[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

THE MOLECULAR WEIGHT OF EGG ALBUMIN. II. IN THE PRESENCE OF ELECTROLYTES[1,2]

BY J. B. NICHOLS

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The investigation of the molecular weight of egg albumin in salt-containing solutions to be reported in this paper is a continuation of the ultracentrifugal study of egg albumin described in 1926.[3] The experimental data were obtained in 1927, but certain points arose which were difficult to explain and which were thought to be due either to experimental error or to abnormalities in the behavior of the protein. The data were therefore set aside to await confirmation.

Although the sedimentation-equilibrium method had given very concordant results and had indicated that egg albumin was composed entirely of molecules 34,500 in weight the diffusion constant from the sedimentation-velocity runs, even in electrolyte-free solution, was only about six-tenths of the required value calculated from the molecular weight and the sedimentation velocity and was fairly irregular from run to run. On the other hand, the specific sedimentation velocity gave reasonable values throughout.

At first it was thought that a slight temperature inequality was present leading to a small convection current which made the diffusion constant appear smaller than it actually was but had no marked effect on the sedimentation velocity.[4] However, a more detailed study of other abnormal cases that have since appeared suggests that the egg albumin centrifugings were not in error and that the material had not undergone harmful decomposition. In a recent paper on Bence-Jones[5] protein a discussion has been given of these abnormalities in the diffusion constant. Since then more examples of abnormal diffusion have been met with. For many of the proteins thus far studied, the values of molecular weights obtained from the sedimentation-equilibrium and the sedimentation-velocity methods have agreed within the experimental error. This fact signifies that the molar frictional coefficient active in free diffusion is often the same as that effective in sedimentation—especially at the isoelectric point. However, hemo-

[1] Presented at the Minneapolis (September, 1929) meeting of the American Chemical Society.

[2] The author wishes to acknowledge the aid given him by E. I. du Pont de Nemours & Company, which rendered possible the completion of this work.

[3] Svedberg and Nichols, THIS JOURNAL, **48**, 3081 (1926).

[4] Cf. Rinde, "The Distribution of the Sizes of Particles in Gold Sols," Dissertation, Upsala, 1928, pp. 85–87.

[5] Svedberg and Sjögren, THIS JOURNAL, **51**, 3594 (1929).

cyanin[6] and cuprammonium cellulose[7] have shown abnormalities due to hindered diffusion in the more concentrated solutions, the first on account of its large size and the second probably because of its thread-like nature. This hindrance to diffusion caused by the too close proximity of the molecules in the more concentrated solutions disappears in sufficiently dilute systems. The same explanation does not suffice for egg albumin, Bence-Jones protein and some other proteins because the molecules are small compared with hemocyanin and regular compared with cuprammonium cellulose. Further consideration must be given to this problem before it will be possible to state with certainty what the cause is of the abnormally small diffusion of the proteins with small molecules.

On the other hand, the sedimentation velocity behaved normally for egg albumin and gave consistent results under conditions which have been found to produce normal behavior in other proteins: (1) sufficiently salt-free, stable solutions as prepared by electrodialysis; (2) well-buffered solutions in the neighborhood of the isoelectric point; (3) solutions containing buffering or neutral salts in quantity insufficient to cause "salting-out." Therefore only determinations of the sedimentation constant will be used to characterize the egg albumin in different ionic environments, but it is well to mention in passing that the value found for the diffusion constant of egg albumin at 30° is roughly 0.065 sq. cm./day or 0.076×10^{-5} sq. cm./sec., which is of the same order as Herzog's value,[8] 0.087×10^{-5} sq. cm./sec. when the latter is adjusted to the same temperature, 30°.

Experimental

The egg albumin used in the experiments was crystallized according to the method of Sørensen,[9] and dialyzed for fifteen days in flowing distilled water at 0°, saturated with toluene. The material was further purified previous to the experiments by electrodialysis at a current density of 0.3 ma./sq. cm., bringing down the conductivity of a 1% solution to 1.3×10^{-5} mhos at 18°. The solutions were brought to the desired concentration and salt content or P_H immediately before starting the centrifuge runs. In the neighborhood of the isoelectric point the partial specific volume was taken as 0.754 at 30° except in sodium chloride solutions, where $V = 0.749$.[10] The reason for this depressing action is not clear. Sodium chloride would be expected to show a dehydration effect but it is not evident that such an effect would produce the observed depression of the value of the partial specific volume.

Table I shows that the partial specific volume does not vary over an appreciable P_H range, in fact not until we reach strongly acid solutions.

All of the experiments were carried out in the high-speed oil-turbine type

[6] Svedberg and Chirnoagă, THIS JOURNAL, **50**, 1401 (1928).

[7] Stamm, *ibid.*, **52**, 3047 (1930).

[8] Herzog, *Z. Elektrochem.*, **13**, 539 (1907).

[9] Sørensen, *Medd. Carlsberg Lab.*, **12**, 12 (1917).

[10] The value of 0.749 for egg albumin solutions containing sodium chloride has also been obtained at the du Pont Experimental Station.

TABLE I

VARIATION OF PARTIAL SPECIFIC VOLUME IN DIFFERENT SOLVENTS AT 30°

Solvent	P_H of soln.	Partial sp. vol.
0.102 *N* HCl	1.16	0.759
0.010 *N* HCl	2.44	..
Acetate buffer 0.025 *N* with respect to sodium	3.6	.755[a]
0.02 *N* acetate buffer	4.7	.754
1.0% NaCl solution	4.75	.749
Electrolyte-free	4.67	.754[a]
Acetate buffer 0.025 *N* with respect to sodium	5.4	.754[a]
0.017 *M* primary–secondary phosphate buffer	7.3	.749

[a] These values are adjusted to 30° from a temperature of 19.2°.

of ultracentrifuge[11] with the exception of the one sedimentation-equilibrium experiment. As shown in previous communications the molecular weight is determined from the sedimentation equilibrium [3,12] by the relation

$$M = \frac{2\,RT \ln (c_2/c_1)}{(1 - V\rho)\,\omega^2\,(x_2 + x_1)(x_2 - x_1)} \qquad (1)$$

and from the sedimentation velocity[10,13] by the expression

$$M = \frac{RT\,s}{(1 - V\rho)D} \qquad (2)$$

R is the gas constant, T the absolute temperature, V the partial specific volume of the solute, ρ the density of the solvent, ω the angular velocity, c_2 and c_1 the concentrations at the distances x_2 and x_1 from the center of rotation, D the diffusion constant and $s = (dx/dt)\ (1/\omega^2 x)$, the specific sedimentation velocity or sedimentation constant.

Since, however, the diffusion constant for egg albumin is abnormal, Equation 2 is not valid as it stands, and the results will be left in terms of s, the sedimentation constant, which will be shown later to be equal to the value calculated from the molecular weight determined by sedimentation-equilibrium measurements.

The solutions were subjected to a centrifugal force approximately 100,000 times that of gravity. Exposure time was from twenty to forty seconds and the usual time of centrifuging was three to four hours. Pictures of the sedimenting system were taken every half hour. The length of column of solution used was from 13 to 15 mm. and the thickness 2 mm. All values refer to 1% egg albumin at 30°. The absorption band of egg albumin in the short ultraviolet region of the spectrum was employed in making the concentration determination in the centrifuge by isolating the wave length range 290–240 mμ from the mercury arc by means of gaseous chlorine and bromine filters. Imperial Process plates were used on account of their

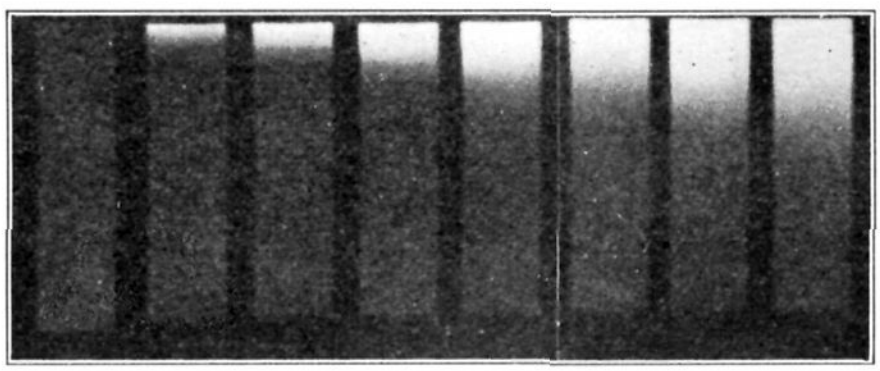
[11] Svedberg and Nichols, THIS JOURNAL, **49**, 2929 (1927).

[12] Svedberg and Fåhraeus, *ibid.*, **48**, 430 (1926).

[13] Svedberg and Lysholm, *Nova Acta Reg. Soc. Scient. Upsaliensis*, Vol. ex. ord., ed. 1927.

thin, uniform emulsion and were developed with Eclipse metol–hydroquinone developer.

Figure 1 gives a reproduction of the photographic record of the centrifuging of an acetate buffer solution of 1.0% egg albumin at a P_H of 4.7. This experiment was performed at 30° and at a speed of 41,900 r. p. m. corresponding to a centrifugal force of nearly 100,000 times that of gravity. The first exposure to the left shows the condition of the solution at the start of the run, and succeeding exposures show it after one-half, one hour, etc., up to three and one-half hours of centrifuging.



0 0.5 1.0 1.5 2.0 2.5 3.0 3.5

Hours.

Fig. 1.—Sedimentation of egg albumin at 4.7 P_H.

Table II contains typical data describing a centrifuge run. It refers to a 1.0% solution of egg albumin 0.0102 N with respect to hydrochloric acid and gives results from one of the three plates used in calculating the s-value for this solution.

TABLE II

SPECIFIC SEDIMENTATION VELOCITY OF EGG ALBUMIN IN 0.01 N HYDROCHLORIC ACID

Egg albumin concn., 1 g. per 100 cc.; HCl concn., 0.0102 N; P_H of solution, 2.44; T, 303.1°K; length of column, 1.39 cm.; thickness of column, 0.20 cm.; Imperial Process plates; short ultraviolet illumination (290–240 mμ region isolated from the mercury arc by gaseous chlorine and bromine filters); exposure time, 20 sec.; Eclipse metol–hydroquinone developer, 2 min.

Time interval, hr.	Δx, cm.	$x_{med.}$, cm.	Speed, r. p. m.	Centrifugal force $\omega^2 x$	Sp. sedimentation velocity, $s_{30°}$ cm./sec.
0.5–1.0	0.044	4.625	41,880	8.90×10^7	2.81×10^{-13}
1.0–1.5	.048	4.671	41,780	8.94	2.98
1.5–2.0	.044	4.717	41,650	8.98	2.69
2.0–2.5	.046	4.762	41,380	8.94	2.81
2.5–3.0	.048	4.809	41,370	9.03	2.89
3.0–4.0	.102	4.884	41,280	9.13	3.03
					Mean $s = 2.87 \times 10^{-13}$

Table III gives the values for the sedimentation constant obtained in the isoelectric region of the protein. In common with the findings on the majority of the proteins thus far studied, the addition of salts or buffer

solutions of the same P_H as the isoelectric point of the protein does not affect the molecular condition of the protein, as evidenced by the constancy of the sedimentation velocity. Furthermore, the mean value obtained for the sedimentation constant, 4.06×10^{-13} cm./sec., is nearly the same as the s-value for Bence-Jones protein.[14] Reduced to the same temperature, 20°, the sedimentation constant of egg albumin is 3.25×10^{-13} cm./sec., while that for Bence-Jones protein is 3.55×10^{-13} cm./sec.

TABLE III

SEDIMENTATION VELOCITY OF ISOELECTRIC AND OF SALT-FREE EGG ALBUMIN

Solvent	P_H of soln.	Speed, r. p. m.	Mean centrifugal force	Sp. sedimentation velocity, s_{20} in cm./sec.
Water	4.67	41,800	9.61×10^7	4.03×10^{-13}
Water	4.67	41,430	9.04	3.94
0.02 N acetate buffer	4.70	41,370	9.63	4.07
0.017 N acetate buffer	4.70	41,900	9.16	4.12
0.7% NaCl	4.73	41,290	9.12	4.15
			Mean s =	4.06×10^{-13}

By the sedimentation-equilibrium method both proteins have been found to possess a molecular weight of approximately 34,500. Using the s- and M-values determined, respectively, by the sedimentation-velocity method and by the sedimentation-equilibrium, we may determine whether these proteins consist of spherical molecules.[5] The molar frictional constant calculated from the sedimentation velocity was found to agree closely with that calculated for a substance of molecular weight 35,000 and possessing spherical molecules of the same specific volume as the two proteins. As mentioned in the article cited, the radius of the egg abumin molecule is 2.17 mμ as compared with 2.18 mμ for Bence-Jones protein.

In a recent paper on the osmotic pressure of egg albumin, Marrack and Hewitt[15] determined the molecular weight of egg albumin to be 43,000 in salt solutions as compared with the present determination of 34,500 both in salt-containing and in salt-free solution. Unpublished data tend to support their consideration that a small amount of impurity in constant amount might be formed in salt-containing solutions on standing. This small amount of material is perhaps the first step in the slight precipitation of denatured egg albumin that always occurs on standing. An electrodialyzed, salt-free solution of egg albumin is relatively stable. In the high-speed type of ultracentrifuge the whole experiment is completed in three or four hours after the addition of the salt or buffer, so there is much less chance for the formation of the approximately 5% of aggregated material required to give the average value of 43,000 found by the osmotic method that requires from one to two days to reach equilibrium. Under

[14] Svedberg and Sjögren, Ref. 4, p. 3603.

[15] Marrack and Hewitt, *Biochem. J.*, **23**, 1082 (1929).

conditions comparable to the osmotic method, however (*e. g.*, in the sedimentation-equilibrium method at ordinary temperatures, which requires two days of centrifuging), a change in the egg albumin frequently occurs, causing an increase in light absorption and the appearance of a small amount of aggregated material at room temperature.

As the isoelectric region is left, the sedimentation velocity seems to be decreased slightly more than the experimental error would account for.

TABLE IV

DEPRESSING EFFECT ON THE SEDIMENTATION CONSTANT IN ACID AND ALKALINE EGG ALBUMIN

Solvent	P_H of solution	Sp. sediment. velocity s in cm./sec.	s/s_0
0.34 N acetate buffer	3.53	3.59×10^{-13}	0.89
0.017 M double phosphate buffer	7.4	3.57×10^{-13}	.885

Table IV shows the possible slight depressing effect occurring in an egg albumin solution buffered at a P_H of 3.53 with 0.34 N acetate buffer and in a solution buffered at a P_H of 7.4 with 0.017 M primary–secondary phosphate buffer. The normal sedimentation velocity, s_0, has been reduced by nearly the same ratio, respectively, 0.89 and 0.885, in each case.

Table V gives evidence from the sedimentation equilibrum of 3.59 P_H egg albumin in 0.2 N acetate buffer, that the possible slight depression of the s-value in moderately acid egg albumin is not due to a slight Donnan effect since the buffer concentration is actually lower for the sedimentation-equilibrium run than for the sedimentation-velocity run. The value for the molecular weight is normal; therefore, it follows that the depression of the s-value did not arise from an electrical effect produced by a partial separation of charges in the centrifuge.

TABLE V

SEDIMENTATION EQUILIBRIUM OF 3.59 P_H EGG ALBUMIN IN 0.2 N ACETATE BUFFER

Concentration, 0.97 g./100 cc.; speed, 10,900 r. p. m. ($\omega = 363.3\pi$); mean centrifugal force, 5.84×10^6 dynes; length of column, 0.53 cm.; $V = 0.749$ at 18°; $T = 291°$; density of solution, 1.0019; exposure times 29, 36.5, 41.5 hours after the start; lengths of exposure, 20, 40, 80 and 160 seconds; Imperial Eclipse Plates, metol developer, 3 minutes' development

Distances, Cm. x_2	x_1	Mean concn. % c_2	c_1	Number of exposures	M
4.68	4.63	1.555	1.397	8	34,800
4.63	4.58	1.397	1.266	7	32,200
4.58	4.53	1.266	1.144	7	33,750
4.53	4.48	1.144	1.041	9	31,600
4.48	4.43	1.041	0.943	7	33,500
4.43	4.38	0.943	.849	8	35,750
4.38	4.33	.849	.772	8	33,100
4.33	4.28	.772	.701	8	33,950
4.28	4.23	.701	.635	7	35,000
				Mean	33,740

Repression of Donnan Effect.—If we add acid or alkali to a salt-free protein and subject the solution to a high centrifugal force, a partial separation is produced of the large protein ion from the small inorganic ions of opposite sign. An electrical potential acting in opposition to the centrifugal potential is thereby set up similar in character to the Donnan membrane potential. In the simplest case—no foreign ions present—which we very likely approximate for unbuffered solutions, the effect of the electrical potential is to reduce the true value of the molecular weight by the factor[16] $(n + 1)$ or

$$M = \frac{(n + 1)RT\,s}{(1 - V\rho)\,D} \tag{3}$$

where n is the mean valence of the protein ion. Thus it is possible to gain some information about the mean valence of a protein by first centrifuging in unbuffered acid or alkaline solution and then centrifuging another sample to which has been added sufficient electrolyte to repress the electrical potential to a negligible value. The latter determination is made chiefly for the purpose of showing that no actual change has occurred in the molecular weight at the given P_H value.

In order to give some idea of the action of the Donnan effect on the diffusion constant as well as on the sedimentation constant, the values of D as well as those of s are given in Table VI. It is evident that the electrical effect produced by the separation of the ions has a much greater effect on the diffusion constant than it has on the sedimentation constant. The values of D given in the table should be considered only apparent as mentioned earlier, but they at least illustrate the action of the Donnan effect. No attempt has been made to calculate the valence of the protein ion from the changes produced in the D and the s-values by the free acid or alkali because relatively minute quantities of decomposition products largely repress the true Donnan effect. For the 0.01 N HCl solution, the amount of free acid present amounts to approximately 36% of that added, thus

TABLE VI

REPRESSION OF DONNAN EFFECT

Expt.	Solvent	P_H of solution	Apparent diffusion constant, $D_{30°}$, sq. cm./day	Sedimentation constant, $s_{30°}$ cm./sec.
1	0.001 N HCl	3.94	0.111	3.19×10^{-13}
2	0.34 N acetate buffer	3.53	.061	3.59[a]
3	0.01 N HCl	2.44	.103	2.86
4	0.01 N HCl + 0.7% NaCl	2.68	.069	4.01
5	0.001 N NaOH	5.13	.144	2.85
6	0.1 N acetate buffer	5.44	.061	3.96

[a] s depressed by hydration, possibly.

[16] Svedberg, *Kolloid-Z.* (Ergänzungsband) **36**, 63, 64 (1925); Nichols, "Sixth Colloid Symposium," **1928**, p. 298.

accounting for the extremely small change from 0.001 N HCl solutions to 0.01 N HCl solutions. The apparently greater effect in alkaline solution is in line with the higher osmotic pressure found by Loeb[17] on the alkaline side of the isoelectric point as compared with that on the acid side.

Figure 2 represents the variation of the sedimentation constant with P_H for unbuffered solutions compared with the variation in osmotic pressure over the same range found by Loeb.[17] The agreement of maxima and minima is fairly good. On the acid side of the isoelectric point the maximum osmotic pressure occurs at a P_H of 3.4. It probably agrees within experimental error with the minimum sedimentation constant which occurs

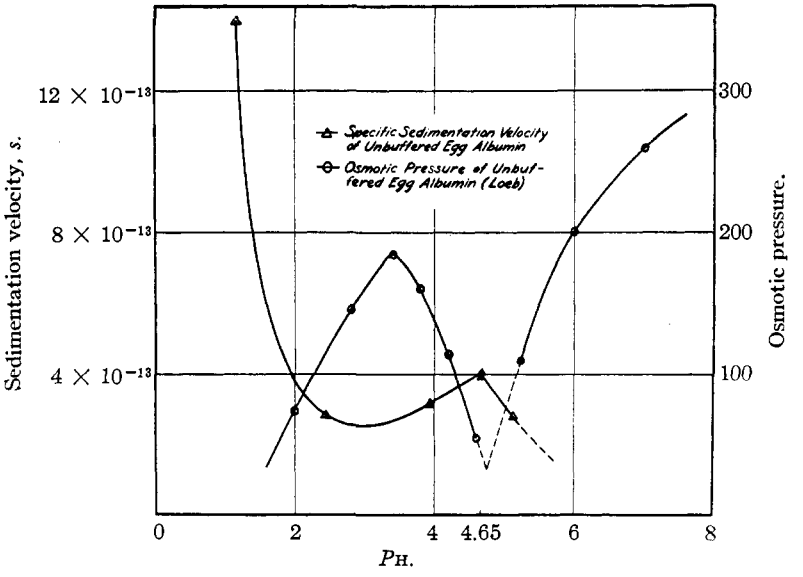


Fig. 2.—Donnan effect on sedimentation velocity of egg albumin.

at a P_H of 3.0 approximately; the minimum osmotic pressure in the isoelectric region appears to be at a slightly higher P_H than the maximum sedimentation constant, which occurs at a P_H of 4.65, corresponding closely to Tiselius' value of 4.6 from cataphoresis measurements. The significance of the rapid increase of the sedimentation constant below a P_H of 2 will be explained further on. It should also be noticed that both the s-values and the osmotic pressures experience a greater change on the alkaline side than on the acid side.

Figure 3 shows the variation of the sedimentation constant with P_H for buffered and unbuffered solutions of egg albumin. The s-value for buffered solutions as drawn appears to be nearly constant over a considerable

[17] J. Loeb, "Proteins and the Theory of Colloidal Behavior," McGraw-Hill Book Co., New York, 1st ed., **1922**, p. 75, Fig. 18 and p. 118, Fig. 39.

range but may decrease slightly (possibly owing to hydration if this decrease is subsequently verified) on the acid side of the isoelectric point, return to the normal value found for the isoelectric region at a P_H of about 2.4 and then join the upward arm of the unbuffered curve at some point; that is, denaturation may be taking place to an increasing extent from a P_H of 2.5 down.

Acid-Denaturation.—When the egg albumin is made still more acid, 0.102 *N* HCl solution, P_H 1.16, it is evident (Fig. 3) that a profound change occurs. In a solution of 0.1 *N* HCl the protein grows more and more viscous on standing and the opalescence increases as well. A 4.0% solution

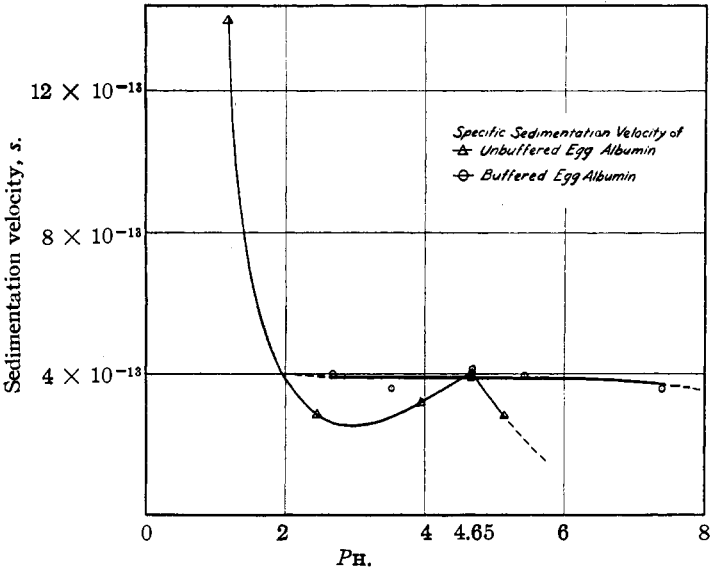


Fig. 3.—Repression of Donnan effect on sedimentation velocity of egg albumin by addition of salts.

of egg albumin sets in a few hours to a stiff gel. In a 1.0% solution the viscosity at 30° increases from 0.00853 to 0.01057 in eleven hours. The solution centrifuged was considered to have an age of three hours or a viscosity of 0.00908 at 30°.

The extreme difference in character of the concentration curves for the egg albumin in 0.1 *N* HCl solution from those obtained for egg albumin in isoelectric buffer is shown in Fig. 4. The time interval between successive curves was half an hour in each case and the experimental conditions were practically the same for the two solutions. There was some increase in light absorption in the highly acid protein and about 10% of uncentrifugible material was present. The rapid sedimentation indicates a rather large increase in size of the effective particle; yielding a rough *s*-value of 14.0 ×

10^{-13} cm./sec. as compared with the normal value of 4.0×10^{-13} for a molecular weight of 34,500. The protein has evidently been denatured by the strong acid and has aggregated to gel clumps. Any Donnan effect may be neglected on account of the large excess of hydrochloric acid present. Diffusion should also be small enough to be neglected after incipient gelling has set in because of the increase in size of the effective particle, the increase in the gross and the structural viscosity and the relative inaccuracy of the calculations on a changing system.

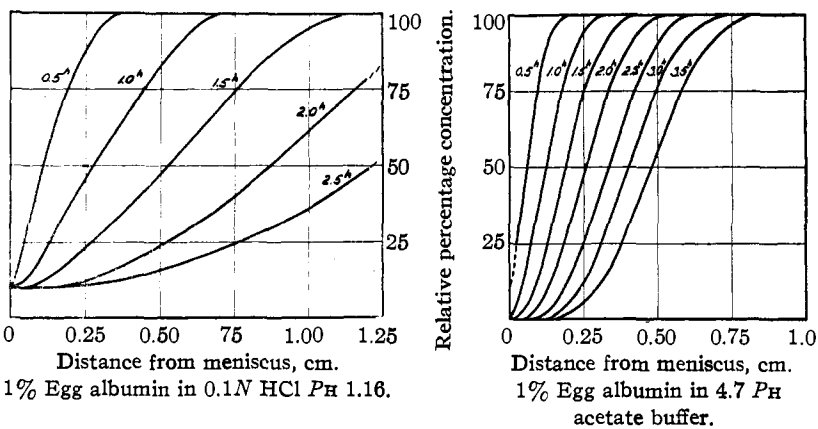


Fig. 4.—Sedimentation curves of acid-denatured and isoelectric egg albumin.

The distribution curve calculated for the run is given in Fig. 5 for the exposures taken after 60, 90, 120 and 150 minutes of centrifuging. The gradual shift of the points obtained from the successive exposures in the direction of larger radii indicates that the system was changing throughout the whole period. Another possible source of error in the calculation of the distribution curve lies in the assumption of a constant light absorption over the range of sizes of particles present. The distribution curve corresponding to thirty minutes of centrifuging is dotted in to indicate that it is far from correct since the limit of the smaller particles comes at such a small radius. It is probable that diffusion was still playing some part at this stage of the gelling and thus that the system was changing the most rapidly during the first hour. The number of molecules per particle is also plotted to give an idea of the range of "molecules" present in the gel clumps formed of the denatured egg albumin.

Thus we see that egg albumin is moderately stable until fairly high acidities and alkalinities are reached and exhibits a strong Donnan effect in the intermediate acidity and alkalinity range if the ionization of the protein is allowed to exert its maximum effect in unbuffered solutions.

A careful investigation of the behavior of egg albumin in the region of

high acidities should throw much light on the acid-denaturation of proteins and on gelling phenomena. Similarly, a study of the conditions at high alkalinities will prove of interest because no information has been gained by osmotic methods in this region on account of the action of strong alkali on the membranes.

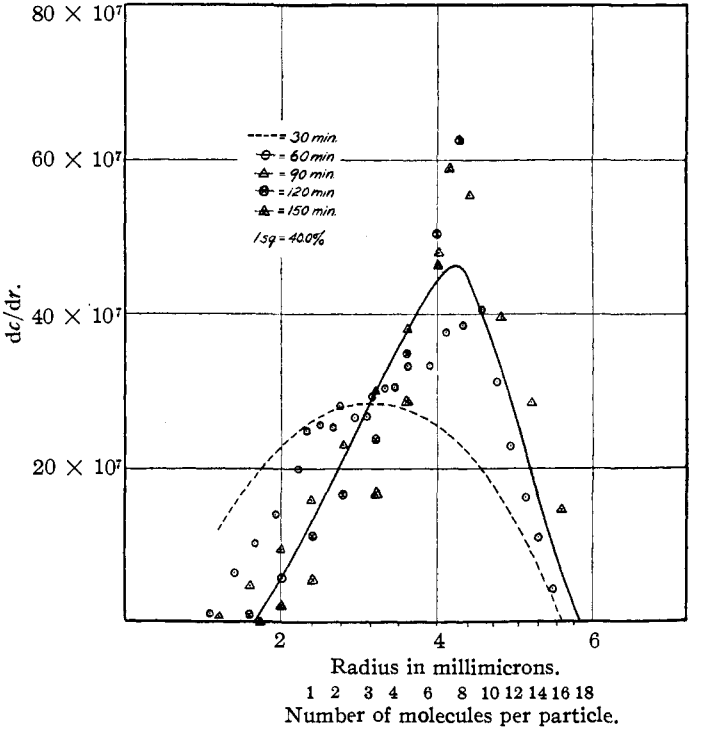


Fig. 5.—Distribution curve for egg albumin in 0.1*N* HCl.

The expenses connected with these experiments have been defrayed by grants from the Nobel Fund of Chemistry and from the foundation "Therese och Johan Anderssons Minne."

Summary

1. Crystallized, electrodialyzed egg albumin has a sedimentation constant of 4.06×10^{-13} cm./sec. at 30° but the diffusion constant is abnormal, approximately six-tenths of the required value on the basis of the sedimentation constant and the molecular weight.

2. The molecule is spherical and has a radius of 2.17 mμ; it is practically identical in size and mass with that of Bence-Jones protein, although entirely different in chemical composition.

3. The depression of the sedimentation constant arising from the Donnan effect in unbuffered solutions on either side of the isoelectric point of

egg albumin has been investigated; the maximum depression occurs in the neighborhood of 3.0 P_H.

4. At a P_H of 1.16 in 0.1 N hydrochloric acid, egg albumin is completely denatured and gradually forms a gel showing a distribution curve of gel clumps; the mean size corresponds to about seven molecules per particle after three hours.

WILMINGTON, DELAWARE

[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

THE P_H-STABILITY REGION OF EGG ALBUMIN

BY BERTIL SJÖGREN AND THE SVEDBERG

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An ultracentrifugal study of the molecular weight of egg albumin in electrolyte-free condition near its isoelectric point was carried out by Svedberg and Nichols in 1926.[1] This protein when properly purified was found to be homogeneous with regard to molecular weight. The value arrived at, 34,500 ± 1000, is in good agreement with the result of Sørensen's osmotic measurements, *viz.*, 34,000.[2] In 1927 Nichols made a series of determinations of the molecular weight and the sedimentation constant of egg albumin in the presence of electrolytes within the P_H region 1.2–7.4.[3] His measurements showed that egg albumin is probably stable from P_H 3 to 7. The value of the sedimentation constant arrived at was 4.06×10^{-13} at 30°, which corresponds to 3.31×10^{-13} at 20°. In acid solution (P_H 1.16) this protein was found to be completely denatured, gradually forming a gel and showing a distribution curve of gel clumps, the mean size of which corresponded to about seven molecules per particle after three hours.

Since the time when Nichols' determinations were carried out, the ultracentrifugal technique has been further developed and it was, therefore, considered of importance to supplement his measurements by a new and more detailed study of the behavior of egg albumin within a wider P_H range.

Preparation of Material and Light Absorption

The egg albumin was prepared according to Sörensen's method[2] with some slight modifications. The material was crystallized three times, the isoelectric state of the crystallizing liquid being checked directly by means of P_H determinations. The crystals were dissolved in water, dialyzed in the ice box against water and finally electrodialyzed; concentration of stock solution 5.25%. At the time when the determinations were made the material was about three months old.

[1] T. Svedberg and J. B. Nichols, THIS JOURNAL, **48**, 3081 (1926).

[2] S. P. L. Sørensen, *Medd. Carlsberg Lab.*, **12**, 348 (1917).

[3] J. B. Nichols, THIS JOURNAL, **52**, 5176 (1930).