THE COLLOIDAL BEHAVIOUR OF SERICIN. III.*

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

Sec. VIII. Electro-cataphoresis of the Sericin Solution.

In electro-cataphoresis, an electromotive force acts on an interface, the electric current flowing tangentially to the plane of the interface and causing a movement of a solid with respect to the liquid. Concerning electric properties of sericin particles there has been little work. But, it is long recognized that sericin micellæ possess a negative charge. The charge on protein molecules in solution on either side of the isoelectric point is due to the ionization of protein salts.

Having a light passing through the cataphoretic tube at right angle to the plane of the tube, I measured the migration of the boundary between the sericin sol and the dispersing medium under the influence of electric potential. The cataphoretic velocities of proteins obtained recently by many authors are quoted in Table 46.

Table 46.

Protein	Cataph. velocity	*p*H	Author
Gelatin	$-0.43\ \left(\frac{\mu}{\text{sec.}}\right)$	5.4	Mattson, 1932(24)
Egg albumin	−0.35	5.0	Abramson, 1928(25)
,, ,,	−0.38	5.1	Mattson, 1932
Casein	−1.89	5.2	König, 1932(26)
Serum albumin	−1.11	4.02	,, ,,
Pseudo-globulin	−2.15	3.73	,, ,,

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* Continued from page 260 of this volume.

(24) Mattson, *Soil Science*, **33** (1932), 43.

(25) Abramson, *J. General Physiol.*, **12** (1929), 711–725.

(26) König, *Biochem. Z.*, **252** (1932), 325–342.

The cataphoretic velocity of the protein solution is much smaller than that of hydrophobic colloids as seen from Table 46.

(1) **The Migration Velocity of Sericin Particles.** In the case of dilute solutions of sericin I added small definite amounts of pumice suspension in order to make the migration of the boundary easily observable. As shown by Abramson and Mattson it appeared that the pumice particles were coated with the protein and behaved electrokinetically as well as the protein, and no difference in cataphoresis was observed on addition of pumice.

A sericin solution (5 c.c.) containing small amounts of pumice suspension was mixed with the same volume of a citric acid phosphate buffer solution and the mixture was put in the cataphoretic tube and the migration velocity was measured after passing an electric current for 15 minutes. The experimental results obtained are shown in Table 47 and Fig. 20.

Table 47.

Variation of cataphoretic velocity with concentration at various *p*H values. (For good "kaijo")

*p*H	Concentration of sericin sol.			
	0.542%	0.50%	0.235%	0.18%
	u	*u*	*u*	*u*
3.6	+0.242	+0.270	+0.383	+0.415
3.8	+0.225	+0.255	+0.355	+0.375
4.0	+0.180	+0.207	+0.277	+0.325
4.2	+0.137	+0.154	+0.230	+0.250
4.4	−0.010	−0.020	−0.060	−0.080
4.6	−0.050	−0.085	−0.119	−0.179
5.0	−0.152	−0.190	−0.306	−0.323

u denotes μ/sec. for 1 volt/cm.

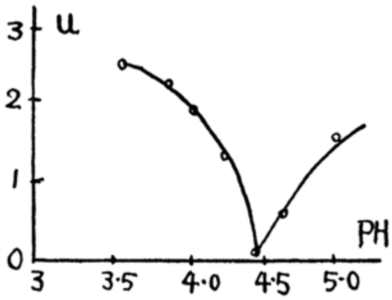

Fig. 20.

Sericin particles prepared by a usual method were transported to the anode in the electric field. The rate of migration, however, is a function of the hydrogen-ion concentration of the solution. It is found that at the isoelectric point there is no charge; on either side of this point there is a charge, positive in acid and negative in alkali, which rises to a maximum till a certain point is reached (Fig. 20). It may be regarded as the result of an increase of charge caused by increased ionization.

At the constant *p*H value the cataphoretic velocity of sericin particles diminishes gradually with increasing concentration and the speed of migration is greatest for the sericin obtained from the cocoon of good "kaijo" and much slower for that obtained from the cocoon of bad "kaijo" under the same conditions. With proteins which form molecular aggregates the potential difference between the particle and liquid in which it lies is influenced by the distribution of diffusible ions around the surface of the particle.

The theory of the electrokinetic phenomena was first given by Helmholtz and he assumed a double layer on the interface between the two phases; for cataphoresis he wrote $u = \frac{\zeta HD}{4\pi\eta}$, where u is the cataphoretic velocity of the particle in centimeter per second, ζ the potential of the double layer, H an external potential gradient, D the dielectric constant of water, and η the viscosity of the fluid. We can, therefore, calculate ζ-potential of sericin micellæ from the above formula:

$$\zeta = \frac{4\pi\eta u}{HD}$$

As shown in Fig. 20 the cataphoretic velocity—*p*H curve is not symmetrical on both sides of the isoelectric point. Isoelectric points obtained from the curves are given in Table 48.

Table 48.

" Kaijo "	*p*H at isoelec. point
Good	4.35
Middle	4.45
Bad	4.45

(2) **Variation of Cataphoretic Velocity with Temperature.** At low temperature the rate of migration of sericin micellæ is proportional to the temperature which causes a change in the state of aggregation of sericin particles. The results obtained are shown, for example, in Table 49.

Table 49.

For good "kaijo" at *p*H 3.6.

Temp.	Conc. 0.50%		Conc. 0.34%	
t (C.)	u (μ/sec.)	log u	u (μ/sec.)	log u
0	+0.215	−0.334	+0.236	−0.373
10	+0.225	−0.352	+0.270	−0.431
20	+0.275	−0.439	+0.340	−0.531
30	+0.402	−0.604	+0.502	−0.701
40	+0.750	−0.875	+1.155	−0.062

Logarithm of cataphoretic velocity of sericin micellæ is directly proportional to the temperature in the range between 10° and 40°C., namely,

$$\log u = A + Bt\ ,$$

where t is the temperature in centigrade, and A and B are constants.

(3) **Effect of Electrolytes on the Cataphoretic Velocity.** It is noticed that the rate of migration under the influence of electric potential is proportional to the ζ-potential of particles under the same conditions. When the ζ-potential is changed by the addition of electrolytes, a change in the cataphoretic velocity may be caused. The experimental results are shown in Table 50.

Table 50.

Effect of electrolytes on cataphoretic velocity.
(Conc. of the salt solution = 0.001 mol. pH = 5.0)

Volume (c.c.)	$NaCl$	KCl	$MgCl_2$	$CaCl_2$	$AlCl_3$	$FeCl_3$
	u (0.22%)	u (0.23%)	u (0.24%)	u (0.27%)	u (0.22%)	u (0.22%)
0	−0.31	−0.32	−0.295	−0.285	−0.31	−0.312
0.1	—	—	—	—	−0.04	−0.094
0.2	−0.38	−0.37	−0.25	−0.255	+0.071	+0.037
0.5	−0.46	−0.46	−0.188	−0.209	+0.179	+0.124
0.7	−0.53	−0.52	−0.16	−0.171	+0.160	+0.142
1.0	−0.59	−0.588	−0.156	−0.154	+0.124	+0.122
1.5	−0.581	−0.580	−0.13	−0.120	+0.081	+0.083
2.0	−0.53	−0.48	−0.12	−0.098	+0.069	+0.069

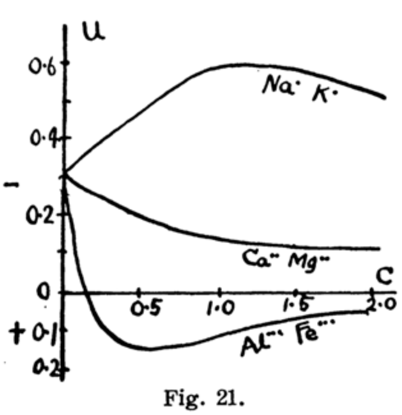

Fig. 21.

Fig. 21 shows a comparison of salts with cations of different valencies in their influences on the cataphoretic velocity of sericin particles. The abscissa is the volume of salt solutions added, the ordinate the cataphoretic velocity or the ζ-potential. The electrolytes like NaCl and KCl cause the increase of the rate of migration at the low concentrations as is the case with other proteins. $AlCl_3$ and $FeCl_3$ change the direction of the cataphoretic velocity in very low concentrations and these salts must be expelled from the water used in the silk industry.

(4) **Effect of Time of Heating on the Cataphoretic Velocity.** When the sericin solution prepared by the usual way is heated again without the cocoon layer in the same flask for a definite time, the viscosity diminishes gradually and at last the capacity of gelatinization is lost. The rate of migration, therefore, is changed by this treatment (Table 51).

Table 51.

Good "kaijo", conc. 0.16%; $pH = 3.8$ (20°C.).

Time of heating in hrs. (T)	Cataph. velocity u (μ/sec.)	T	u
0	0.395	2	0.443
0.5	0.406	3	0.450
1	0.410	4	0.490

The cataphoretic velocity of the sericin solution increases with increasing time of heating. This may be due to increase in the degree of dispersion of sericin micellæ and to decrease in the degree of its hydration by further heating.

(5) **Effect of Time of Standing on the Cataphoretic Velocity.** On standing at room temperature the sericin solution gradually gelatinizes, and the effect on the rate of migration may be contrary to that described in (4).

Table 52. ($pH = 3.8$)

Time of standing (min.)	Good "kaijo"	Bad "kaijo"
	(Conc. 0.306%) Cataph. velocity (μ/sec.)	(Conc. 0.23%) Cataph. velocity (μ/sec.)
0	+0.330	+0.260
60	+0.331	+0.257
120	+0.301	+0.242
180	+0.274	+0.220
240	+0.196	+0.187

As shown in Table 52, the cataphoretic velocity of the sericin solution diminishes with the time of standing. This may be due to increase in the size of aggregates and in the degree of hydration of the sericin micellæ.

Sec. IX. Separation of Sericin into Two Components.

(1) **Separation by Precipitation.** Robin and Verdeil (1853) noticed that magnesium sulphate gave a precipitate with egg-white and with serum. Virchow (1854)(27) found further that, besides $MgSO_4$, other salts, such as K_2SO_4, Na_2SO_4, alum, $CaCl_2$, NaCl, possessed the property of precipitating proteins.

Heynsius called attension to the great precipitating power of ammonium sulphate which precipitates albumin from solution. Hofmeister investigated the individual power of the acid- and the metal-groups of the salt to precipitate proteins. His results are quoted in Table 53. The numbers indicate the number of grams in 100 c.c. of solutions when precipitation of globulin commences in a solution of egg albumin.

Table 53.

	Na	K	NH_4	Mg
Sulphate	11.39	No ppt.	13.39	15.93
Acetate	13.83	16.38	No ppt.	No ppt.
Chloride	21.21	26.28	,, ,,	,, ,,
Nitrate	46.10	No ppt.	,, ,,	,, ,,

It is noticed from Table 53 that the capacity of precipitation depends on both the acid- and the metal-groups of a salt. The precipitating power can be arrranged in the following order: SO_4>citrate>tartrate>acetate>chloride>nitrate. This order of the effective influence of the neutral salts has become known as Hofmeister's series.

The precipitation of sericin from solution has been carried out in a variety of ways. Cramer (1965)(28) and Wetzel (1898)(29) precipitated sericin with lead acetate. Anderlini (1887)(30), Türk (1920)(31), K. Kondo (1921) and T. Watanabe (1928) took advantage of the insolubility of sericin in alcohol.

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(27) Virchow, "Über ein eingenthümliches Verhalten albuminöser Flüssigkeiten bei Zusatz von Salzen."

(28) Cramer, *J. prakt. Chem.*, **96** (1865), 76.

(29) Wetzel, *Z. physiol., Chem.*, **26** (1898), 535.

(30) Anderlini, *Chem. Zentr.*, 1887, 941; ibid., 1888, 795.

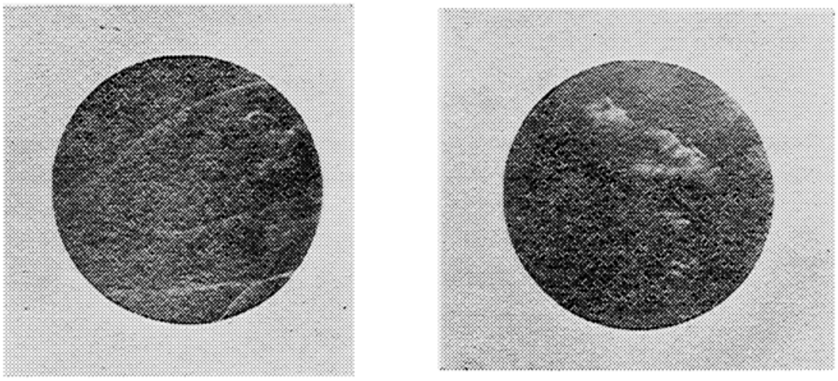
(31) Türk, *Z. physiol. Chem.*, **111** (1920), 69.

I noticed that the sensibility of the sericin solution to the electrolyte is different with "kaijo" of cocoons and that in low concentrations salts do not always precipitate the whole of sericin existing in the solution, but a part of it remains in solution after the addition of salts.

Then it can be considered that sericin must exist in solution in different states of aggregation. So I intended to separate components of sericin by the addition of ammonium sulphate which causes a comparatively slight denaturation of the protein. The application of ammonium sulphate precipitation method to the separation of the sericin components was done by Shelton and Johnson in 1925.(32) They found that varying concentration of ammonium sulphate gave two precipitates; the first of these being a gelatinous substance insoluble in cold water and to obtain it they added ammonium sulphate crystals to the solution.

If a lukewarm sericin solution freshly prepared be one-third saturated with ammonium sulphate, the fibrous precipitation called sericin A is obtained; by half-saturation of the filtrate the amorphous sericin called sericin B can be obtained. In practice, to a lukewarm sericin solution an equal volume of a saturated ammonium sulphate solution is added gradually and the mixture is allowed to stand for some time, sericin A first flocculating on the surface of the solution and then sericin B slow precipitating from the solution and setting down on the bottom of the vessel.

Sericin A and sericin B precipitated by $(NH_4)_2SO_4$



Sericin A (×124) Sericin B (×124)

Fig. 22.

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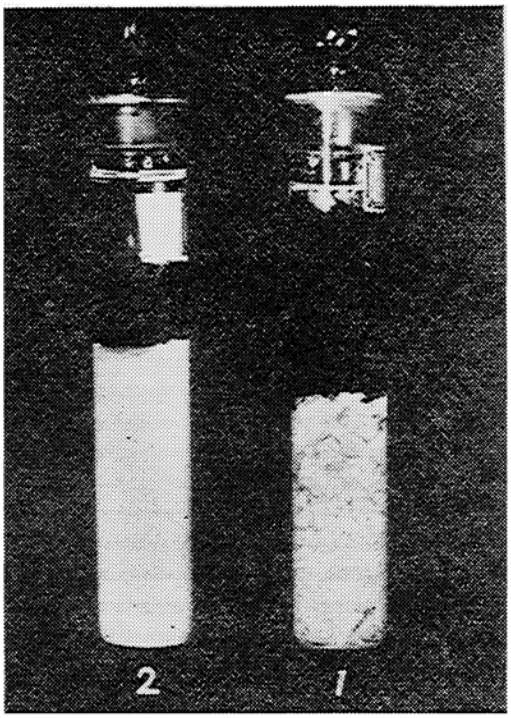
(32) Shelton and Johnson, *J. Am. Chem. Soc.*, **42** (1925), 412–418.



1···Sericin A, 2···Sericin B.

Fig. 23.

The non-spherical colloids, such as casein, fibrinogen or muscle globulin, are more easily precipitated with electrolytes than the spherical. In the case of sericin, sericin A is transparent, elastic, long fibers and easily aggregates together to a mass, but sericin B is white, small granules (Fig. 22 and 23).

After separated from the mother liquor, the precipitate, sericin A and sericin B, was suspended in water and freed from ammonium sulphate by dialysis for a few days and dried slowly over calcium chloride in a desiccator after washing it with alcohol and ether.

Two fractions can be separated from sericin solutions by means of ammonium sulphate, but this separation is difficult for cold or aged sericin solutions. The proportion of two sericin components is different with "kaijo" of cocoons as shown in Table 54.

Table 54.

	Good "kaijo"	Mid. "kaijo"	Bad "kaijo"
Sericin A: Sericin B	61.97 : 38.03 61.93 : 38.07	55.17 : 44.83 55.27 : 44.73	43.48 : 56.52 43.16 : 56.84
Nitrogen content of sericin A (%)	16.35 16.37 } 16.41 16.50	16.33 16.28 } 16.33 16.39	16.25 16.40 } 16.33 16.36
Nitrogen content of sericin B (%)	16.17 16.14 } 16.15 16.14	16.07 16.10 } 16.10 16.12	16.04 16.09 } 16.09 16.13

Table 54 shows that the sericin solution obtained from cocoons of good "kaijo" contains more sericin A than the solution obtained from cocoons of bad "kaijo," and that the nitrogen content is on an average 16.36 per cent. for sericin A, and 16.11 per cent. for sericin B.

I noticed further that, besides with ammonium sulphate, the separation of sericin into two components can be effected with many other salts and acids. Sericin A is precipitated at low concentrations of precipitating agents, while sericin B remains in solution at such concentrations but is precipitated from the filtrate of A on adding more precipitating agents. In practice, I precipitated sericin B by addition of equal volume of 90 per cent. alcohol after separating sericin A from the solution. For precipitation of sericin A I added aqueous solutions of electrolytes to the sericin solution until the solution remained clear after the precipitation of sericin A.

The nitrogen contents of sericin A and sericin B thus prepared are given in Table 55.

Table 55.

Coagulant	Nitrogen content (%)		Colour of coagulated sericin
	Sericin A	Sericin B	
$HgCl_2$	16.42	16.19	Colourless
$AlCl_3$	16.37	16.17	,,
$AuCl_3$	16.47	16.28	Reddish violet
$PtCl_4$	16.42	16.18	Slightly yellow
$La(NO_3)_3$	16.38	16.16	Colourless
$Er(NO_3)_3$	16.35	16.17	,,
$Th(NO_3)_4$	16.39	16.16	Slightly yellow
$Zr(NO_3)_4$	16.38	16.16	,, ,,
$CuSO_4$	16.36	16.15	Slightly blue
Alum	16.38	16.17	Colourless
Uranyl acetate	16.38	16.16	Slightly yellow
HCl	16.40	16.16	Colourless
H_2SO_4	16.40	16.16	,,
CCl_3CO_2H	16.37	16.14	,,
$(CO_2H)_2$	16.40	16.16	,,
Phosphotungstic acid	16.37	16.15	Grayish white
Phosphomolybdic acid	16.39	16.15	Grayish blue
Tannic acid	16.35	16.10	Colourless
$(NH_4)_2SO_4$	16.36	16.11	

The *p*H of sericin solutions is commonly on the alkaline side of isoelectric point, so sericin forms salts with salts of heavy metals, some of which have a characteristic colour.

The isoelectric point of sericin A and sericin B are respectively *p*H 3.8 and *p*H 4.5. When these two sericins are mixed, the isoelectric point may depend on the proportion of the mixture. The optimal *p*H value for precipitation by electrolytes must be, therefore, varied with "kaijo" of cocoons.

(2) **Separation by the Action of Electric Current.** One hundred c. c. of the sericin solution was subjected to electrodialysis for one hour, whereby a sharp separation of solid sericin A at the anode resulted, leaving a clear liquor containing sericin B, which could be driven out of the solution with alcohol (Table 56).

Table 56.

Nitrogen contents (%) of separated sericins.

"Kaijo"	Sericin A	Sericin B
Good	16.41	16.14
Middle	16.38	16.11
Bad	16.37	16.08

(3) **Separation by Freezing the Sericin Solution.** As it will be described later, two sericins were separated by first gelatinizing the sericin solution at room temperature for two hours and then freezing it. The frozen mass was then melted at 20°C. and filtered through filter paper. The solid residue was sericin A and the filtrate contained sericin B, obtainable in solid form by precipitation with alcohol.

Sec. X. The Precipitability of Sericin on the Action of Reagents.

It has already been mentioned that in dilute solutions salts act as solvents for isoelectric proteins, and that in concentrated solutions near the isoelectric point they act as protein precipitants. The amount needed to initiate precipitation depends on the nature of the added salts. Recent work by Lachs and Chwalinski (1932), showing that in many cases the influence of capillary-active non-electrolytes is to lower the coagulation values of univalent electrolytes and to raise the coagulation value of multivalent ions, is interpreted in terms of charges in the thickness of the electric double layer and the dielectric constant.

The thickness of the layer is affected only slightly by univalent ions and the dielectric constant then determines the charge in coagulation value, but multivalent ions have a strong influence on the thickness of the double layer. Kruyt and de Jong (1922)(33) have developed a general theory of charge and hydration as dual stability factors, which enables both hydrophilic and hydrophobic sols to be considered from the same point of view. They have shown that when the charge on the particles of an agar hydrosol is removed by electrolytes the sol remains stable, but the sol is flocculated if it also contains a dehydrating substance, such as alcohol or acetone.

Then a hydrophilic sol can be converted into a hydrophobic sol by adding alcohol or acetone and the resulting system can then be precipitated by small quantities of electrolytes.

These phenomena can be also seen in the sericin hydrosol. The molecules of the water of hydration are believed to be oriented around the particle in such a way that the first layers are firmly attached, whilst succeeding layers are less oriented and less firmly attached. The outer layers of less oriented water molecules are removed by dehydration at the isoelectric point.

The precipitation of proteins from solutions by the salts of the Hofmeister series is attributed to the so-called lyophilic action. The action of electrolytes on the hydrophilic colloids is generally very complex and depends upon the valency of cation of the salts, effect of anion, degree of hydration of the protein, and its adsorption capacity, etc.. To some extent Hofmeister's lyotropic series holds good for it.

Gortner(34) found that Hofmeister's series exists for the precipitation of wheat flour and that hydrogen- and hydroxyl-ions are adsorped on the surface of protein colloids. The effects of anions are as follows:

$$F > SO_4 > Cl > \text{tartrate} > Br > I\,.$$

With regard to the precipitation of proteins by salts of heavy metals, T. Geill (1929)(35) observed that there was generally two precipitation ranges, one in very weak concentration (about 10^{-4} N), and the other in strong concentration (about 1 N).

I found also that there was two precipitation ranges for sericin when the electrolytes, such as $Th(NO_3)_4$, $FeCl_3$, $AlCl_3$, and lead acetate, were added to the sericin solution. The experimental results obtained are given in Table 57.

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(33) Kruyt and de Jong, *Z. physik. Chem.*, **100** (1922), 250.

(34) R. A. Gortner, *Kolloid-Z.*, **44** (1928), 97.

(35) T. Geill, *Biochem. Z.*, **216** (1929), 165–178.

Table 57.

Flocculation of sericin with $Th(NO_3)_4$.
Conc. of sericin solution = 0.37%

Conc. of electrolyte (mol.)	X c.c.	Flocculation
0.01	0.04	− (no floc.)
”	0.07	−
”	0.11	+ (slight floc.)
”	0.14	++ (floc.)
”	0.16	+++ +++ (most floc.)
”	0.20	++ (floc.)
”	0.30	+
”	0.50	−
0.1	0.15	+
”	0.17	++
”	0.20	+++ +++

Flocculation of
sericin with $FeCl_3$.

Conc. of electrolyte (mol.)	X c.c.	Flocculation
0.01	0.3	−
”	0.4	++
”	0.7	+++
0.1	0.1	+++ +++
”	0.5	++
”	0.6	−
5.0	0.15	+
”	0.20	+++ +++

X denotes the volume of the electrolyte added to 5 c.c. of the sericin solution.

Pauli(36) considered that the precipitation occurring in very weak concentrations of salts of heavy metals was due to the formation of a complex salt of the protein with the cation of a salt, and that in strong concentrations it was due to the formation of a complex salt of the protein with the anion.

Silbermann states in his book, "Die Seide, II", that the aqueous solution of sericin is precipitated by alcohol, ether, metallic salts, and tannin, but not by potassium ferrocyanide nor acetic acid. At 20°C. I measured the flocculation values of the sericin solution, namely the concentrations of electrolytes in solution in millimoles per litre when the sericin is precipitated completely in two hours.

The influence of the nature of the electrolytes, as shown by H. Freundlich(37), proves that we are dealing with an electric phenomenon. The ions which are the most effective are those having a charge opposite in sign to that of the colloidal particles and the adsorbility and valency of these ions are of great importance.

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(36) Pauli, *Biochem. Z.*, **233** (1931), 87–112.

(37) Freundlich, *Z. physik. Chem.*, **114** (1924), 65.

(1) Flocculation Values for the 0.2 per cent. Sericin Solution. As already shown, the sericin sol possesses a negative charge like arsenic trisulphide sol, so the cations of salts must influence remarkably the flocculation values for sericin sol. Table 58 gives the flocculation values for sericin sol.

Table 58.

Flocculation value r for 0.2% solution.

Cation	Salt	r	Salt	r	Salt	r
Mono-valent	$AgNO_3$	12.28	$C_6H_5NH_2\cdot HCl$	2.84		
Bi-valent	$HgCl_2$	6.53	$Zn(NO_3)_2$	5.66	$CuCl_2$	3.84
	$(CH_3CO_2)_2Cu$	1.07	$(CH_3CO_2)_2UO_2$	1.07	$Cu(NO_3)_2$	1.07
	$CuSO_4$	0.91				
Trivalent	$FeCl_3$	0.740	$[Co(NH_3)_6]Cl_3$	0.740	$(CH_3CO_2)_3Fe$	0.740
	$AlCl_3$	0.570	$Al(NO_3)_3$	0.566	$La(NO_3)_3$	0.566
	$Fe(NO_3)_3$	0.384	$Er(NO_3)_3$	0.360	$\frac{1}{2}\ Al_2(SO_4)_3$	0.470
Tetra-valent	$Zr(NO_3)_4$	0.384	$Th(NO_3)_4$	0.196		
Acids	HCl	1.57	H_2SO_4	0.785	$(CO_2H)_2$	0.590
Alkaloidal reagents	Tannin	37.03	CCl_3CO_2H	0.740	Lead acetate	0.384
	P. T. acid	0.122	P. M. acid	0.566		

P. T. acid = phosphotungstic acid. P. M. acid = phosphomolybdic acid.

(2) Flocculation Values for the 0.4 per cent. Solution.

Table 59.

Cation	Salt	r	Salt	r	Salt	r
Mono-valent	$AgNO_3$	13.79	$C_6H_5NH_2\cdot HCl$	3.84		
Bi-valent	$HgCl_2$	7.40	$Zn(NO_3)_2$	7.40	$CuCl_2$	5.66
	$(CH_3CO_2)_2Cu$	1.37	$Cu(NO_3)_2$	1.379	$(CH_3CO_2)_2UO_2$	1.228
Trivalent	$La(NO_3)_3$	1.66	$FeCl_3$	1.071	$[Co(NH_3)_6]Cl_3$	0.91
	$AlCl_3$	0.740	$Al(NO_3)_3$	0.740	$(CH_3CO_2)_3Fe$	0.740
	$Fe(NO_3)_3$	0.566	$\frac{1}{2}\ Al_2(SO_4)_3$	0.940	$Er(NO_3)_3$	0.91
Tetra-valent	$Zr(NO_3)_4$	0.91	$Th(NO_3)_4$	0.31		
Acids	HCl	1.96	H_2SO_4	1.20	$(CO_2H)_2$	1.07
Alkaloidal reagents	Tannin	74.07	CCl_3CO_2H	0.91	Picric acid	2.06
	P. M. acid	0.384	Lead acetate	0.375		

From the figures, we find the following facts. Sericin sol is negatively charged; the added anion, which also has a negative charge, apparently plays a subordinate rôle in the flocculation process. On the other hand, the valence of the cation governs, to a great extent, the flocculating power of the added electrolyte. It is noticed that $HgCl_2$ and $Zn(NO_3)_2$ give flocculation values about 6.0 milli-moles, while aluminium, iron, and lanthanum give values in the neighborhood of 0.6 milli-mole. The hydrogen-ion and ions of heavy metals have a greater flocculating power than is expected from their valences. But, the salts having cations of the same valence do not show the same flocculation value. The effects of anions are in the order given below:

$Cl < NO_3,\ CH_3CO_2 < SO_4$ (cupric salt);
$Cl < CH_3CO_2 < NO_3$ (ferric salt);
$Cl < NO_3 < SO_4$ (aluminium salt).

It can been seen that Hofmeister's series holds goods here.

(3) **Flocculation Value and Concentration of Sol.** When I investigated the flocculation values for sericin sols of different concentrations, the following results were obtained.

Fig. 24 shows that dilution of the sol lowers the flocculation values of many salts. Like the case of the adsorption formula, the logarithm of flocculation value is a linear function of the logarithm of the concentration of sericin sol (Fig. 25).

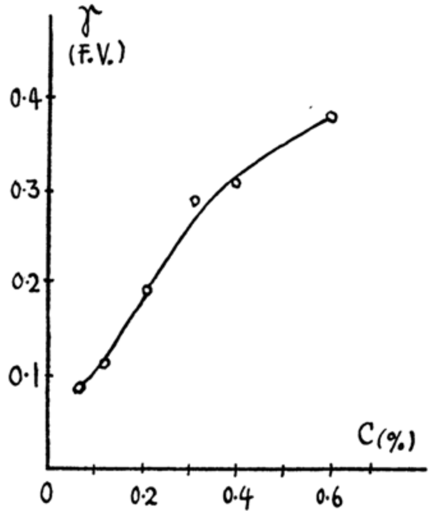

Fig. 24.

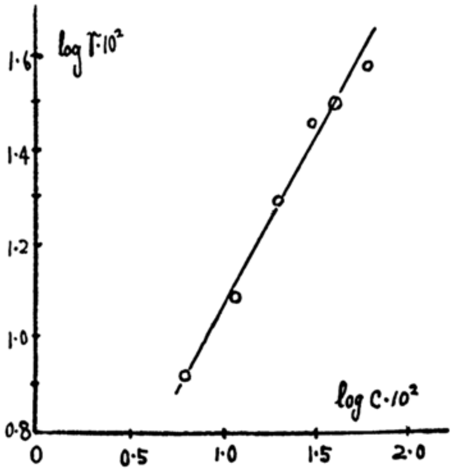

Fig. 25.

$$r = k.c^{\frac{1}{n}} \quad \text{or} \quad \log r = \log k + \frac{1}{n} \log c$$

(4) **Flocculation value and *p*H value.** Sericin has a wide coagulation range in *p*H values, for it is a mixture of at least two components. And the coagulation range is varied slightly with electrolytes (Table 60).

Table 60.

	Floc. range (*p*H)	Optimal floc. (*p*H)
(a) Acid (HCl, H_2SO_4)	3.5–4.4	3.9
(b) Salt ($FeCl_3$, $AlCl_3$, $CuSO_4$)	3.6–4.6	4.2
Salt (nitrate)	3.8–4.8	4.6

In the presence of citric acid-phosphate buffer solution, the coagulation range is extended to more acid side and the optimal coagulation point exists near *p*H 4.0.

(5) **Flocculation of Sericin with Alcohols and Acetone.** As alrealy shown, not only proteins are held in solution by their electric potential, but also their stability is influenced by association with water. Even electrically charged proteins can in some cases be driven out of solution by substances, such as alcohols and acetone, which have the power of extracting this water from the particles. Sericin is, therefore, precipitated from solution by addition of alcohols and acetone. The two constituents of sericin are precipitated by different concentrations of an alcohol. With increasing disaggregation of the micellæ, the precipitability by alcohols decreases so that a fractionation of sericin is possible on the basis of their alcoholic solutions as done by T. Watanabe.(38)

When the dielectric property of the sericin solution is changed by addition of some organic substances, like chloroform, carbon tetachloride, benzene, and aniline, sericin is also flocculated from solution.

(a) **Alcohols.** Ethyl alcohol in 80 per cent. concentration precipitates sericin from solution and the *p*H value of the maximum precipitability is varied in the presence of other substances. The optimal *p*H values for precipitation of dialysed sericin by ethyl alcohol are as follows:

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(38) T. Watanabe, loc. cit.

*p*H 4.50 (+ 0);
*p*H 3.8–3.9 (+ citric acid-phosphate buffer solution);
*p*H 3.75 (+ small amount of ammonia);
*p*H 4.60 (+ small amount of CO_2).

One c.c. of an alcohol was added to the mixture of 2 c.c. of the sericin solution (0.41%) and 3 c.c. of the buffer solution and the mixture was shaken throughly. After two hours' standing at room temperature the optimal *p*H values for precipitation of sericin was determined, which are shown in Table 61.

Table 61.

Alcohol	Opt. floc. (*p*H)	Alcohol	Opt. floc. (*p*H)
CH_3OH (85%)	4.0	C_4H_9OH (iso.)	4.2
C_2H_5OH (95%)	3.8	$C_5H_{11}OH$	4.4
C_3H_7OH (90%)	3.6		

The flocculation power of alcohols decreases with increasing dilution of them and at the same time the *p*H value of the maximum precipitability is shifted to the more acid side.

Jirgensons (1931)$^{(39)}$ showed that the coagulating influence of phenol, acetone, and certain alcohols on hydrosols of starch or egg-albumin rose with increasing concentration, attaining a maximum at a certain concentration which is a linear function of the dielectric constant of the coagulants. On adding an alcohol to the discharged sericin sol to which a minute quantity of an electrolyte had been added, an immediate flucculation occurred.

The precipitability of sericin sol by alcohols was sensitized by addition of minute quantities of $AlCl_3$ (0.0001–0.005 mol.). $FeCl_3$ had also a sensitizing effect at very weak concentrations (0.0001–0.005 mol.), but a stabilizing effect at strong solutions (0.01–0.4 mol.). Glycocoll (0.0008 mol.) and ammonium oxalate (0.005–0.1 mol.) acted as stabilizer in the flocculation of sericin by alcohols.

(b) **Acetone.** Acetone had a similar action on the sericin solution. In the low concentrations sericin A was apt to flocculate easily with acetone, for its isoelectric point lies near *p*H 3.7.

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(39) B. Jirgensons, *Biochem. Z.*, **240** (1931), 218-231.

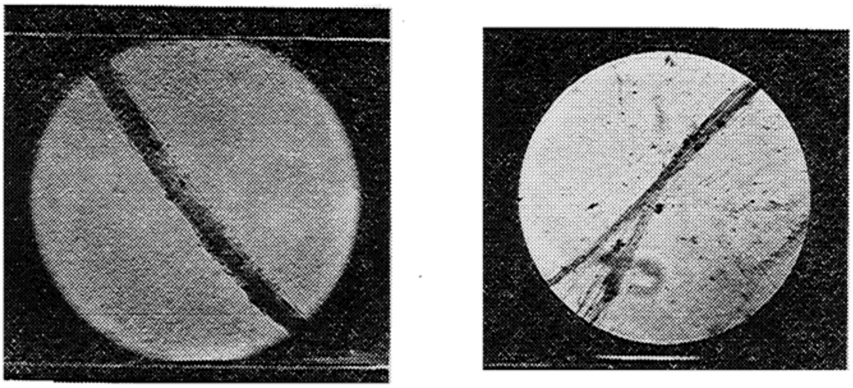
Table 62.

(2 c.c. Sericin solution + 3 c.c. Buffer solution + 1 c.c. Acetone).

Conc. of acetone (Merck) (%)	Opt. floc. (*p*H)	Floc. range (*p*H)	Precipitate
100	4.0	3.6–4.8	Floats, voluminous
83	3.8	3.6–4.2	Large form
50	3.6	3.4–3.8	Small form
33	3.6	3.4–3.8	,, ,,
10	3.6	3.4–3.7	,, ,,

The change in the concentration of acetone caused also the variation in the *p*H value of the maximum precipitability of sericin. Most of the aging phenomena of sols are in general characterized by the fact that the particles of a highly dispersed solution gather to form larger particles, and that their sensitiveness to flocculation is increased.

(6) **Form of Coagulated Sericin.** When electrolytes, such as thorium nitrate, zirconium nitrate, ammonium sulphate, mercuric chloride, phosphomolybdic acid, and uranyl acetate, were added to the sericin solution, the long, fibrous precipitates were obtained, which are illustrated in Fig. 26.



A. $(NH_4)_3SO_4$ B. $HgCl_2$

Fig. 26.

Especially sericin A gave easily a fibrous coagulant which had elasticity and shrank to mass on stirring. It can be, therefore, considered that sericin A has a micellar structure like fibroin, and sericin B an amorphous structure. By the optical method K. Obara (1932)(40) showed that sericin coating the silk fiber might have also a micellar structure. Then the high viscosity of sericin A may be explained by this consideration. On freezing the solution, sericin precipitated also in the form of fiber.

Sec. XI. The Distribution of Sericin Particles in the Electric Field.

If the particles combine with alkali, they would migrate to the anode, and if with acid, to the cathode. I observed the behaviour of sericin particles in Burton's cataphoretic apparatus. Towards the end of five minutes of passing the electric current under 110 volts, a white thin coagulant was produced near the boundary between sericin sol and water layer at the anodic side. The sericin solution in the anodic side gradually became more turbid and that in the opposide side was tinged with slight yellowish colour.

The anodic layers were acidic, the cathodic alkaline as in the case of the cocoon layer used as the diaphragm in distilled water. The sericin layer obtained from the cocoons of bad "kaijo" showed deeper yellow colour in the cathodic side.

(1) **Reactions of Each Liquid Layer.** Sericin particles in the cathodic side were easily coagulated with copper sulphate or lead acetate, but those in the anodic side were not flocculated with them, though both were precipitated with alcohol or ammonium sulphate.

Though the change of colour in the biuret reaction depends on the concentration of the sericin solution, sericin in the anodic side gave always a more intense tinge of red than that in the cathodic side. Then we can presume the degree of "kaijo" of cocoons from the colour reaction of the anodic layer. The relation between biuret reaction and the concentration of sericin are shown in Table 63.

Table 63. (Good "kaijo").

Conc. (%)	Anodic side		Cathodic side	
	Sericin layer	Water layer	Sericin layer	Water layer
Above 0.35	Brownish red	Violet	Violet red	Slightly blue violet —Colourless
0.35–0.25	Pink	Slightly violet	Violet red	Colourless

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(40) K. Obara, *Bull. Inst. Phys. Chem. Research, Japan*, **11** (1932), 466.

Table 63.—(*Concluded*)

Conc. (%)	Anodic side		Cathodic side	
	Sericin layer	Water layer	Sericin layer	Water layer
0.25–0.15	Violet red	Slightly violet	Red violet	Colourless
0.15–0.05	Red violet	Slightly blue violet	Violet	,,
Below 0.05	Red violet	Colourless	Violet	,,

The change of the colour of the biuret reaction depends not only on the concentration of sericin, but also on other soluble matters which exist with sericin in the cocoon fiber and become free in the electric field.

(2) **Isoelectric Point of Sericin Solutions in Cathodic and Anodic Sides.** The isoelectric point was measured by the change of surface tension. For the anodic sericin solutions the isoelectric point existed nearly at *p*H 3.80 and for the cathodic sericin at *p*H 4.40, although they had considerably different "kaijo" (Table 64).

Table 64.

"Kaijo"	*p*H at isoelectric point			
	Anodic sericin		Cathodic sericin	
Very good	3.70	3.80	4.36	4.40
Common	3.80		4.45	
Bad	4.00		4.50	

(3) **The Nitrogen Contents of Sericin in Both Solutions.** Though the amounts of sericin existing in both sides varied with the "kaijo" of cocoons, the nitrogen contents of sericin in the solutions were nearly equal and were found on an average 16.15 per cent. for sericin in the anodic solution and 16.01 per cent. for sericin in the cathodic solution (Table 65).

Table 65.

"Kaijo"	N (%) anodic		N (%) cathodic	
Good	16.156	16.15	16.08	16.03
Common	16.156		16.01	
Bad	16.143		16.01	

From the nitrogen content it can be considered that the anodic sericin is rich in sericin A and the cathodic in sericin B. The outer, middle, and inner layers of cocoons have different " kaijo ", but their anodic and cathodic sericins showed nearly the same nitrogen contents as described above. Both sericin solutions were also found already in the liquid silk in the silkworm. It is, therefore, clearly understood that the " kaijo " of cocoons is ascribed first to the varieties of the silk-worm, secondly to the physiological values of mulberry leaves and the external effects on the gelatinization of liquid silk.

(*To be continued.*)