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.

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[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).

Some measurements were also carried out on a similar material which had been kept in the ice box for sixteen months.

The former material did not show any decomposition when submitted to the ultracentrifugal analysis, whereas the latter substance was found to be decomposed to a considerable extent.

The light absorption of solutions of egg albumin at three different hydrogen-ion concentrations was measured by means of the Judd-Lewis

TABLE I

LIGHT ABSORPTION OF SOLUTIONS OF EGG ALBUMIN

	M	Solvent	M	P_H of soln.	Wave length of max. mμ	Wave length of min. mμ	ϵ/c at max.	ϵ/c at min.
HCl	0.007	KCl	0.093	2.2	279	253	8.5	5.0
KH_2PO_4	.095	Na_2HPO_4	.005	5.5	280	255	5.7	2.7
Na_2HPO_4	.026	NaOH	.014	11.2	280	254	10.8	5.6

spectrophotometer as described in previous communications. The thickness of layer was 2.0 cm. in all measurements and the protein concentration 0.10% at P_H 2.2 and 5.5 but 0.05% at P_H 11.2. In Table I the position of the maximum and the minimum of absorption and the values of the extinction coefficients for the maximum and the minimum are recorded. Fig. 1 gives the absorption curves.

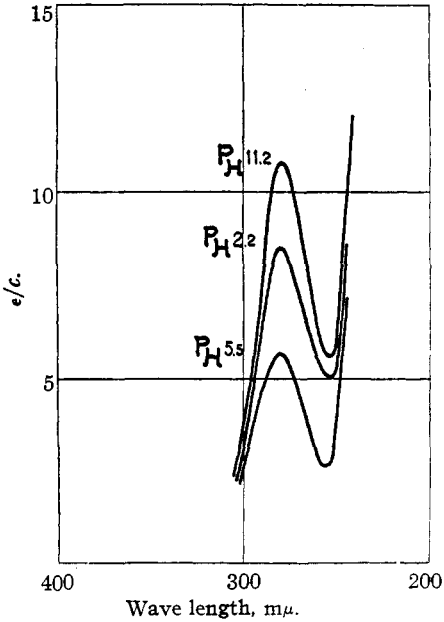

Fig. 1.

As seen from the table the position of the maximum and the minimum does not change with P_H. The value of the extinction coefficient, however, is much higher in the acid and alkaline solutions than in the solution of P_H 5.5. The ultracentrifugal analysis described below gives the result that at P_H values lower than 4 and higher than 9 some of the egg albumin molecules are broken up into a non-centrifugible substance. The increase in light absorption is probably due to the presence of this decomposition product.

Determination of the P_H-Stability Region.—A number of sedimentation velocity runs were made in the oil-turbine ultracentrifuge as described in previous communications. The speed was 40,000–45,000 r. p. m., the temperature in the solution 20–23° and the time of centrifuging three to

five hours. The solutions studied were brought to the desired P_H immediately before starting the run. In Table II and Fig. 2 the results are summarized.

TABLE II

EGG ALBUMIN, SEDIMENTATION VELOCITY MEASUREMENTS

Solvent				P_H of soln.	Concn. of protein, %	$s_{20°} \times 10^{13}$ cm./sec.	Non-centrifugible products, %
	M		M				
HCl	0.095	KCl	0.005	1.1	0.25	6.60	55
HCl	.046	KCl	.054	1.4	.20	5.20	35
HCl	.007	KCl	.093	2.15	.20	3.98	10
HAc	.017	NaAc	.003	3.80	.20	3.65	0
HAc	.01	NaAc	.01	4.61	.18	3.43	0
KH_2PO_4	.095	Na_2HPO_4	.005	5.5	.20	3.39	0
KH_2PO_4	.095	Na_2HPO_4	.005	5.5	.45	3.61	0
KH_2PO_4	.095	Na_2HPO_4	.005	5.5	.45	3.53	0
KH_2PO_4	.03	Na_2HPO_4	.03	6.74	.15	3.59	0
KH_2PO_4	.03	Na_2HPO_4	.03	6.74	.15	3.67	0
KH_2PO_4	.005	Na_2HPO_4	.062	7.76	.18	3.37	0
NaOH[a]	.0007	Na_2HPO_4	.03	8.67	.20	3.66	0
NaOH[a]	.001	Na_2HPO_4	.032	9.16	.20	3.77	0
NaOH[a]	.005	Na_2HPO_4	.03	10.28	.12	3.39	20
NaOH[a]	.014	Na_2HPO_4	.026	11.16	.12	3.45	25
NaOH[a]	.017	Na_2HPO_4	.05	11.32	.11	3.31	30
NaOH[a]	.036	Na_2HPO_4	.01	12.46	.07	2.91	40
NaOH[a]	.05			12.63	.05	2.03	50

[a] 1% in NaCl.

The sedimentation constant is independent of P_H within the range 3 to 11 with a mean value of 3.54×10^{-13} at 20°, which is slightly higher than

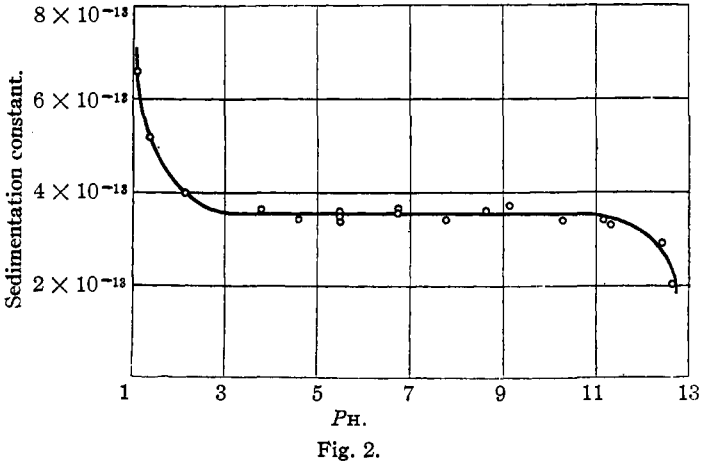

Fig. 2.

the value 3.31×10^{-13} calculated from Nichols' determination at 30°. Downward from a P_H of 4 and upward from a P_H of 9 the egg albumin

molecules begin to dissociate into low-molecular decomposition products. The stability region is, therefore, somewhat smaller than the range within which normal molecules still exist. At high acidities the sedimentation rises rapidly owing to the formation of condensation products as already observed by Nichols. At high alkalinities the sedimentation drops, indicating the breaking up of the protein molecule.

In order to ascertain whether the centrifugible part of the material was homogeneous with regard to molecular weight at the ends of the P_H-stability range, two sedimentation equilibrium runs were made at P_H 4.0 and 9.2. In Table III the result is recorded.

TABLE III

EGG ALBUMIN, SEDIMENTATION EQUILIBRIUM MEASUREMENTS

Solvent	P_H of soln.	Molecular weight
0.016 M in HAc .004 M in NaAc	4.0	33,800, no drift in the values with distance from center of rotation
.032 M in Na_2HPO_4 .001 M in NaOH 1% in NaCl	9.2	34,600, no drift

A sedimentation equilibrium run performed by Nichols at a P_H of 3.59 gave the value 33,700 for the molecular weight and likewise no drift with distance from center of rotation. It is obvious, therefore, that the centrifugible material is quite homogeneous at the ends of the stability region.

The homogeneity of a sample of egg albumin which had been kept in electrolyte-free solution with toluene as a preservative for sixteen months in the ice box was tested at a P_H of 5.5 in a sedimentation velocity run and a sedimentation equilibrium run. The sedimentation constant had increased to about 4×10^{-13} cm./sec. The molecular weight showed strong drift with distance from the center of rotation, the values ranging from 18,100 to 52,000. From these measurements it follows that egg albumin undergoes decomposition and aggregation with time. The same material when new had given a sedimentation constant of 3.4×10^{-13} cm./sec. (O. Lamm).

Discussion of Results

It is of interest to compare the ultracentrifugal behavior of egg albumin with that of Bence-Jones protein using the new value for the sedimentation constant recorded above. As already pointed out by the present authors in a previous communication,[4] egg albumin and Bence-Jones protein are closely related with regard to molecular weight, sedimentation constant s, molar frictional constant f and molecular radius r, the said constants being identical within the limits of experimental error. In Table IV the values of these constants are recorded, the new s-value for egg albumin being used for the calculation; f_s denotes the value of the molar frictional constant to be expected for a spherical molecule of the same density.

[4] T. Svedberg and B. Sjögren, THIS JOURNAL, 51, 3594 (1929).

TABLE IV

MOLECULAR CONSTANTS FOR EGG ALBUMIN AND BENCE-JONES PROTEIN (20°)

Protein	Mol. wt.	$s \times 10^{13}$	$f \times 10^{-16}$	$f_s \times 10^{-16}$	r, mμ.
Egg albumin	34,500	3.54	2.47	2.47	2.17
Bence-Jones protein	35,000	3.55	2.48	2.49	2.18

The coincidences borne out by Table IV are even closer than could be expected when taking into account the probable experimental errors of the data. The P_H-stability regions of egg albumin and Bence-Jones protein are similar but show some distinct differences. Egg albumin has a somewhat wider stability range than Bence-Jones protein, the stability extending further into the acid as well as into the alkaline regions. The acid decomposition of Bence-Jones protein consists in the splitting-up of some of the molecules into a non-centrifugible substance, leaving some molecules intact, while the decomposition of egg albumin in acid solution is accompanied by the formation of a highly aggregated and rapidly sedimenting substance. In electrochemical respect egg albumin and Bence-Jones protein are quite different, the former having its isoelectric point at P_H 4.6 and the latter at 5.2 according to recent determinations by A. Tiselius. The two proteins, although not separable by centrifuging, may easily be separated by means of cataphoresis.

The expenses connected with these experiments have been defrayed by a grant from the foundation "Therese och Johan Anderssons Minne."

Summary

1. The ultracentrifugal methods have been applied to the study of the P_H-stability region of egg albumin.

2. In the P_H-range 4–9 the protein is stable and homogeneous with regard to molecular weight. The mean value 34,200 is in good agreement with the previous determination by Svedberg and Nichols, *viz.*, 34,500.

3. Below P_H 4 and above 9 some of the molecules are split up into a non-centrifugible substance. The sedimentation constant of the centrifugible material is independent of P_H within the range 3–11 with a mean value of 3.54×10^{-13} at 20°. The molar frictional constant is 2.47×10^{16} at 20°, which value is identical with the molar frictional constant calculated for a spherical molecule of the same molecular mass and density. The egg albumin molecule, therefore, is spherical and has a radius of 2.17 mμ.

4. At P_H values lower than 3, the sedimentation increases, indicating the formation of aggregates of denatured protein; at P_H values higher than 9 the sedimentation decreases, indicating the breaking up of the whole material. Both the acid and the alkaline decomposition is accompanied by an increase in the light absorption.

5. A comparison of egg albumin and Bence-Jones protein using the new value of the sedimentation constant for egg albumin shows a still closer

agreement than was formerly found for the molecular constants of these proteins.

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[A COMMUNICATION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

THE HYDROGENATION OF ACETOACETIC ESTER AND CERTAIN OF ITS DERIVATIVES OVER NICKEL

BY HOMER ADKINS, RALPH CONNOR AND HOWARD CRAMER

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The hydrogenation of acetoacetic ester in the liquid phase with a nickel catalyst has apparently not previously been investigated although Sabatier and Mailhe[1] obtained ethyl butyrate, acetone, isopropyl alcohol, propionic and dehydroacetic acids in a vapor phase reduction over nickel. The methods used in the present investigation were essentially the same as those previously described.[2]

Acetoacetic ester absorbed one mole equivalent of hydrogen when reduced over a nickel catalyst. There were produced small quantities of alcohol and dehydroacetic acid, two parts of ethyl β-hydroxybutyrate and one part of a derivative of this ester, ethyl β-(β′-hydroxybutyryloxy)-butyrate, $CH_3CHOHCH_2CO_2CH(CH_3)CH_2COOC_2H_5$. The ratio of the mono-ester (I) to the di-ester (II) when the reduction was carried out in various solvents is as indicated: no solvent 67% I, 33% II; in ethanol 100% I; in diethyl ether 68% I, 32% II; in methylcyclohexane 68% I, 32% II; in ethyl β-hydroxybutyrate 69% I, 31% II; in *tert.*-butyl alcohol 83% I, 17% II; in *n*-butanol 66% I and 34% *n*-butyl β-hydroxybutyrate, b. p. 96–97 (13 mm.). Ethyl α-methylacetoacetate upon reduction gave a mono- and di-ester (ethyl α-methyl-β-(α′-methyl-β′-hydroxybutyryloxy)-butyrate), in the ratio of approximately two parts by weight of the latter to five parts of ethyl α-methyl-β-hydroxybutyrate. However, the dialkyl substituted derivatives of acetoacetic ester, ethyl α,α-dimethylacetoacetate and ethyl α-methyl-α-ethylacetoacetate, gave quantitative reduction to the corresponding α,α-dialkyl-β-hydroxybutyric ester. The formation of the di-esters may be avoided by the use of ethanol, for a quantitative yield of ethyl α-methyl-β-hydroxybutyrate was obtained by reducing ethyl α-methylacetoacetate in that solvent.

The salient facts seem to be: (1) the two beta ketonic esters capable of enolization upon hydrogenation in the absence of a solvent or in the presence of the ethyl-β-hydroxybutyrate or of ether or methylcyclohexane gave large amounts of di-esters along with the simple hydroxy ester.

[1] Sabatier and Mailhe, *Bull. soc. chim.*, [4] **3**, 232 (1908).

[2] Adkins and Cramer, THIS JOURNAL, **52**, 4349 (1930).