CLIV. THE PREPARATION AND PHYSICO-CHEMICAL PROPERTIES OF SERICIN.

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It is usually accepted that silk is in the main composed of two proteins, designated as sericin and fibroin. A fairly large number of investigations of the chemical composition of these proteins have been carried out, though their physico-chemical properties have been little studied. As silk is not only a very important material in the textile industry but also a substance of biological interest, it is desirable to obtain information concerning these properties. The present paper is chiefly concerned with the study of the physico-chemical properties of sericin.

I. PREPARATION OF SERICIN.

Several methods of preparing sericin have been proposed, but none seems to be satisfactory. Cramer [1865] extracted sericin with boiling water and precipitated it with lead acetate. The precipitate was decomposed by hydrogen sulphide and the protein separated by alcohol.

Bondi [1902] has described two methods. In the first the sericin was extracted by boiling the cocoons in water for 1 hour. This extraction was repeated three times, the water each time being renewed. All the extracts were collected and evaporated to dryness. The residue was powdered, treated with water repeatedly to free it from impurities soluble in water, then dissolved in alkali and precipitated by alcohol. The second method differs from the previous one in precipitating the sericin in the aqueous extract by acetic acid and then treating the precipitate thus obtained with alcohol and ether to reduce it to powder. Fischer [1908], Türk [1920], Kondo [1921] and several other investigators have extracted sericin from the cocoons in an autoclave at a temperature of 118° or more, and precipitated the sericin with alcohol of a concentration not less than 50 %.

Quite recently Shelton and Johnson [1925], after a careful survey of the previous methods, concluded that the best way to extract is at 105° in the autoclave. They asserted also that there are two kinds of protein, one soluble in water and the other insoluble.

EXPERIMENTAL.

1. *Influence of heating at various temperatures on the precipitability of sericin at a certain acidity.*

Since there is a wide divergence of opinion among many investigators with regard to the extraction-temperature, it is necessary to decide first of all the most suitable temperature in order to get the greatest yield and also to extract the protein in its natural state.

20 g. of cocoon, freed from the chrysalides, were soaked in water for a few hours and then extracted with 500 cc. of distilled water at 100° and 105° in an autoclave for 1 hour. The extractions were repeated three times, the water each time being renewed. The nitrogen content in each extract was determined and compared with that of the filtrate which was obtained by filtering off the precipitate caused by the addition of acetate mixture of p_H 3.8, which seems to give the most copious precipitation, as shown later. The difference of the two values gives the amount of protein precipitated at the above acidity. In the same way the amount of the protein precipitable by 25 % alcohol in the filtrate was calculated. The results are given in Table I (where quantities are expressed in milligrams).

Table I.

Extraction-temperature	Number of extraction	N in 100 cc. of extract	N precipitated by acetate mixture	N precipitated by 25 % alcohol
105°	1	146.4	9.4	58.1
	2	16.8	2.4	6.7
	3	2.0	0	0.8
100°	1	68.3	39.2	21.0
	2	66.0	54.0	1.5
	3	15.3	13.8	0.2

The table clearly shows that, though extraction at 105° in the autoclave was much more effective than at 100°, it induced an alteration in the nature of the protein, *i.e.* the portion precipitated by the acetate mixture becomes very much reduced and that by alcohol increased. To verify this fact the following experiment was carried out. The aqueous extract at 100°, which contained 52.8 mg. nitrogen in 100 cc., of which 32.1 mg. belonged to the portion precipitated by the acetate mixture, was heated in the autoclave for 1 hour at a high temperature, and nitrogen determinations were made on the filtrate in the same way as in the foregoing experiment. The results are given in Table II (quantities of nitrogen are expressed in milligrams).

We see from this experiment that, as the temperature was raised, the amount of the protein precipitated by the acetate mixture decreased and a higher concentration of alcohol was required for the precipitation of the protein in the filtrate. It may be concluded, therefore, that heating at a high temperature causes some change in or decomposition of the protein molecule (or protein aggregate).

Table II.

The original solution contained 52.8 mg. N in 100 cc.

Temperature of autoclave	N precipitated by acetate mixture	N precipitated by alcohol of the final concentration			
		25 %	40 %	50 %	75 %
100°	32.1	20.0	20.4	20.4	20.4
105°	12.4	36.1	37.5	38.0	38.0
110°	10.2	29.9	40.6	41.0	41.0
112°	9.4	29.9	40.0	41.0	41.0
125°	7.1	26.8	31.1	36.4	44.0
130°	6.2	7.0	16.9	25.2	40.2
140°	6.2	0.6	6.6	10.2	14.6

2. *Influence of heating on the chemical character of sericin.*

The typical properties of the proteins are due to the presence of the amino-acid groups which build up the protein molecule. In the case of sericin, whether the alteration of its physical properties by heating is to be ascribed merely to the splitting of the carbamino-linkage, or is accompanied by the destruction of certain amino-acids is an interesting question. The writer does not touch this problem here, but one fact must be emphasised in connection with the method of preparation, namely, that the Hopkins-Cole glyoxylic acid reaction for tryptophan is positive with the protein precipitated by the acetate mixture, but negative with the protein precipitated by alcohol.

3. *The optimal H ion concentration for the precipitation of sericin.*

The aqueous extract was rendered acid by adding an acetate mixture of varying H ion concentration and the amount of the precipitate observed after the lapse of 1 hour. In the same way the precipitate produced by alcohol in the filtrate from the above precipitate was noted. The results are indicated in the following table:

Table III.

p_H of the acetate mixture	The amount of the first precipitate	The amount precipitated by alcohol
3.2	++	++
3.4	++	++
3.6	+++	+++
3.8	++++	+++
4.0	++	++
4.2	++	-
4.4	-	-

After standing overnight there was no difference between the range from p_H 3.4-4.0.

4. *Discussion of the methods of various investigators.*

On the basis of the above experiments it is now apparent that the method of using an autoclave in the extraction of sericin is least to be recommended. Especially should such a temperature as 118°, introduced by Fischer, be avoided. On the other hand, the method adopted by Anderlini [1887, 1888],

namely, to extract in water at 50–60° for 25 days, is very tedious. By Cramer's method we can get a mixture of two proteins, one precipitable by the acetate mixture and the other not. Bondi's first method also gives such a mixture. Shelton and Johnson's second method of fractionating by means of ammonium sulphate is good, but Bondi's second method seems to be the best, which the writer has modified as follows.

5. *The writer's method of preparing sericin.*

The cocoon used was the Italian yellow Lombardi.

100 g. of cocoon, carefully freed from the chrysalides, were soaked in water for 6 hours (excessive soaking without antiseptics must be avoided), washed thoroughly with water several times, and extracted by boiling for 90 minutes in 20–30 volumes of distilled water. The extraction was repeated three times. All the extracts were collected and filtered through a thick paper mat in a Büchner funnel. A clear yellow solution was obtained. To this solution *N* acetic acid was added drop by drop with good stirring until the p_H of the whole solution attained about 3.9–3.8, about 100 cc. of the acid being necessary. Soon a thick yellow precipitate appeared and settled down to the bottom. After standing a few hours, the supernatant fluid was decanted and the precipitate washed several times with water. The decanted solution and the washwaters were collected and kept for the isolation of the water-soluble portion of sericin. The precipitate was transferred to a flask and boiled with a sufficient amount of 25 % alcohol for a few minutes. This process was repeated until the alcohol was colourless. Then the precipitate was brought to the state of a dry powder by means of alcohol and ether. The yield was about 27 g. For further purification this crude sericin was dissolved in dilute ammonia, warming to 60–70°. The solution was filtered through a clean paper mat in a Büchner funnel and the sericin reprecipitated by adding acetic acid. The process was repeated twice, the last precipitation being produced by dilute hydrochloric instead of acetic acid. The precipitate was washed with water until there was no chloride reaction in the wash-water and then reduced to powder by treatment with aqueous alcohol solutions of increasing strength and finally with dry ether. The sericin thus obtained is a somewhat yellowish coloured powder, soluble with difficulty in water, but soluble in alkali and in acid of a certain concentration. Its ash content is 0.173 % and N content 16.04 %.

The solution kept for the isolation of the water-soluble sericin was evaporated under diminished pressure to a thick syrup, which was poured into 25 % alcohol. The greater part of the protein was precipitated and filtered through a hard filter paper. The precipitate was dissolved in distilled water, filtered through the paper mat and, after having been reprecipitated by alcohol, was brought to a state of powder by treatment with alcohol and ether. The substance thus obtained is pure white and easily soluble in water. Its N content is 17.05 %. The writer designates this substance *sericin peptone*. Whether this is solely a decomposition product of the protein precipitable by acetic acid

or a mixture containing also a different kind of protein existing *ab initio* in the cocoon, must be ascertained by further researches.

II. PHYSICO-CHEMICAL PROPERTIES OF SERICIN.

1. *The solubility of sericin.*

The perfectly dried sericin, prepared as above, was very slightly soluble in water; but when boiled with water, it went into solution to a slight extent, perhaps owing to the alkali from the glass. As will be seen later, the sericin is a protein of marked acid character so that it dissociates in water as an acid and becomes soluble. Its solubility, therefore, depends solely upon the degree of dissociation, which is again a function of the acidity of the medium. The following experiments give data concerning its solubility at different H ion concentrations.

0.2 g. portions of the powdered sericin, which passed through a No. 40 sieve, were weighed out into a series of flasks. To each of them 20 cc. portions of 0.2 *M* acetate mixture of varying H ion concentrations, and of the compositions shown in the following table, were added. The flasks were put into a thermostat at 25° and occasionally stirred. After the lapse of 2 hours the contents were filtered and the amount of protein in the filtrates determined by means of Kingslake's nephelometer. The turbidity was produced by bringing the solutions to the acidity of p_H 3.8 (which was shown to be the best for producing precipitation in the previous experiment) and compared with that of a standard solution, which contained 50 mg. of sericin and 0.01 mol NaOH in 100 cc. The results are given in Table IV and Fig. 1.

Table IV. *Solubility of sericin at different acidities.*

Acetic acid 0.2 <i>M</i> cc.	Na-acetate 0.2 <i>M</i> cc.	p_H (electrometric)	Amount of sericin dissolved in 100 cc. mg.
20	0	2.64	31.1
19.5	0.5	3.16	25.5
19.0	1.0	3.43	11.7
18.5	1.5	3.60	9.6
17.6	2.4	3.86	0
16.4	3.6	4.07	5.8
14.7	5.3	4.27	23.9
12.6	7.4	4.46	31.0
10.6	9.8	4.68	51.8
5.9	14.1	5.07	53.6
2.9	17.1	5.42	60.7

The above table and the accompanying figure (Fig. 1) clearly demonstrate that the solubility of the sericin is a minimum near p_H 3.9 and increases on both sides from this point. Since it is usually assumed that a protein, as an ampholyte, is least soluble at its isoelectric point, we can conclude that this point lies near p_H 3.9 in the case of sericin. Some other physico-chemical properties also lead to the same conclusion.

Fig. 1. Milligrams of sericin dissolved (ordinate) in 100 cc. of acetate mixture (p_H abscissae).

2. Swelling of sericin.

Procter and Wilson and J. Loeb have developed a theory of the mechanism of swelling upon the basis of Donnan's membrane equilibrium theory. According to them the swelling of the protein must be a minimum at its isoelectric point. This is actually the case with sericin.

To 0.1 g. portions of sericin in a number of test-tubes of uniform size (7×50 mm.) 2 cc. portions of the acetate mixtures of different H ion concentrations were added. After being well mixed they were put into a thermostat at 25° , and at the end of 24 hours the height of the swollen sericin was read. The results are illustrated in Fig. 2.

Fig. 2. Swelling of sericin in acetate mixture.

The same experiment was repeated, using HCl and NaOH of varying concentrations, instead of the acetate mixture, the result of which is shown in Fig. 3.

Fig. 3. Swelling of sericin in HCl and NaOH.

The curves show apparently a drop near the isoelectric point. Moreover, it is noticeable that in the region of greater acidity the curve falls again after attaining a maximum at $1/32$ M HCl. Though the amount of sericin dissolved,

which was determined by the micro-Kjeldahl method of Folin in an aliquot portion of the supernatant clear fluid, increased with the increase of acid as shown in Table V, we can still recognise that the swelling of sericin is retarded at the higher acidities.

Table V. *The amount of sericin dissolved in 2 cc. of various concentrations of HCl.*

The total concentration of sericin = 100 mg.

Conc. of HCl (M)	2/1	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
Sericin dissolved (mg.)	10.0	8.9	8.3	6.5	8.0	—	—	—	7.0	3.0	2.4	0

3. *The viscosities of sericin-HCl and sericin-NaOH mixtures.*

To a certain extent the viscosity of a colloidal solution is defined by the magnitude of the micelle. In this sense it must run parallel to the swelling.

0.2 g. portions of powdered sericin were weighed out into a series of test-tubes, to which 20 cc. portions of HCl of concentrations varying from 1/5 to 1/640 M were then added. To facilitate the dissolution the tubes were put into a water-bath at 80° for 20 minutes, the contents being stirred at times with a glass rod. After this procedure, one portion of the mixture was filtered through filter paper. Both mixture and filtrate were cooled in running water and transferred to a thermostat at 25°, in which the viscosity was measured at the end of 30 minutes. A viscometer of Ostwald's type was used. The above procedure was followed strictly, because the viscosity varied with the time and the temperature and duration of dissolution. The viscosity was expressed as the ratio t/t_0 , where t and t_0 are the times for the flow of the sericin solution and water respectively. In the same solution the H ion concentration and the conductivity were measured. The calculation of the former was carried out by the following formula,

$$p_H = \frac{E - 0.2464}{0.05911},$$

where E is the E.M.F. observed against the saturated calomel electrode at 25°. No correction was made for liquid potential. Further, the amount of sericin dissolved in the filtrate and the non-precipitable portion in it, when neutralised by adding the same amount of acid or base used to dissolve the protein, were determined and are given in columns 5 and 6 in Tables VI *a* and *b* respectively. The difference of these two values gives the amount of sericin precipitable at the isoelectric point.

Table VI *a*. *The viscosity of the sericin-HCl mixtures.*

HCl added M	p_H	Relative viscosity of		The amount of sericin (mg.) dissolved in 100 cc.		
		Mixture	Filtrate	Total	Non-precip.	Precip.
1/5	0.804	1.10	1.10	—	—	—
1/10	1.17	1.32	1.30	0.367	0.145	0.222
1/20	1.43	2.24	2.20	0.700	0.080	0.620
1/40	1.78	1.55	1.51	0.773	0.038	0.735
1/80	2.17	2.72	1.74	0.850	0	0.850
1/160	2.65	2.09	1.65	0.745	0	0.745
1/320	3.22	1.77	1.50	0.667	0	0.667
1/640	3.57	1.08	1.08	0.024	0	0.024

Table VI b. *The viscosity of the sericin-NaOH mixtures.*

NaOH added <i>M</i>	<i>p_H</i>	Relative viscosity of		The amount of sericin (mg.) dissolved in 100 cc.		
		Mixture	Filtrate	Total	Non-precip.	Precip.
1/10	12.71	1.19	1.18	1.000	0.330	0.670
1/20	12.33	1.24	1.23	1.000	0.218	0.792
1/40	11.75	1.53	1.53	1.000	0.008	0.992
1/80	10.83	2.27	2.18	1.000	0	1.000
1/160	7.64	3.10	2.90	0.934	0	0.934
1/320	5.78	2.27	1.98	0.840	0	0.840
1/640	5.18	1.45	1.40	0.608	0	0.608
Aq.	4.48	1.07	1.07	—	—	—

Fig. 4. Viscosity of sericin-HCl and -NaOH.

From the shape of the curve it will be seen that the viscosity is at a minimum near p_H 3.9 and attains a maximum on both sides, as is usually the case with other proteins. One peculiar fact is that there are two maxima on the acid side, the one which occurred at the lower acidity, however, became less marked in the filtrate. This is no doubt caused by the highly swollen micelle. The second maximum is difficult to explain. As a matter of fact, in such a peculiar heterogeneous solution as sericin-HCl, the viscosity is a very complicated thing. Adolf [1923] observed a similar rise of viscosity in the case of globulin-oxalic acid, but her explanation does not appear to be satisfactory.

4. *The amount of HCl or NaOH with which sericin combines.*

This amount was calculated from the values in Table VI a by the expression $a - b/a$, where a is the concentration of total HCl added, b that calculated from the p_H of the solution (free HCl) and a the degree of dissociation calculated from the ratio of the equivalent conductivities. A similar calculation holds for NaOH.

Table VII a. *The number of mols of HCl combined per 0.2 g. sericin in solutions of the given HCl concentrations containing 1 g. sericin per 100 cc.*

Total HCl (a)	Free HCl from p_H			Combined HCl	
	p_H	(b)	$1/a$	b/a	
0.2011	0.80	1.57×10^{-1}	1.095	17.24×10^{-2}	2.87×10^{-2}
0.1	1.17	6.74×10^{-2}	1.059	7.14×10^{-2}	2.86×10^{-2}
0.05	1.43	3.76×10^{-2}	1.035	3.82×10^{-2}	1.18×10^{-2}
0.025	1.78	1.67×10^{-2}	1.025	1.72×10^{-2}	7.86×10^{-3}
0.0125	2.17	6.84×10^{-3}	1.016	6.95×10^{-3}	5.55×10^{-3}
0.00625	2.65	2.24×10^{-3}	1.010	2.27×10^{-3}	3.98×10^{-3}
0.00313	3.22	6.00×10^{-4}	1.000	6.00×10^{-3}	2.53×10^{-3}
0.00157	3.57	2.70×10^{-4}	1.000	2.70×10^{-4}	1.30×10^{-3}

Table VII b. *The number of mols of NaOH combined per 0.2 g. sericin in solutions of the given NaOH concentrations containing 1 g. sericin per 100 cc.*

All concentrations are given in mols per litre.

Total NaOH (a)	p_{OH}	Free NaOH from p_{OH} (b)	$1/a$	b/a	NaOH combined
0.09146	1.29	5.13×10^{-2}	1.095	5.61×10^{-2}	3.54×10^{-2}
0.05	1.74	1.82×10^{-2}	1.055	1.91×10^{-2}	3.09×10^{-2}
0.025	2.35	4.47×10^{-3}	1.024	4.58×10^{-3}	2.04×10^{-2}
0.020	2.51	3.09×10^{-3}	1.018	3.15×10^{-3}	1.68×10^{-2}
0.0125	3.28	5.31×10^{-4}	1.000	5.31×10^{-4}	1.20×10^{-2}
0.01	4.54	2.88×10^{-5}	1.000	2.88×10^{-5}	9.72×10^{-3}
0.00625	6.43	3.74×10^{-7}	1.000	3.74×10^{-7}	6.25×10^{-3}
0.005	7.53	2.69×10^{-8}	1.000	2.69×10^{-8}	5.00×10^{-3}
0.00313	8.29	5.14×10^{-9}	1.000	5.14×10^{-9}	3.13×10^{-3}
0.00157	8.89	1.29×10^{-9}	1.000	1.29×10^{-9}	1.57×10^{-3}

Fig. 5. The number of mols of HCl or NaOH combined per 0.2 g. sericin in solutions of the given p_H values containing 1 g. sericin per 100 cc.

As shown in the above experiment, there seems to exist no saturation limit in the combining capacity of sericin for HCl and NaOH as far as the concentrations of this experiment are concerned. At higher concentrations, namely, beyond p_H 1.43 and p_{OH} 2.35, a sudden increase occurred, perhaps owing to the splitting of the protein molecule. At the lower concentrations both curves show that the minimum of combining capacity lies near p_H 3.9.

5. The conductivity of the sericin-HCl solutions.

The measurement of the electrical conductivity was made at 25° as rapidly as possible (1–5 minutes) after that of the viscosity, using a vessel of the Arrhenius type. The specific conductivity due to the sericin-HCl (K_s) was calculated by the expression

$$K_s = K_t - K_f,$$

where K_t = the total specific conductivity, obtained from the experiment.

K_f = the specific conductivity due to free HCl or NaOH calculated by multiplying the concentration of free HCl or NaOH by 0.422 or 0.22, which are the specific conductivities of M HCl or M NaOH solution at 25° respectively.

Table VIII a. *The conductivities of sericin-HCl mixtures.*

HCl added (mol)	K_i	HCl from p_H (b)	0.422.b K_j	K_s
0.1	3.776×10^{-2}	6.74×10^{-2}	2.846×10^{-2}	9.30×10^{-3}
0.05	1.866×10^{-2}	3.76×10^{-2}	1.587×10^{-2}	2.76×10^{-3}
0.025	7.615×10^{-3}	1.67×10^{-2}	7.052×10^{-3}	5.36×10^{-4}
0.0125	2.957×10^{-3}	6.84×10^{-3}	2.887×10^{-3}	8.00×10^{-5}
0.00625	1.056×10^{-3}	2.24×10^{-3}	9.457×10^{-4}	1.11×10^{-4}
0.00313	3.455×10^{-4}	6.00×10^{-4}	2.531×10^{-4}	9.24×10^{-5}
0.00157	2.249×10^{-4}	2.70×10^{-4}	1.156×10^{-4}	1.09×10^{-4}

Table VIII b. *The conductivities of sericin-NaOH solutions.*

NaOH added (mol)	K_i	NaOH from p_{OH} (b)	0.22.b K_j	K_s
0.09146	1.623×10^{-2}	5.13×10^{-2}	1.128×10^{-2}	4.950×10^{-3}
0.05	8.121×10^{-3}	1.82×10^{-2}	4.004×10^{-3}	4.117×10^{-3}
0.025	3.027×10^{-3}	4.47×10^{-3}	9.837×10^{-4}	2.043×10^{-3}
0.0125	8.135×10^{-4}	5.31×10^{-4}	1.195×10^{-4}	6.940×10^{-4}
0.01	4.972×10^{-4}	2.88×10^{-5}	6.350×10^{-4}	4.910×10^{-4}
0.00625	3.200×10^{-4}	3.74×10^{-7}	8.230×10^{-6}	3.192×10^{-4}
0.005	2.451×10^{-4}	2.69×10^{-8}	5.918×10^{-9}	2.451×10^{-4}
0.00313	1.753×10^{-4}	5.14×10^{-9}	1.131×10^{-9}	1.753×10^{-4}
0.00157	1.070×10^{-4}	1.29×10^{-9}	2.838×10^{-10}	1.070×10^{-4}

Fig. 6. The conductivity of sericin solution and of sericin-peptone solution at different p_H .

6. *The osmotic pressure of the sericin solution.*

The apparatus used was of Sørensen's type. The capacity of the collodion sack was 15 cc. The outside solution was 80 cc. of pure HCl or NaOH of such a concentration as to be half that used to make the sericin solution. The measurement was carried out in a thermostat at 25° and the reading was taken when the level of the liquid in the capillary no longer moved under a fixed opposing pressure. It took about 24–30 hours to attain equilibrium. At the same time the concentration of H ion in the outside and inside solutions was determined electrometrically. The pressure was expressed in millimetres of

water (see Fig. 7). The p_H values for the inside and outside solutions became equal at the point of minimum osmotic pressure, whilst the difference between these values changed sign on either side of this minimum. This held good both for sericin and sericin-peptone.

Fig. 7. The osmotic pressure of 1 % sericin and sericin-peptone solutions at different p_H .

7. *The change of some physico-chemical properties of sericin-HCl or -NaOH with time.*

In the investigation of the physico-chemical properties of proteins carried out by many investigators, little attention has been paid to the influence of time. Since the state of aggregation of the protein and the hydrolysis by acid or base are functions of time as well as of concentration, we must take the factor of time into consideration. This is especially the case when the solution of the protein is made from the solid state, as in the case of gelatin, caseinogen, sericin, etc. In the following experiments (Table IX) the change of the viscosity, the conductivity, the p_H and the precipitability at the isoelectric point with time were determined in solutions of 1 % sericin in 1/200 *M* HCl and 1/100 *M* NaOH (which were prepared as already described).

It is noticeable that the most remarkable change occurred in the viscosity. The acid sericin solution turned almost to a gel, while this was not the case with the sericin-NaOH solution.

III. THE PHYSICO-CHEMICAL PROPERTIES OF SERICIN-PEPTONE.

1. *The solubility of sericin-peptone.*

Sericin-peptone is readily soluble in hot water and is precipitated by 25 % alcohol or by half-saturation with ammonium sulphate. But in this process

of precipitation the H ion concentration plays a predominant rôle, as will be seen in the following experiment.

Table IX. *The change of some physico-chemical properties of sericin solutions with time at 25°.*

Hours	Viscosity	Conductivity (0.5 g. sericin + 50 cc. 1/200 HCl)	p_H	The amount not precipitated in 100 cc.
0	1.41	1.869×10^{-2}	1.45	74 mg.
$\frac{1}{2}$	1.42	1.866×10^{-2}	1.43	74
1	1.43	1.866×10^{-2}	1.42	80
2	1.45	1.866×10^{-2}	1.39	79
4	1.56	1.866×10^{-2}	1.38	78
8	1.83	1.867×10^{-2}	1.38	82
16	2.96	1.869×10^{-2}	1.36	84
32	4.12	1.902×10^{-2}	1.32	86
(0.5 g. sericin + 50 cc. 1/100 NaOH)				
0	2.63	4.972×10^{-4}	9.46	0
$\frac{1}{2}$	2.67	4.972×10^{-4}	9.46	0
2	2.68	4.942×10^{-4}	9.45	0
4	2.70	4.930×10^{-4}	9.43	0
8	2.74	4.925×10^{-4}	9.43	0
16	2.80	4.912×10^{-4}	9.42	8.6
32	2.86	4.832×10^{-4}	9.40	10.9

A 1 % solution of sericin-peptone was prepared and dialysed against distilled water for 2 days, followed by electrodialysis¹. The solution thus purified showed a specific conductivity of 0.756×10^{-4} mho. During the electrodialysis some flocks appeared. They were filtered off so that the protein concentration was reduced to 0.953 %.

Into a series of test-tubes 1 cc. portions of the above sericin-peptone solution were pipetted out and diluted with 5 cc. portions of the acetate mixture of varying acidity (p_H 2.65–4.68) followed by the addition of 2 cc. portions of absolute alcohol. After being well stirred the turbidities were compared in the Kingslake nephelometer. The values in the following table were obtained by recalculating the nephelometrical readings on the basis that the precipitation of the protein at p_H 3.86 was complete (compare Table III).

Table X. *The precipitability of sericin-peptone by 25 % alcohol at different acidities.*

p_H	2.65	3.43	3.65	3.86	4.07	4.26	4.46	4.68	4.87
Sericin-peptone precipitated	0	0.824	0.886	0.953	0.913	0.834	0.620	0.552	0.0383

2. *The conductivity, p_H , viscosity and osmotic pressure of sericin-peptone-HCl and sericin-peptone-NaOH solution.*

The experiments and calculations were carried out (in the same way as described above) on the solutions prepared by mixing 25 cc. portions of sericin-peptone solution with 25 cc. portions of HCl or NaOH of various concentrations.

¹ The dialyser consisted of a U-tube made of hard glass. The limbs were connected by a ground joint and equipped with a collodion membrane at the bottom.

Table XI a. *The number of mols of HCl combined per 0.2 g. sericin-peptone in solutions of the given HCl concentrations containing 1 g. sericin-peptone per 100 cc.*

All the concentrations are given in mols per litre.

Total HCl conc. (a)	p_H	Free HCl from p_H (b)	$1/a$	b/a	HCl combined
0.05	1.39	4.11×10^{-2}	1.035	4.25×10^{-2}	7.50×10^{-3}
0.025	1.75	1.76×10^{-2}	1.025	1.80×10^{-2}	7.00×10^{-3}
0.0125	2.16	6.90×10^{-3}	1.016	7.01×10^{-3}	5.49×10^{-3}
0.00625	2.64	2.27×10^{-3}	1.010	2.29×10^{-3}	3.96×10^{-3}
0.008	2.43	3.73×10^{-3}	1.013	3.78×10^{-3}	4.22×10^{-3}
0.0057	2.69	2.06×10^{-3}	1.006	2.07×10^{-3}	3.63×10^{-3}
0.004	3.01	9.68×10^{-4}	1.004	9.72×10^{-4}	3.03×10^{-3}
0.002	3.52	3.02×10^{-4}	1.000	3.02×10^{-4}	1.70×10^{-3}
0.001	3.84	1.44×10^{-4}	1.000	1.44×10^{-4}	0.865×10^{-3}
0.0005	4.04	9.20×10^{-5}	1.000	9.20×10^{-5}	0.403×10^{-3}
0	4.36	4.42×10^{-5}	1.000	4.42×10^{-5}	—

Table XI b.

The number of mols of NaOH combined per 0.2 g. sericin-peptone in solutions of the given NaOH concentrations containing 1 g. sericin-peptone per 100 cc.

All the concentrations are given in mols per litre.

Total NaOH conc. (a)	p_H	p_{OH}	Free NaOH from p_{OH} (b)	$1/a$	b/a	NaOH combined
0.458	12.32	1.57	2.69×10^{-2}	1.055	2.84×10^{-2}	1.74×10^{-2}
0.025	11.92	1.97	1.07×10^{-2}	1.024	1.09×10^{-2}	1.41×10^{-2}
0.0125	11.38	2.51	3.09×10^{-3}	1.000	3.09×10^{-3}	9.41×10^{-3}
0.00625	10.12	3.77	1.70×10^{-4}	"	1.70×10^{-4}	6.08×10^{-3}
0.008	10.58	3.31	4.90×10^{-4}	"	4.90×10^{-4}	7.51×10^{-3}
0.0057	9.96	3.93	1.18×10^{-4}	"	1.18×10^{-4}	5.58×10^{-3}
0.004	8.78	5.11	7.76×10^{-6}	"	7.76×10^{-6}	3.99×10^{-3}
0.002	5.22	8.47	3.39×10^{-9}	"	3.39×10^{-9}	2.00×10^{-3}
0.001	4.87	9.02	9.55×10^{-10}	"	9.55×10^{-10}	1.00×10^{-3}
0.0005	4.53	9.36	4.37×10^{-10}	"	4.37×10^{-10}	5.00×10^{-4}

Fig. 8 The HCl and NaOH combination curve of sericin-peptone. Ordinates denote mols HCl or NaOH (multiplied by 10^3) which combine with 0.2 g. sericin-peptone.

Table XII. *The viscosities of sericin-peptone-HCl and -NaOH solutions.*

Sericin-peptone-HCl.									
p_H	1.39	1.75	2.16	2.64	2.43	3.01	3.52	3.84	4.04
Viscosity	1.03	1.03	1.04	1.03	1.03	1.05	1.05	1.04	1.03
Sericin-peptone-NaOH.									
p_H	4.36	4.53	4.87	5.22	8.78	9.96	10.58	11.38	12.32
Viscosity	1.04	1.03	1.04	1.04	1.04	1.05	1.05	1.04	1.04

The viscosity of the sericin-peptone solution showed no variation with change of acidity. These results seem to show that the sericin-peptone solutions are much more highly dispersed or more nearly molecular than the sericin solutions.

Table XIII a (Fig. 6).

The specific conductivities of sericin-peptone-HCl solutions.

Total HCl conc. mol	K_i	Free HCl conc. (b)	K_f	K_s due to sericin-peptone HCl
0.05	2.168×10^{-2}	4.11×10^{-2}	1.734×10^{-2}	4.340×10^{-3}
0.025	8.333×10^{-3}	1.76×10^{-2}	7.427×10^{-3}	9.060×10^{-4}
0.0125	3.185×10^{-3}	6.90×10^{-3}	2.916×10^{-4}	2.730×10^{-4}
0.00625	1.195×10^{-3}	2.27×10^{-3}	9.579×10^{-3}	2.381×10^{-4}
0.008	1.841×10^{-3}	3.73×10^{-3}	1.574×10^{-4}	2.670×10^{-4}
0.004	6.674×10^{-4}	9.68×10^{-4}	4.085×10^{-4}	2.589×10^{-4}
0.002	2.867×10^{-4}	3.02×10^{-4}	1.275×10^{-4}	1.592×10^{-4}
0.001	1.515×10^{-4}	1.44×10^{-4}	6.080×10^{-5}	9.070×10^{-5}
0.0005	9.740×10^{-5}	9.20×10^{-5}	3.882×10^{-5}	5.858×10^{-5}
0	5.730×10^{-5}	—	—	5.730×10^{-5}

Table XIII b (Fig. 6).

The specific conductivities of sericin-peptone-NaOH solutions.

Total NaOH conc. mol	K_i	Free NaOH conc. (b)	K_f	K_s due to sericin-peptone NaOH
0.04579	8.700×10^{-3}	2.69×10^{-2}	5.919×10^{-3}	2.781×10^{-3}
0.025	4.161×10^{-3}	1.07×10^{-2}	2.354×10^{-3}	1.807×10^{-3}
0.0125	1.276×10^{-3}	3.09×10^{-3}	6.798×10^{-4}	5.962×10^{-4}
0.00625	4.183×10^{-4}	1.70×10^{-4}	3.736×10^{-5}	4.023×10^{-4}
0.008	6.101×10^{-4}	4.90×10^{-4}	1.077×10^{-4}	5.024×10^{-4}
0.004	2.500×10^{-4}	7.76×10^{-6}	1.708×10^{-6}	2.482×10^{-4}
0.002	1.173×10^{-4}	3.39×10^{-9}	7.454×10^{-10}	1.173×10^{-4}
0.001	8.620×10^{-5}	9.55×10^{-10}	2.100×10^{-10}	8.620×10^{-5}
0.0005	7.680×10^{-5}	4.37×10^{-10}	9.923×10^{-11}	7.680×10^{-5}

DISCUSSION.

The most characteristic property of sericin is that the isoelectric point lies further on the acid side than in the case of any other protein. The solubility-, viscosity-, and swelling-curves all point to a value near p_H 3.9. The experiments with the osmotic pressure, the combining capacity and the electrical conductivity also confirm the above statement, each being a minimum near that point.

As to the isoelectric point of sericin-peptone, there is a difference between the results obtained with the solubility in 25 % alcohol and with the osmotic pressure. In the former case the minimum occurs near p_H 3.9, in the latter

near p_H 4.4. Pauli [1922] observed also that the optimal precipitability of serum albumin and of gelatin by alcohol occurred at a little higher acidity than the minimum of the osmotic pressure. He explained this fact by assuming that the number of neutral particles of protein, which can be precipitated by alcohol, reaches a maximum at an acid concentration greater than the isoelectric point. But at the same time the osmotic pressure increases, because "the ionisation of $A^{n+}nCl^-$ (protein-chloride) gives rise to an increase in osmotic pressure which counteracts and finally exceeds the decrease caused by the formation of neutral particles." Besides this explanation it must be taken into consideration that the basic dissociation of an ampholyte like a protein is strongly suppressed in the presence of alcohol. Moreover, it has been confirmed that the greater the molecule of an ampholyte, the more it is possible to suppress the basic dissociation with diluted alcohol [Willstätter, 1922]. Therefore it may well be that the basic dissociation of sericin-peptone is diminished and the substance is more acidic even in so dilute an alcohol solution as 25 %. These considerations support the view derived from the experiment on the osmotic pressure that the true isoelectric point lies near p_H 4.4. It has been also asserted by many authors that the osmotic pressure of a protein solution can be mainly caused by the unequal distribution of electrolytes, which falls under the Donnan membrane equilibrium theory. If the protein molecule on one side of the membrane exists at its isoelectric point, there can be no unequal distribution of electrolytes. This was actually the case with sericin-peptone at p_H 4.4, where both the osmotic pressure and the unequal distribution vanished.

SUMMARY.

- (1) The extraction of sericin in the autoclave is proved to deprive the protein molecule of its precipitability by dilute acid, accompanied by the disappearance of the glyoxylic acid reaction for tryptophan.
- (2) A new method for the preparation of sericin is given.
- (3) Several physico-chemical properties of sericin-HCl, sericin-NaOH, sericin-peptone-HCl and sericin-peptone-NaOH were examined, and it was found that the isoelectric points of sericin and sericin-peptone exist near p_H 3.9 and 4.4 respectively.

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