	0.4513	0.4608
70	0.4915	0.4735	0.4687

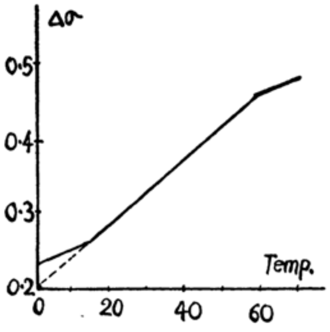

Fig. 10.

It can be seen that the curve is in three branches. This is due to the fact that below 18°C. sericin exists as gel form, above 60°C. as sol form, and between these temperatures as a mixture of two forms. This result is coincident with that obtained in the change of viscosity with temperature.

If the free energy expressed by Gibbs-Helmholz' equation, F, is substituted by the surface tension, free energy per unit area, the following equation results:

$$U_s = \sigma - T\frac{\mathrm{d}\sigma}{\mathrm{d}T}\,.$$

$T\frac{\mathrm{d}\sigma}{\mathrm{d}T}$ is the heat energy which is necessary to form unit area at constant temperature.

Differentiating the above equation:

$$\frac{\mathrm{d}U_s}{\mathrm{d}T} = \frac{\mathrm{d}\sigma}{\mathrm{d}T} - \frac{\mathrm{d}\sigma}{\mathrm{d}T} - T\frac{\mathrm{d}^2\sigma}{\mathrm{d}T^2} = -T\frac{\mathrm{d}^2\sigma}{\mathrm{d}T^2} = 0$$

Therefore, $\frac{\mathrm{d}\sigma}{\mathrm{d}T}$ will be constant for the true solution. But, for the colloidal solution it is not constant as shown in Table 18 and Fig. 11.

Table 18.

Values of $\Delta\sigma/\Delta T$ (good "kaijo.")

Temp. (C.)	0.48%	0.304%	0.228%	0.075%
0–10	0.211	0.209	0.270	0.170
10–20	0.267	0.241	0.170	0.168
20–30	0.189	0.269	0.135	0.231
30–40	0.284	0.281	0.214	0.251
40–50	0.271	0.250	0.221	0.117
50–60	0.233	0.255	0.183	0.268
60–70	0.143	0.170	0.061	0.129

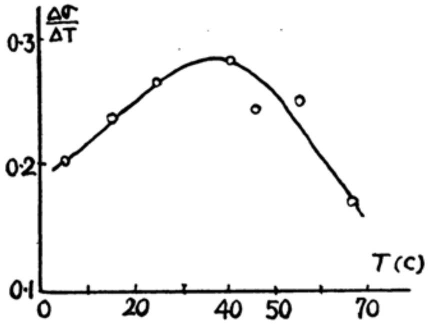

Fig. 11.

The $\frac{\Delta\sigma}{\Delta T}$–temperature curves have a maximum near 45°C.. This will be due to the variation of state of sericin particles with temperature.

(2) **Variation of the Surface Tension with Time.** As the solutions age, the actively moving submicrons aggregate to larger immobile submicrons, the surface-active solutions becoming meanwhile more surface-active. Ghosh and Noth⁽¹³⁾ found that the surface tension of colloidal solutions changes irregularly with time. At three different temperatures I measured the variation of surface tension with time (Table 19.)

Table 19.

Surface tension in dyne per cm.

	15°C.			40°C.		
Time (min.)	Good (0.409%)	Mid. (0.302%)	Bad (0.433%)	Good (0.236%)	Mid. (0.250%)	Bad (0.270%)
10	56.17	55.36	52.78	50.18	49.70	48.00
30	55.79	55.04	52.54	49.75	49.73	48.83
60	55.48	54.60	52.48	55.00	51.60	49.96
120	55.45	54.06	52.29	55.00	54.52	52.50
180	55.35	54.01	52.12	54.65	54.18	52.03

---

(13) Ghosh and Noth, *J. Phys. Chem.*, **36** (1932), 1916–1927.

60°C.

Time (min.)	Good (0.323%)	Mid. (0.310%)	Bad (0.300%)
10	44.75	44.05	45.70
30	44.34	43.73	45.46
60	43.51	43.35	45.41
120	43.45	43.05	44.95
180	43.38	42.92	44.70

At 40°C., where the greatest variation of $\frac{\Delta\sigma}{\Delta T}$ occurs, the variation of surface tension with time is also the greatest as shown in Table 19.

(3) **Variation of Surface Tension with Concentration.** Bottazi⁽¹⁴⁾ (1911) drew the general conclusion that proteins in a state of perfect solution lower the surface tension of water, and that the reduction is, to a certain extent, proportional to their concentrations. From my experimental results it can be seen that the surface tension of sericin solution diminishes with increasing concentration and that it has the following relation with concentration:

$$0.1\sigma + \log 100.c = \text{constant} = k,$$

where σ is the surface tension of sericin sol and and c is its concentration in gram per 100 c.c.. The results are summarized in Table 20 and plotted in Fig. 12. The value of constant K is about 6.7 and it varies slightly with "kaijo" of cocoons.

(4) **Effect of Salts, Acids, and Alkalis on the Surface Tension.** (a) **Salts.** By a series of investigations on gelatin solutions, W. Frei⁽¹⁵⁾ (1908) found that the anions, SO_4'', Cl', and NO_3', raise the surface tension of neutral gelatin in the order given, while the cations, $Na^{\cdot}$, $K^{\cdot}$, and $Mg^{\cdot\cdot}$, raise it about in the same order as they increase the surface tension of water, and that the anions raise it in the order NO_3', SO_4'', and Cl', if the solution is acid. The surface tension of neutral solutions is raised

Table 20.

Surface tension (dyne/cm.) of sericin sol (good "kaijo.")

Conc. % / Temp.	0.383	0.256	0.191	0.127	0.096	0.048	0.024	0.012
20	53.39	54.53	55.56	57.21	58.38	60.62	62.95	65.95
30	51.20	52.47	53.23	55.06	56.26	60.01	61.98	65.06
40	48.86	50.01	51.20	52.61	54.15	58.16	60.23	64.11
50	47.05	48.62	49.25	51.18	52.83	57.03	58.31	62.08
60	45.58	46.00	46.93	49.01	50.78	55.42	57.00	60.55

---

(14) Bottazzi, "Viscosity and surface tension of suspensions and solutions of muscle proteins under the influence of acid and alkali."

(15) W. Frei, "Physical chemistry and veterinary science."

"Kaijo"	Const. K (20°C.)
Good	6.792
Middle	6.783
Bad	6.678

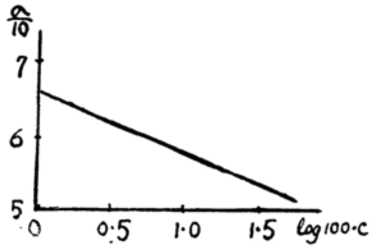

Fig. 12.

by OH-ions and lowered by H-ions. The surface tension of protein solutions may be raised by the addition of electrolytes which can increase the degree of dispersion of the protein.

The results obtained are collected in Table 21.

Table 21. Influence of salt solutions (0.1 N).

V	NaCl	$NaNO_3$	NH_4Cl	$CaCl_2$	$FeCl_3$	$Th(NO_3)_4$
c.c.	0.15%	0.083%	0.083%	0.106%	0.07%	0.07%
0.0	55.26	57.83	57.23	58.91	57.00	57.00
0.05	55.89	—	—	58.24	57.52	61.32
0.1	56.21	58.12	57.00	58.12	57.56	62.63
0.2	55.83	58.44	56.91	57.61	60.62	63.20
0.3	55.64	58.73	56.85	57.21	61.83	62.68
0.5	55.58	58.38	56.34	57.42	60.60	62.30
0.7	55.70	58.06	55.65	57.17	60.63	62.35
1.0	55.76	58.00	55.21	56.66	60.36	62.32

(1) When an electrolyte like NaCl, $NaNO_3$, KI, KNO_3, NH_4SCN, $MgCl_2$, $FeCl_3$ or $Th(NO_3)_4$, is added to a sericin solution the surface tension rises to a maximum and then diminishes. (2) In the cases of NH_4Cl, $CaCl_2$, $CaSO_4$, $Ca(NO_3)_2$, or $Ca_3(PO_4)_2$ the surface tension goes on decreasing if the amount of the electrolyte is increased. (3) The surface tension increases with increasing concentration of an electrolyte like Na_2CO_3, Na_2SiO_3, etc.. The electrolytes belonging to the first class give also a maximum or a minium point to the electrokinetic potential of sericin sol

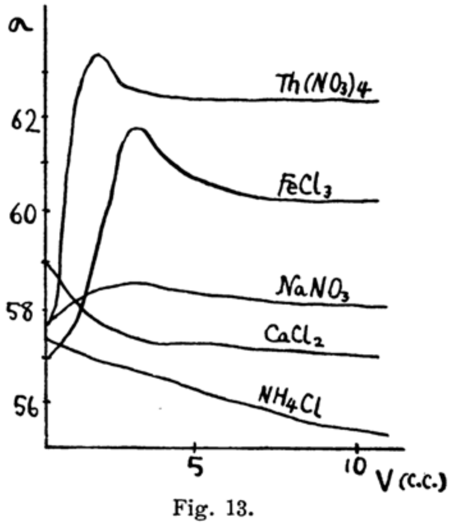

Fig. 13.

as shown later. The surface tension, therefore, have an intimate relation with the electrokinetic phenomena.

If σ_m be the maximum lowering value of the surface tension of sericin sol containing a salt and σ_0 the surface tension of it with no salt, the difference of these two values, $\sigma_0-\sigma_m$, is shown in the Table 22.

The sericin solution obtained from cocoons of good "kaijo" is more affected by the monovalent cations and less by the polyvalent cations than that obtained from cocoon of bad "kaijo." By a series of investigations with sericin solutions, I found that the cations, $Na^{\cdot}$, $Mg^{\cdot\cdot}$, and $Fe^{\cdot\cdot\cdot}$, raise the surface tension of sericin in the order given at low concentrations.

Table 22.

"Kaijo"	$NaNO_3$ $\frac{\sigma_0-\sigma_m}{C}$	$FeCl_3$ $\frac{\sigma_0-\sigma_m}{C}$	$Th(NO_3)_4$ $\frac{\sigma_0-\sigma_m}{C}$
Good	18.07	69.0	88.6
Middle	14.00	76.9	108.2
Bad	3.60	82.4	103.8

(b) **Acids and alkalis.** W. Frei noticed that the surface tension of neutral gelatin solutions is raised by small amounts of hydroxyl-ions and depressed by hydrogen-ions. The values of the surface tension of sericin sol at various *p*H values effected by addition of various amounts of 0.1 *N* hydrochloric acid given in Table 23.

Table 23. (at 20°C.)

Good (0.092%)		Mid. (0.072%)		Bad (0.081%)	
*p*H	σ	*p*H	σ	*p*H	σ
6.07	56.70	6.36	59.20	6.53	57.41
5.21	54.20	5.40	57.21	5.46	55.12
4.69	52.96	4.63	55.96	4.90	54.33
4.00	53.08	4.11	55.70	4.30	54.08
3.75	53.30	3.75	56.10	3.80	54.62
3.54	54.45	3.25	57.86	3.46	55.38
3.32	55.05	3.17	59.11	3.28	55.75
3.25	56.51	3.05	60.38	—	—

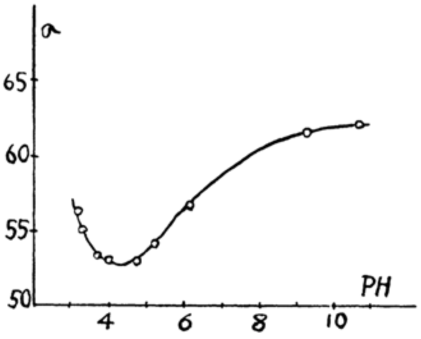

Fig. 14.

Fig. 14 shows that the addition of the acid first lowers the surface tension, and then increases it, and that the addition of the alkali gradually raises the surface tension.

The maximum lowering of the surface tension of the sericin solution corresponds to the isoelectric point of the dissolved sericin, because molecules are more efficacious than ions in lowering the surface tension of water. So we can determine the isoelectric point of a soluble protein by measuring the surface tension of its solution.

Table 24.

"Kaijo"	Isoelec. point (maximum lowering point)	Conc. (%)
Good	pH 4.25	0.092
Middle	4.30	0.072
Bad	4.40	0.081

The isoelectric point of the sericin solution obtained from cocoons of good "kaijo" exists at a more acid side than that of the sericin solution obtained from cocoons of bad "kaijo" (Table 24). It depends, therefore, upon the "kaijo" of cocoons and also upon the concentration of the sericin solution.

The effect of sulphuric acid on the surface tension of the sericin solution is similar to that of hydrochloric acid, but the former shows the slower change of the surface tension with pH values than the latter. For the organic acids, succinic, tartaric, and citric acids, the degree of the lowering of the surface tension, $\Delta\sigma$, is given in Table 25.

Table 25.

"Kaijo"	Tartaric acid $\Delta\sigma = \frac{\sigma_0 - \sigma_m}{C}$	Succinic acid $\Delta\sigma$	Citric acid $\Delta\sigma$
Good	72.2	88.2	87.8
Middle	89.1	126.5	112.4
Bad	104.3	143.3	133.2

The effects of these acids are remarkable in the case of the sericin solution obtained from cocoons of bad "kaijo." The effects of fatty acids on the surface tension of sericin sol obtained from outer layer of cocoons are shown in Table 26 and Fig. 15.

Table 26. (Conc. = 0.07%).

Vol. of acid added in c.c.	Formic acid (dyne/cm.)	Acetic acid (dyne/cm.)	Butyric acid (dyne/cm.)
0.	56.17	56.17	56.17
0.1	56.05	55.42	53.69
0.2	55.82	54.84	45.59
0.3	55.50	54.26	48.42
0.5	55.15	52.65	46.00
0.7	54.97	51.92	43.37
1.0	54.26	50.76	42.05

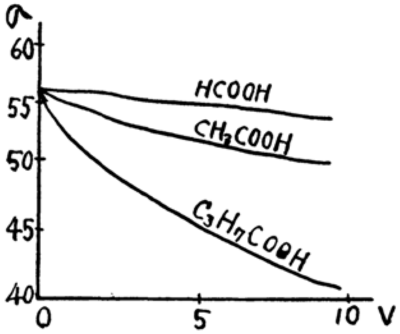

Fig. 15.

I. Traube⁽¹⁶⁾ showed that capillary-active organic substances lower the surface tension to an increasing degree as the length of the carbon chain increases in the homologous series. This regular change in the surface tension can be seen in Table 26.

On the addition of alkali like 0.1 *N* NH_4OH, NaOH, etc., the surface tension of the sericin solution increases to a maximum at a certain alkali concentration as shown in Table 27.

Table 27.

Variation of surface tension with alkali concentration.

Vol. of alkali added in c.c.	0.1 *N* NH_4OH		0.1 *N* NaOH	
	$\sigma\left(\frac{\text{dyne}}{\text{cm.}}\right)$	*p*H	σ	*p*H
0.0	56.70	6.07	56.70	6.07
0.05	58.63	7.39	61.55	9.29
0.1	58.81	8.72	62.01	10.59
0.2	59.70	10.21	61.86	—
0.3	59.05	—	61.63	—

At the isoelectric point the sericin solution becomes most turbid and upon the addition of sodium hydroxide the dispersed phase passes gradually into a state of solution and at the same time the solution turns to yellow.

Sec. IV. The Probable Dimension of the Sericin Particle on the Surface of the Sericin Solution.

P. L. du Noüy⁽¹⁷⁾ (1925) presumed that the time drop of the surface tension of a solution was due to the variation in arrangement of dispersed particles on the surface and he calculated the probable dimension of molecules from the relation between the maximum position of time drop of the surface tension and the space property of protein molecules. He considers that in high concentrations the protein molecules aggregate irregularly on the surface of the solution and exert only a part of their polar groups to lower the surface tension and at low concentrations they have a free space (gap) between them and move freely through the surface. When the protein molecules closely contact with together at an adequate concentration, the time drop of the surface tension will be strong. By this con-

---

(16) I. Traube, *Ber.*, **17** (1884), 2294; *J. prakt. Chem.*, **34** (1886), 292.

(17) P. L. du Noüy, *J. Biol. Chem.*, **64** (1925), 595-613.

sideration he found that egg albumin formed a monomolecular film on the surface of the solution. F. Hervik (1931)⁽¹⁸⁾ also showed that serum albumin existed in a similar condition.

Two c.c. of sericin solution of various concentrations were poured into watch-glasses of the same radius of curvature and the surface tensions were measured with du Noüy's tensiometer immediately and after two hours at room temperature. In Table 28 $\Delta\alpha$ expresses the difference of the rotation angle of the tensiometer.

Table 28. Good "kaijo."

Conc. (%)	Time drop (2 hours later) $\Delta\sigma$	Conc. (%)	Time drop (2 hours later) $\Delta\alpha$
0.52	−0.4	0.392	0.3
0.052	0.1	0.0392	0.0
0.0052	4.3	0.00392	5.8
0.00104	6.0	0.000784	6.1
0.00052	4.3	0.000392	4.0
0.000052	3.0	0.0000392	0.1
0.0000052	1.1	0.00000392	2.7

The concentration of sericin corresponding to the maximum time drop of surface tension are shown in Table 29.

Table 29.

Original conc. (%)	Conc. at max. drop. (%)	Quantity of sericin in 2 c.c. (g.)
0.52	0.00104	2.08×10^{-5}
0.392	0.001306	2.612 ,,
0.34	0.001133	2.266 ,,
0.22	0.00110	2.20 ,,
0.214	0.00107	2.14 ,,

Table 30.

Original conc. (%)	Width of surface layer (A. U.)
0.52	53.8
0.392	58.5
0.34	59.5
0.22	56.4
0.214	57.9

If the sericin particles aggregate throughout on the surface (between air and the solution, and between solution and the glass surface), the quantity of sericin must be equal to $\frac{G}{S}$ in cm., where G is the weight of sericin contained in the surface and S is the total area of the surface of the solution and equals to 26.53 sq. cm. in this case. The probable dimension of sericin particles therefore, is $\frac{G}{S}\cdot\frac{1}{d}$, where d is the specific gravity of sericin.

---

(18) F. Hervik, *Kolloid-Z.*, **56** (1931), 1–7.

The width of sericin layer calculated from the above assumption are shown in Table 30.

The probable dimension of the sericin layer on the surface of the sericin solution corresponds in average to 57.2 A. U. for sericin obtained from cocoons of good "kaijo."

Sec. V. The Dispersity of Sericin Particles in the Aqueous Solution.

The sericin solution is a poly-dispersed system of sericin particles, so the rates of diffusion may vary within wide limits. The sericin solutions obtained from cocoons of various "kaijo" are different in viscosity, coagulation by electrolytes, or Tyndall's phenomena. This may be partly due to the difference in size of sericin particles.

In 1929 J. H. Northrop and M. L. Anson⁽¹⁹⁾ gave a method for the determination of diffusion constants in the case of substances which cannot be obtained in a pure solution. The diffusion constant, D, is defined as the quantity of material that diffuses across a plane of unit dimensions in unit time under unit concentration gradient. The speed of diffusion is governed by the law of Fick, which is expressed in the equation:

$$dQ = DS\frac{dc}{dx}\,dt \qquad \text{or} \qquad D = \frac{dQ}{S\,dt\,\frac{dc}{dx}},$$

where dQ is the quantity which passes across the plane of area S in time dt under a concentration gradient of $\frac{dc}{dx}$. The solute diffuses from concentration C to concentration zero (pure solution) and if the volume of these solutions is relatively large and the experiment is carried on for a short time, the concentration may remain practically constant. Let the effective area of the pores be S, the effective length be l and the quantity diffusing in time t be Q, then above equation becomes simply

$$D = \frac{Q}{St\,\frac{c}{l}} = \frac{l}{S}\cdot\frac{Q}{tc} = K\,\frac{Q\,(\text{sq. cm.})}{t\,(\text{day})},$$

where Q is the number of c.c. of the concentrated solution that contains the amount of the substance diffused, and K is l/S. In order to obtain the value of constant K it is necessary to standardize the apparatus with hydrochloric acid solution the diffusion coefficient of which is known.

---

(19) J. H. Northrop and M. L. Anson, *J. General Physiol.*, **12** (1929), 543.

$$K = \frac{D\,t}{Q}$$

After this constant has been determined for a definite membrane it may be used to determine the diffusion coefficient of sericin particles.

The apparatus was similar to that used by Northrop and Anson except that the diffusion membrane used was hard filter paper (Carl Schleicher and Schüll, No. 597). The cell contained 17 c.c. and the outside liquid, pure water, was 50 c.c.. The cell was filled with the sericin solution and rinsed with water. The diffusion was allowed to continue until the desired amount has passed through the membrane at 18°C..

The amount of sericin diffused into the outside liquid in a definite time was determined by Kjeldahl's method.

	Exp. 1	Q (equivalent c.c.)		Exp. 2	Q	
$t = 15$ hours	No. 1	2.85	mean 2.87	No. 1	2.90	mean 2.89
	No. 2	2.97		No. 2	2.83	
	No. 3	2.79		No. 3	29.4	

Total average = 2.88 c.c.

Therefore, the diffusion coefficient is

$$D = K\frac{Q}{t} = 0.02873 \times \frac{2.88}{\frac{15}{24}} = 0.1240\,\frac{\text{sq. cm.}}{\text{day}}.$$

According to Einstein the diffusion coefficient is related to the radius of the molecule, r, by the following equation:

$$D = \frac{RT}{N}\cdot\frac{1}{6\pi r\eta},$$

where R is the gas constant in erg/deg. mole, N Avogadro's constant, η the viscosity of water at 18°C., and T absolute temperature.

$$r = \frac{RT}{N}\cdot\frac{1}{6\pi D\eta} = 1.430 \text{ m}\mu. \text{ for } 0.198\% \text{ solution.}$$

The molecular weight of the sericin particle may be calculated from the radius r by the following equation provided the specific gravity, S, is known:

$$M = \frac{4}{3}\pi r^3 SN \text{ (if the particle be spherical),}$$

$$M = 10618.91 \text{ for } 0.198\% \text{ solution } (S = 1.43).$$

From this results I can presume that readily diffusible sericin particles are molecular colloids.

By the optical method H. R. Bruins(20) showed that the hydrophilic colloids like starch, gum arabic, etc. have an anomalously large diffusion velocity and that the radius is nearly equal to that of a molecularly dispersed substance. The relations between the concentration of the sericin solution and the diffusion coefficient thus calculated are shown in Table 31.

Table 31.

Conc. (%)	Time (hours)	Volume (c.c.) of sericin diffused	D (sq. cm./day)	$r \times 10^7$ cm.	M
0.383	16	2.05	0.0882	2.009	29440.57
0.294	16	2.58	0.1110	1.596	14774.19
0.266	16	2.85	0.1228	1.444	10922.41
0.198	15	2.88	0.1239	1.430	10618.91
0.142	15	3.05	0.1400	1.266	7366.62

In low concentrations the size of sericin particles increases with increasing concentration and above 0.5 per cent. the rate of increase becomes very small. In the case of the same concentration sericin particles obtained from cocoons of good "kaijo" are larger than those obtained from cocoons of bad "kaijo."

Table 32.

Conc. (%)	$\ln r$.	$\frac{\ln r}{C} = A$
0.388	0.6973	1.796
0.294	0.4653	1.566
0.266	0.3678	1.383
0.198	0.3572	1.803
0.142	0.2355	1.658

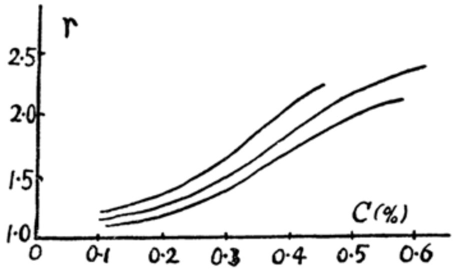

Fig. 16.

The logarithm of the radius of sericin particles is approximately proportional to the concentration. (Table 32 and Fig. 16.)

---

(20) H. R. Bruins, *Kolloid-Z.*, **54** (1931), 265; ibid. **57** (1931), 152.

As shown later the radius of sericin particles is proportional to the specific conductivity k in low concentration (Table 33.)

Table 33.

Conc. (%)	r(mμ.)	Sp. cond. $k\times10^3$	$\frac{k\times10^3}{r}$
0.636	2.372	0.636	0.268
0.40	1.786	0.530	0.296(?)
0.266	1.423	0.368	0.259
0.25	1.401	0.350	0.250
0.22	1.351	0.330	0.244
0.15	1.189	0.299	0.251
0·132	1.189	0.298	0.250

The calculated weight of micellæ in similarly prepared solutions varies with the concentration and with "kaijo" of the cocoon. Thus in a 0.3 per cent. solution the mean micellar weight is about 15000 for sericin obtained from cocoon of good "kaijo."

Effect of Acid and Alkali. It is well known that the micellar size of protein particles varies with the pH value of the solution. Then, the diffusion coefficient of sericin must decrease on addition of small amounts of acid and increase on addition of alkali. The experimental results are given in Table 34.

Table 34. $C = 0.388\%$ (good "kaijo," 18°C.)

	pH	D	r(mμ.)	M
Original solution	5.53	0.0882	2.009	29440.58
+ 0.01 N HCl	4.88	0.0796	2.226	40061.38
+ 0.01 N NaOH	6.02	0.0903	1.963	27453.60

Table 35.

Relative viscosity η	Refractive index n (18°C.)	$\frac{n}{\eta}$
1.147	1.34828	1.17
1.141	1.34812	1.18
1.136	1.34802	1.18
1.129	1.34796	1.19
1.121	1.34784	1.20
1.109	1.34773	1.21

Sec. VI. The Optical Properties of the Sericin Solution.

(1) The Refractive Index of the Sericin Solution. D. von Dusöe⁽²¹⁾ measured optically the concentration of proteins as the function of refractive index. I found that in low concentrations the refractive index of the sericin solution is proportional to its viscosity, and depends on the concentration, hydration, the form and size of sericin particles, etc.. And at the same concentration the refractive index of sericin sol obtained from the cocoons of good "kaijo" is greater than that of sericin sol

---

(21) D. von Dusöe, *Biochem. Z.*, **230** (1931), 373, 383; **244** (1932), 382.

obtained from the cocoons of bad "kaijo." The relation between the relative viscosity and the refractive index is shown in Table 35.

(2) **The Optical Activity of the Sericin Solution.** Sericin solutions are lævorotatory. Vignon(22) observed that both sericin and fibroin, two constituents of silk, were lævogyrate and their rotatory powers were about equal and approximately −35°.

Sericin	$[\alpha]_D$
From Japanese cocoons	-31.3
From Chinese cocoons	-33.9
From Tonking cocoons	-43.6

These value were obtained with the solution of sericin in 3 per cent. NaOH, but in such a strongly alkaline solution the optical activity of sericin may be reduced as is the case with gelatin. In researches made by T. Watanabe(23) it was observed that the specific rotation of sericin dissolved in 0.3 per cent. NaOH solution is on an average about −80°. I measured the rotatory power of the sericin solution with Landolt's polarimeter under the light of the sodium flame and the experimental materials were the following:

Variety of cocoons	"Kaijo"
"Nichi" No. 1 × "Shi" No. 4 (1930)	good
"Nichi" No. 107 × "Shi" No. 101 (1930)	middle
"Nichi" No. 110 × "Shi" No. 103 (1930)	bad

(a) **Variation of Optical Activity with Concentration.** The specific rotation of sericin is not constant, but it varies with concentration (Table 36). This may be due to the variation of aggregation of sericin micellæ.

Table 36.

Good "kaijo"		Mid. "kaijo"		"Bad kaijo"	
Conc. (%)	$[\alpha]_D$	Conc. (%)	$[\alpha]_D$	Conc. (%)	$[\alpha]_D$
0.14	-118.10	0.12	-123.15	0.13	-124.22
0.16	-110.55	0.15	-111.00	0.16	-105.83
0.18	-105.23	0.18	-103.56	0.18	- 96.33
0·20	-102.20	0.20	-100.10	0.20	- 88.40
0.22	- 95.82	0.24	- 90.67	0·24	- 78.04
0.26	- 93.50	0.28	- 83.16	0.26	- 74.38
0.29	- 91.72	0.30	- 80.00	0.31	- 70.49
0.33	- 89.10	0.35	- 79.05	0.34	- 64.11
0.35	- 88.71	0.40	- 78.62	0.40	- 62.40
0.40	- 86.25	0.42	- 78.19	—	—

---

(22) Vignon, *Compt. rend.*, **103**, 802.

(23) T. Watanabe, *J. Sakurakai, Japan*, December, 1929.

As shown in Table 36, the specific rotation of sericin is lævorotatory and diminishes with increasing concentration. The sericin solution obtained from the cocoon of good "kaijo" shows greater specific rotation than that obtained from the cocoon of bad "kaijo" at the same concentration.

The optical activity of the sericin solution dialyzed for 15 hours in running water is approximately equal to that of the sericin solution not dialyzed.

Table 37.

Good (0.21%)		Mid. (0.22%)		Bad (0.13%)	
*p*H	[α]	*p*H	[α]	*p*H	[α]
5.83	98.71	5.75	94.93	5.35	120.00
5.13	94.62	5.33	96.40	4.81	116.17
4.50	69.05	4.50	75.45	4.30	103.00
4.24	52.24	4.50	55.00	4.14	97.70
4.05	61.12	4.05	55.50	4.00	110.15
3.95	73.10	3.87	83.81	3.93	116.17
3.46	101.50	3.62	93.30	3.50	124.41
3.11	101.68	3.11	106.81	3.06	126.53

Table 38.

Vol. added c.c.	Good (0.18%)	Mid. (0.15%)	Bad (0.11%)
0	105.78	113.73	125.89
0.05	125.22	128.93	152.64
0.1	141.67	154.80	171.18
0.3	100.83	133.60	146.64
0.5	122.78	129.61	137.89
0.7	132.22	162.23	—

(b) **Effect of Acid and Alkali on Optical Activity.** Under the influence of hydrogen-ions the rotation of sericin decreases and it shows a minimum value at the isoelectric point of sericin.

(i) **Hydrochloric acid** (0.01 *N*). In the 0.2 per cent. sericin solution the isoelectric point corresponds approximately to *p*H 4.20. With sulphuric acid and oxalic acid the same phenomena can be seen.

(ii) **Sodium hydroxide** (0.1%). It can be seen that on addition of alkali to the sericin solution the rotatory power increases to a maximum and then diminishes. The maximum value corresponds nearly to the neutral point of the solution. The effect of alkali on the optical activity of sericin is the greatest for the sericin solution obtained from the cocoon of good "kaijo" (Table 38).

(c) **Effect of Salts on the Optical Activity.** The asymmetry of the sericin micellæ may vary with concentration, with different "kaijo" of the cocoon, and on addition of electrolytes. The rotation of sericin increases if the hydration is raised, for example, by the addition of alkali or salts with a pronounced lyotropic action.

Table 39.

Volume added in c.c.	$(NH_4)_2CO_3$	CH_3CO_2Na	Na_2CO_3	Na_3PO_4	NaCl	$C_4H_4O_6K.Na$
	(0.26 %)	(0.3%)	(0.32%)	(0.2%)	(0.21%)	(0.18%)
0	93.54	90.67	89.81	105.00	98.43	105.33
0.05	100.33	135.80	99.81	97.71	85.09	140.67
0.1	134.38	108.49	83.56	90.53	101.76	140.11
0.3	103.54	126.80	111.56	127.13	110.00	134.59
0.5	88.54	151.00	98.52	134.50	100.61	—
0.7	70.33	126.11	91.50	125.46	—	—

(Conc. of salt solutions is 0.1 per cent.)

The specific rotation of the solution attains a maximum on increasing the amount of the salt, the effect of Rochelle salt being the strongest (Table 39). Potassium bitartrate, glycocoll, etc. affect also strongly the rotation.

Sec. VII. The Electric Conductivity of the Sericin Solution.

Proteins differ from ordinary electrolytes in having a large cumbersome ion which may arise from several aggregated molucules. According to the investigation by Whitney and Blake (1904) the specific conductivity k is 68×10^{-6} mho for 0.2 per cent. gelatin sol. Although the protein solution is purified by dialysis, the state of aggregation of sericin micellæ changes at the same time and the variation of its electric conductivity may be caused by this treatment. For this reason, I employed the non-dialyzed sericin solution for measurement of its electric conductivity. The electric conductivity was measured by Kohlrausch's method and the cell constant was 0.1578.

(1) **Variation of Specific Electric Conductivity with Concentration.** The experimental results are shown in Table 40 and in Fig. 17.

Table 40.

Good "kaijo"		Middle "kaijo"		Bad "kaijo"	
Conc. C (%)	Sp. cond. $k \times 10^3$	Conc. C (%)	Sp. cond. $k \times 10^3$	Conc. C (%)	Sp. cond. $k \times 10^3$
0.800	0.6326	0.771	0.6481	0.861	0.6502
0.588	0.6070	0.625	0.6362	0.680	0.6452
0.578	0.5955	0.548	0.6340	0.580	0.6341
0.508	0.5718	0.538	0.6173	0.532	0.6251
0.480	0.5636	0.480	0.6014	0.470	0.6056
0.427	0.5091	0.430	0.5641	0.390	0.5537
0.412	0.4931	0.380	0.5349	0.374	0.5232
0.370	0.4265	0.360	0.4849	0.335	0.4622
0.300	0.3981	0.320	0.4157	0.265	0.3945
0.270	0.3431	0.281	0.3857	0.250	0.3670
0.234	0.3260	0.170	0.3051	0.194	0.3076
0.160	0.3086	0.104	0.2982	0.140	0.3006
0.000	0.3564	0.000	0.3564	0.000	0.3564

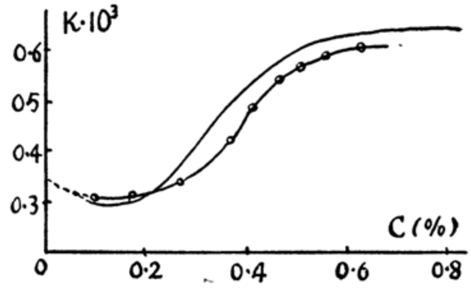

Fig. 17.

The sericin solutions prepared by boiling the cocoon layer with water contain a very small amount of electrolytes as impurities and combined form, so the specific electric conductivity has a remarkably high value. The degree of aggregation of sericin micellæ and the amount of electrolytes present in it may vary with increasing concentration, so the electric conductivity will increase with increasing concentration. But, the rate of variation, $\frac{dk}{dc}$ is the greatest near the concentration of 0.35 per cent. The specific electric conductivity k of the sericin solution is a linear function of the logarithm of the concentration, C, as shown in the following formula:

$$k = \log 100.C + B,$$

where B is constant but varies with "kaijo" of the cocoon. The value of constant B for the sericin solution obtained from the cocoon of good "kaijo" is larger than that for the sericin solution obtained from the cocoon of bad "kaijo."

"Kaijo"	Const. B
Good	1.117
Middle	1.096
Bad	1.077

I already found the same relation in the case of surface tension of the sericin solution.

(2) **Variation of Electric Conductivity with Temperature.** The colloidal state and adsorption capacity of sericin micellæ are affected by the variation of temperature. On raising temperature sericin particles transform gradually from gel state into sol state and the adsorption capacity diminishes. So the electric conductivity of the sericin colloid increases with rise in temperature (Table 41).

Table 41. Sp. elec. conductivity at various temperatures.

0°C.		20°C.		40°C.	
C (%)	$k \times 10^3$	C (%)	$k \times 10^3$	C (%)	$k \times 10^3$
1.154	0.4971	1.100	0.6210	1.154	1.1435
0.718	0.5058	0.771	0.6481	0.718	1.0944
0·580	0.4439	0.548	0.6340	0.500	0.8125
0.386	0.3507	0.380	0.5349	0.364	0.5844
0.200	0.2700	0.281	0.3857	0.200	0.4331

When potassium chloride is added to the sericin solution, the electric conductivity has a maximum and then diminishes with increasing concentration of the salt and the effect of the salt is the greatest for bad "kaijo."

(3) **Variation of Electric Conductivity with Time.** The specific electric conductivity of the sericin solution varies irregularly with time and diminishes during about two hours and then gradually increases. The minimum point corresponds approximately to a maximum point of viscosity varying with time (Table 42).

Table 42. The variation of k with time.

Time (min.)	Good (0.530%) $k \times 10^3$	Mid. (0.562%) $k \times 10^3$	Bad (0.390%) $k \times 10^3$
0	0.5802	0.6321	0.5516
15	0.5888	0.6363	0.5537
30	0.6069	0.6641	0.5845
45	0.5845	0.6117	0.5396
60	0.5636	0.5845	0.5095
75	0.5442	0.5856	0.4931
90	0.5260	0.5537	0.4856
105	0.5091	0.5296	0.4891
120	0.4931	0.5296	0.5179
150	0.5349	0.5380	0.5759
180	0.5618	0.5537	0.6117

The conductivity increases under the same condition as the number of ions. This variation may be due to the change of the number of ions and also of surface property of sericin micellæ.

(4) **Effect of Acid and Alkali on Electric Conductivity.** It is probable that the conductivity of the protein is due mostly to combined or adsorbed salts. If a strong acid be gradually added to a sericin solution, the volume of the solution being kept constant, and the electric conductivity be determined after each addition, it is found that it gradually diminishes, until a certain minimum is reached, and then it gradually increases (Table 43).

Table 43. The influence of 0.1 NHCl on the elec. conductivity.

c. c.	Good (0.465%) $k \times 10^3$	Mid. (0.380%) $k \times 10^3$	Bad (0.371%) $k \times 10^3$
0	0.5845	0.5189	0.5470
0.05	0.6116	0.5603	0.5641
0.1	0.5888	0.5312	0.5408
0.2	0.6164	0.5441	0.5931
0.3	0.6415	0.6171	0.6844
0.5	0.6861	0.7670	0.8267
0.7	0.8219	1.1046	1.1660
1.0	1.1718	1.5968	1.6306

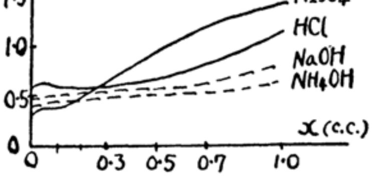

Fig. 18.

The minimum point of the electric conductivity is approximately at *p*H 4.10 which corresponds to the isoelectric point of sericin. Comparing it with the variation of electric conductivity on addition of acid it is the greatest for the sericin solution obtained from the cocoon of bad "kaijo."

If a base be gradually added to the sericin sol, the electric conductivity increases. The effect of alkali on the conductivity is also the greatest for the sericin solution of bad "kaijo." This may be due to the difference of buffer activity of sericin solutions. The buffer activity of sericin will be given later.

(5) Effect of Salts and Pupa on the Electric Conductivity.

Table 44.

The influence of salts on elec. conductivity ($k \times 10^3$).

c. c.	$NaCl$	$CaCl_2$	$MgCl_2$	$AlCl_3$	$FeCl_3$	$Th(NO_3)_4$
	(0.3%)	(0.34%)	(0.36%)	(0.36%)	(0.29%)	(0.39%)
0	0.3636	0.3931	0.4188	0.4188	0.3560	0.4753
0.05	0.3646	0.4759	0.3835	0.4221	0.3536	0.5262
0.1	0.3738	0.5521	0.3947	0.46[illegible]0	0.3969	0.5367
0.2	0.4312	0.6698	0.4023	0.4641	0.4175	0.5442
0.3	0.4861	0.8069	0.4802	0.5845	0.4561	0.5636
0.5	0.5374	0.9662	0.5014	0.8051	0.5741	0.6467
0.7	0.6530	1.2150	0.6865	1.0562	0.7259	0.7239
1.0	0.7988	1.6731	0.9373	1.3722	1.0020	0.9122

(Conc. of salt solutions = 0.1 *M*.)

(a) **Salts.** In the low concentration of salts the changes of the electric conductivity of sericin solutions are shown in Table 44 and Fig. 19. The salts like $CaCl_2$ and $AlCl_3$ which affect remarkably the surface tension and viscosity, give also a stronger effect on the electric conductivity than other salts.

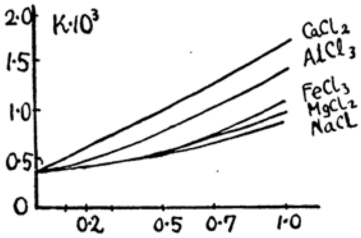

Fig. 19.

(b) **Pupa.** The sericin solution prepared by boiling cocoons containing the pupa with water has a very high value of electric conductivity. If the electric conductivity is only due to the presence of sericin salt and its micellar weight is 12000 for 0.37–0.23 per cent. solution, the degree of dissociation calculated is given in Table 45.

Table 45.

Conc. (%)	Spec. cond. $k \times 10^4$	Dilution V	Equivalent cond. λ_v	Degree of dissoc. $\alpha = \lambda_v / \lambda_\infty$
0.37	4.20	3200	1341	0.54
0.30	3.98	4000	1592	0.64
0.23	3.26	5200	1695	0.68
0.16	3.08	6000	1848	0.74
0.0	3.56	∞	2500	—

In the dilute sericin solution the degree of dissociation corresponds to about 0.60. It was already noticed that the degree of dissociation of sodium caseinate is 0.787 in 0.355 per cent. solution.

(*To be continued*).