less than 0.6. Among the ketones 2-pentadecanone was too low by 1.9 while half of them deviated by 0.4 or less. The B. P. N. of decanoic acid was found to be farthest from the calculated and was 1.57 while half of the acids examined had deviations less than 0.4 unit. Among the esters methyl caprate was too low by 2.28 and half deviated by as much as 0.4 unit.

The amines, cyanides, and isocyanides have been studied among the aliphatic nitrogen compounds. The b. p. n.'s. for the various substitution products containing these characteristic groups may also be found in Table II. Using those values satisfactory results are obtained with the exception of tri-*n*-heptylamine for which a boiling point about 20° too low has been reported. This seems entirely out of line with the other tertiary amines and further investigation may show that a value nearer 350° is correct. The amine that gave the next widest variation from the calculated was di-(α-methylheptyl)-amine with 2.32. It also is high boiling and its boiling point may have been determined incorrectly. With at least half of the amines the deviation amounts to less than 0.4 unit. Among the alkyl cyanides decane nitrile gave the greatest variation of 1.42, while over half of them deviated less than 0.3. Out of ten isocyanides ethyl isocyanide deviated the most with 0.94 while half deviated 0.6 or less.

Other groups of compounds are being studied in the same way and a general survey of the field has been contemplated. The author will welcome data, suggestions, and criticism in the hope that the new method will become a particularly useful tool in organic chemistry and that many new relationships affecting the behavior of compounds will be the result. In developing the method the author is especially indebted to the late Professor E. P. Kohler and to Professors L. Pauling and H. Gilman for many valuable suggestions and encouragement.

Summary

A method has been devised for correlating the structure of organic compounds with their boiling point. Boiling point numbers for a variety of atoms, groups, and molecules have been obtained and the accuracy discussed. Methods for calculating the boiling point of a compound from its structure and the structure from the boiling point have been outlined. The combined effect of several groups in the molecule upon the boiling point has been discussed.

SALT LAKE CITY, UTAH RECEIVED JUNE 9, 1938

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY AND FROM THE DEPARTMENT OF PEDIATRICS, HARVARD MEDICAL SCHOOL]

The Viscosities of Solutions of the Proteins of Horse Serum[1]

BY KATHLEEN R. FAHEY AND ARDA ALDEN GREEN

The viscosity of solutions of proteins is known to vary widely with the character of these solutes. Some, in sufficiently dilute solution, obey approximately the classical Einstein law relating viscosity and concentration. Thus Loeb,[2] in studying egg albumin, observed agreement with the Einstein equation; and Daniel and Cohn[3] noted that "the viscosity of such a protein as egg albumin is not very different from that of glycine when both solutes occupy the same volume fraction of solution. . . . Hemoglobin and serum albumin, whose molecules—according to Svedberg—are larger and not quite spherical, are, however, more viscous than demanded by the coefficient of Einstein's equation. None the less, hemoglobin like egg albumin obeys Poiseuille's law relating viscosity to pressure. A great many protein molecules are, however, highly asymmetrical in shape, and give rise both to anomalous viscosity and to double refraction of flow, in this respect resembling the long chain polymers studied by Staudinger and his collaborators."

The serum protein fractions as then separable were studied carefully by Harriette Chick[4] twenty-five years ago. She investigated their viscosities as affected by temperature and by concentrations of protein, salt and hydrogen ions. She reported, "In all cases, increase in protein concentration is accompanied by a disproportionately great in-

(1) This study was supported in part by a grant from the Commonwealth Fund of New York.

(2) Loeb, *J. Gen. Physiol.*, **4**, 73 (1921-1922).

(3) Daniel and Cohn, THIS JOURNAL, **58**, 415 (1936).

(4) H. Chick, *Biochem. J.*, **8**, 261 (1914); H. Chick and E. Lubrzynska, *ibid.*, **8**, 59 (1914).

crease in the viscosity of the solution. The effect is greatest in case of euglobulin, solutions of which exhibit a high viscosity at a comparatively low protein content. It is least in case of serum albumin, which, for strengths of protein under about 10% behaves almost as a crystalloid. Pseudoglobulin is intermediate between the other two proteins in this respect."

Differences in viscosity of the various serum protein fractions are so great as to be determined readily, and are presumably of significance in regard to the size and shape of the molecules.

Three isoelectrically precipitable globulins, designated PI, PII and PIII, have been separated from normal horse serum.[5] Two of these, PI and PIII, have isoelectric points close to *p*H 5 and one, PII, close to *p*H 6. Since these globulin fractions differ in their method of preparation from those of Chick and other investigators, it was thought advisable to investigate their viscosities and also those of the remaining soluble serum proteins.

Experimental

The isoelectrically precipitable globulins PI, PII and PIII were prepared in the manner described in a previous communication.[5] In some instances the identical preparations were used. The essential characteristic of this method of separation of serum globulins is isoelectric precipitation in a very low concentration of salt.

The proteins precipitated from normal horse serum in solutions one-half saturated with ammonium sulfate were dialyzed against water. After dialysis dilute hydrochloric acid, approximately 0.01 *N*, was added cautiously until a precipitate formed, usually at *p*H 6.5. This precipitate was removed by centrifugation. It contained PIII as well as PII, for although the isoelectric point of PIII is close to *p*H 5, this fraction is nevertheless precipitated with PII at *p*H 6.5 because it is so very insoluble. These globulins were separated from each other by solution in acid and fractional reprecipitation with alkali. To the solution obtained after removal of PII and PIII dilute hydrochloric acid was added again until the *p*H was close to 5, and a certain amount of PI was isoelectrically precipitated. Dilution with water, further dialysis or reprecipitation with ammonium sulfate followed by dialysis brought down a further quantity of PI.

At least three or four solutions in alkali or acid and isoelectric reprecipitations were carried out on each fraction before the proteins were considered to approach homogeneity. The protein remaining in solution after all water insoluble proteins are removed by dialysis and electrodialysis from the total globulin fraction obtained by repeated precipitation with ammonium sulfate at one-half saturation was termed pseudoglobulin.

Since the euglobulins are insoluble at their isoelectric points, viscosities must be determined either in the presence of salt or at a reaction away from the isoelectric point. To obtain solutions in 0.5 *M* saline, precipitates were first dissolved in a small amount of sodium hydroxide; sodium chloride solution was then added, followed by an amount of hydrochloric acid equivalent to the amount of sodium hydroxide used to dissolve the precipitate. To prepare solutions in alkali, dialyzed suspensions of the precipitates in distilled water were treated with the minimum amounts of 0.1 *N* sodium hydroxide to bring the precipitates completely into solution. The resultant *p*H of the PIII solutions was 7.0 and 7.1, of the PII solution 8.0, and of the PI

TABLE I

VISCOSITY OF SOLUTIONS OF PROTEINS OF NORMAL HORSE SERUM

Concn. g./liter	Density ρ	Relative viscosity $\eta/\eta_0 - 1$	Concn. g./liter	Density ρ	Relative viscosity $\eta/\eta_0 - 1$
Globulin PI					
Prepn. 1 at *p*H 6.5 in H_2O			Prepn. 2 at *p*H 5 in 0.5 *M* NaCl		
5.5	0.9983	0.053	10.2	1.0197	0.120
11.4	.9997	.126	20.3	1.0226	.280
16.8	1.0013	.204	30.5	1.0245	.465
22.1	1.0026	.290	40.6	1.0272	.673
27.6	1.0041	.387	50.8	1.0298	.999
Globulin PII					
Prepn. 1 at *p*H 8.0 in H_2O			Prepn. 2 at *p*H 6.4 in 0.5 *M* NaCl		
10.1	0.9999	0.155	2.8	1.0182	0.036
21.0	1.0031	.384	5.7	1.0193	.083
31.0	1.0058	.758	8.5	1.0199	.110
40.8	1.0085	1.400	11.4	1.0207	.160
51.0	1.0116	2.641	14.2	1.0216	.213
Globulin PIII					
Prepn. 1 at *p*H 7.1 in H_2O			Prepn. 2 at *p*H 7.0 in H_2O		
5.8	0.9983	0.125	5.0	0.9983	0.109
12.0	.9997	.267	10.4	.9999	.252
17.7	1.0010	.630	15.3	1.0009	.429
23.3	1.0023	1.224	20.2	1.0023	.716
29.1	1.0036	2.171	25.2	1.0039	1.023
Pseudoglobulin					
Prepn. 1 at *p*H 6.0			Prepn. 2[6]		
20.2	1.0037	0.195	8.2	0.999	0.06
28.7	1.0055	.310	15.0	1.000	.14
35.8	1.0076	.411	28.0	1.003	.30
44.8	1.0101	.570	55.8	1.009	.82
Serum Albumin[8]					
Prepn. 1			Prepn. 2		
29.4	1.0083	0.16	8.35	1.0023	0.046
58.8	1.0166	.37	20.96	1.0059	.116
117.6	1.0438	1.18	41.92	1.0116	.246
235.2	1.0665	9.35	104.80	1.0288	.868
			139.73	1.0394	1.568
			209.60	1.0593	5.191

(5) A. A. Green, THIS JOURNAL, **60**, 1108 (1938).

(6) Published through the courtesy of J. D. Ferry, who made the determinations in conjunction with studies on the dielectric constants of serum proteins.[7]

(7) J. D. Ferry and J. L. Oncley, THIS JOURNAL, **60**, 1123 (1938).

(8) Earlier measurements from the Department of Physical Chemistry, Harvard Medical School,[8] hitherto unpublished in detail.

solution 6.5. The pseudoglobulin solution was electrodialyzed and its *p*H was 6.0. The amount of protein in solution was determined by heat coagulation at the isoelectric point; coagulated protein was washed on sintered glass filters till salt free and was dried at 110° to constant weight.

The viscosities were measured at 25 ± 0.1° in Ostwald viscosimeters. The densities were determined at the same temperature in a pycnometer of approximately 10 cc. volume. The relative viscosities reported are the average of at least three determinations. In calculating the relative viscosity the ratio of the time of flow of 0.5 *M* sodium chloride to water was taken as 1.015 and the density of 0.5 *M* sodium chloride and of water at 25° as 1.0173 and 0.99707, respectively. The data are recorded in Table I.

Discussion

For comparison with the existing data on the viscosities of serum proteins, the data are presented in Fig. 1, in which concentration of protein in grams per liter is plotted against the relative viscosity, $\eta/\eta_0 - 1$, where η is the viscosity of the solution and η_0 the viscosity of the solvent.

The viscosities of the serum proteins studied increase in the order albumin, pseudoglobulin, PI, PII and PIII. The pseudoglobulin and albumin values check satisfactorily with Chick's determinations,[4] and her curve for euglobulin, prepared by acidification of ten-fold diluted horse serum with acetic acid (to *p*H 5.4–6.0) lies between PI and PII.

Chick's data have been recalculated on a volume basis rather than as grams per 1000 grams. Under these circumstances curves for solutions obtained by addition of dilute sodium chloride or dilute alkali appear to be superimposable. Over the range studied our curves are also identical within the limit of error of these measurements whether the protein, PI or PII, was dissolved in 0.5 *M* sodium chloride at the isoelectric point or was in solution in dilute alkali.

Einstein,[9] in 1906, derived an equation for the viscosity of a suspension or solution of spherical particles

$$\eta/\eta_0 = (1 + 2.5\,\varphi) \qquad (1)$$

where φ is the volume fraction occupied by the particles (the solute), and η_0 the viscosity of the solvent at the same temperature. This equation is linear in φ and therefore in the concentration in grams per liter of solution if the partial specific volume of the solute is constant. It has been used by Daniel and Cohn[3] in describing the viscosities of a series of amino acids and related substances in dilute solution. They noted that

(9) A. Einstein, *Ann. Phys.*, **19**, 289 (1906); **34**, 591 (1917).

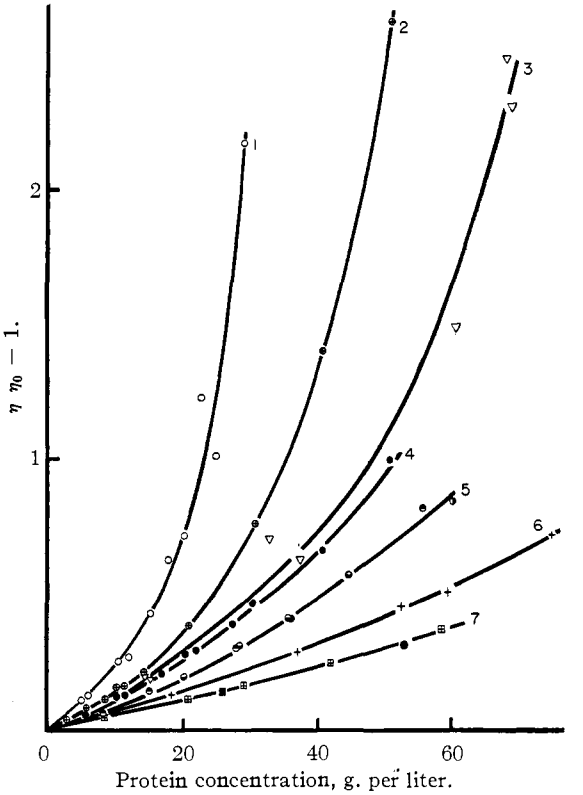

Fig. 1.—Viscosity of serum proteins: curve (1) serum globulin PIII ○; (2) PII ⊕; (3) euglobulin (Chick) ▽; (4) PI ●; (5) pseudoglobulin (this investigation ◓ ◒ and Chick ◑); (6) whole serum (Chick) +; (7) serum albumin (this investigation ⊕, Chick and Lubrzynska ■).

"measurements upon aliphatic amides, amino acids and peptides even up to viscosities more than three times that of water are given by the equation

$$(\eta/\eta_0 - 1) = 2.5\ K\varphi + (2.5\ K\varphi)^{2.8} \qquad (2)''$$

Although the viscosity curves of all proteins deviate in concentrated solutions from the linear relationship demanded by the Einstein equation, in sufficiently dilute solutions the linear relationship is approximated and the value of K may be estimated. The range over which viscosity is roughly linear with concentration is shown graphically for the serum proteins in Fig. 2. The values for K[10] are given in Table II together with

(10) The values of K adopted were obtained from determinations of relative viscosities of solutions of low protein concentration. Even over this range, however, K, calculated by means of equation 1, shows a drift with concentration. Estimated by extrapolation to infinite dilution on the basis of a plot of C against K, the following values of K were obtained and the corresponding values of s/d are also implied:

	Serum albumin	Pseudo-globulin	Serum globulins PI	PII	PIII
K	2.6	4.5	4.6	7.0	10.0
s/d	8.0	11.8	12.0	15.5	19.0

TABLE II

CONSTANTS DESCRIBING VISCOSITY DETERMINATIONS

	V	K[10]	X	Y	s/d[10]
Glycine	0.759	1.07	2.8	1	1.7
α-Alanine	.823	1.48	2.8	1	4.4
Glycylglycine	.706	1.55	2.8	1	4.7
Na ε-aminocaproic acid	.835	2.32	2.8	1	7.3
Lysylglutamic acid	.769	2.26	2.8	1	7.1
Hemoglobin[11]	.75	1.87	...	...	5.9
Egg albumin[4]	.749	2.80[12]	4.0	1.1	8.5
Serum albumin	.725	3.30	4.0	1.7	9.6
Pseudoglobulin	.715	5.70	3.6	1.4	13.7
Euglobulin[4]	.73	7.20	4.0	1.7	15.8
PI	.75	6.6	4.0	1.7	15.0
PII	.72	8.0	4.0	8.0	16.7
PIII	.78	11.5	4.0	8.0	20.5
Casein[22]	.75	18.0	...	...	26.1
Tobacco mosaic virus[18,19]	.73	35.0	...	...	36.9

values for some amino acids and related substances estimated by Daniel and Cohn. The values for the specific volume, V, have been calculated from values for concentration of protein and density in Table I and in Harriette Chick's data. The values calculated from the results of Chick are

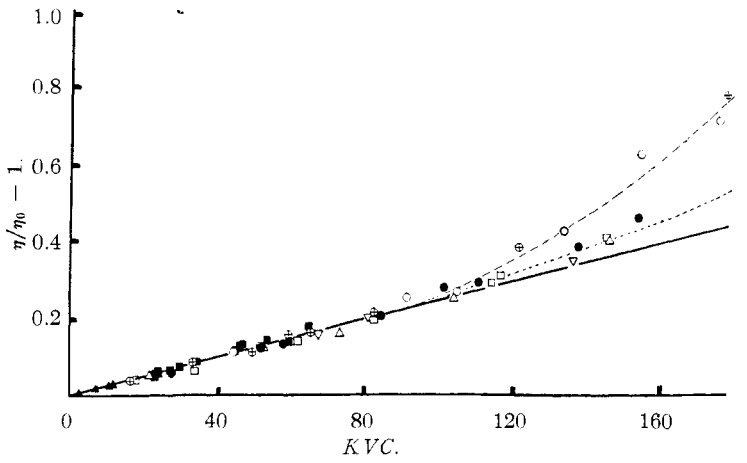

Fig. 2.—Viscosity of dilute solutions of proteins: PI ●; PII ⊕; PIII ○; pseudoglobulin □; serum albumin △; casein ▽ (Sackur[22]); hemoglobin ■ (Cohn and Prentiss[11]); and tobacco mosaic virus ▲ (Lauffer[18] Frampton and Neurath[19]).

0.725, 0.73 and 0.715 for serum albumin, euglobulin and pseudoglobulin, respectively. These are slightly smaller than the values 0.758 for serum albumin and 0.745 for serum globulin, containing both eu- and pseudoglobulin, estimated by Svedberg[13] on solutions which never exceeded 3%.

In order to describe the deviations of viscosities from the linear relationship in the more concentrated solutions of protein, equation (2) may be modified further to give the following

$$\eta/\eta_0 - 1 = \left(\frac{2.5\,KVC}{1000}\right) + Y\left(\frac{2.5\,KVC}{1000}\right)^X \quad (3)$$

The curves in Fig. 2 and the viscosity curves of Chick and collaborators up to $\eta/\eta_0 = 20$ given in Fig. 3 may be described in terms of the above equation using the values for K, X and Y reported in Table II.

The numerical value of K has been calculated from the linear relation in low concentration of protein. The more viscous is the protein the higher the value for K. The values for X for the serum proteins are approximately 4 and, therefore, greater than those for amino acids. The value for Y also tends to increase for the serum proteins and for the more viscous globulins, PII and PIII, is as high as 8.

The peculiarities of protein viscosity have been attributed to hydration of molecules,[4,14,15] to electrical interactions, and to shape of the molecules.

W. Kuhn[16] has developed the following equation for the increase in viscosity of rod-like molecules

$$\eta/\eta_0 - 1 = 2.5\,\varphi + \frac{\varphi}{16}\left(\frac{s}{d}\right)^2 \quad (4)$$

from which a definite relation between length, s, and width, d, of the molecule can be obtained assuming the length is much greater than the width. Guth[17] has presented a more general treatment of the problem in which the Kuhn equation is obtained as a limiting case for molecules which are ellipsoids of revolution. The relation between equation (4) and equation (1) may be expressed as follows

$$K = 1 + \frac{1}{40}\left(\frac{s}{d}\right)^2 \quad (5)$$

The values for K adopted (Table II and footnote 10) lead to values of s/d varying from 1.7 for glycine to 26 for casein and 37 for tobacco mosaic virus.

A similar application of Kuhn's equation to the viscosity measurements on tobacco mosaic virus

(11) Cohn and Prentiss, *J. Gen. Physiol.*, **8**, 619 (1927).

(12) Loeb[2] found that the viscosity of egg albumin follows the simple Einstein equation where K is approximately 1.0. Further determinations on the viscosity of egg albumin will be reported later.

(13) Svedberg and Sjogren, THIS JOURNAL, **50**, 3319 (1928).

(14) M. Kunitz, *J. Gen. Physiol.*, **17**, 365 (1934).

(15) S. Arrhenius, *Medd. Vetenskapakad. Nobelinst.*, **3**, No. 13 (1916); *Biochem. J.*, **11**, 112 (1917).

(16) W. Kuhn, *Z. physik. Chem.*, **A161**, 1 (1932).

(17) E. Guth, *Kolloid. Z.*, **74**, 147 (1936).

by Lauffer,[18] and Frampton and Neurath[19] results in an estimate of s/d as 35 to 36.8. Polson,[20] also, has calculated the relation of length to width of a number of proteins from viscosity measurements using the Kuhn equation in conjunction with the Arrhenius equation for estimating the viscosity increments at infinite dilution. His values of d/s for egg albumin and serum albumin are 0.142 and 0.123, respectively, which yield values of s/d of 7.05 and 8.13 of the same order of magnitude as those calculated above. On the same basis thyroglobulin and Octopus hemocyanin both have values of s/d of 10.

Staudinger[21] exhaustively studied long chained organic compounds of the paraffin series of exceptionally high viscosity and came to the conclusion that their specific viscosities depend on the length of the molecule. In the case of proteins and of acyclic amino acids and their esters with paraffin chains the situation is more complicated. The observed viscosity is higher than that calculated from chain length which he suggests is due to the numerous acid amide linkages.

Whatever the theoretical explanation of the viscosity behavior of protein solutions, it is of significance that these serum proteins vary in range of viscosity from that of the nearly spherical egg albumin molecule to that of the highly viscous casein molecule.[22] Whereas the globulin solutions of normal horse serum are largely homogeneous in the ultracentrifuge, and the titration curves differ definitely but only slightly,[5] the viscosities vary so widely as to be characteristic.

Conclusions

1. The viscosities of solutions of six protein fractions of normal horse serum have been measured and compared with those of other proteins.

(18) M. Lauffer, *Science*, **87**, 469 (1938).
(19) V. Frampton and H. Neurath, *ibid.*, **87**, 468 (1938).
(20) A. Polson, *Nature*, **137**, 740 (1936).
(21) H. Staudinger and H. Becher, *Ber.*, **70B**$_1$, 889 (1937).
(22) O. Sackur, *Z. physik. Chem.*, **41**, 672 (1902).

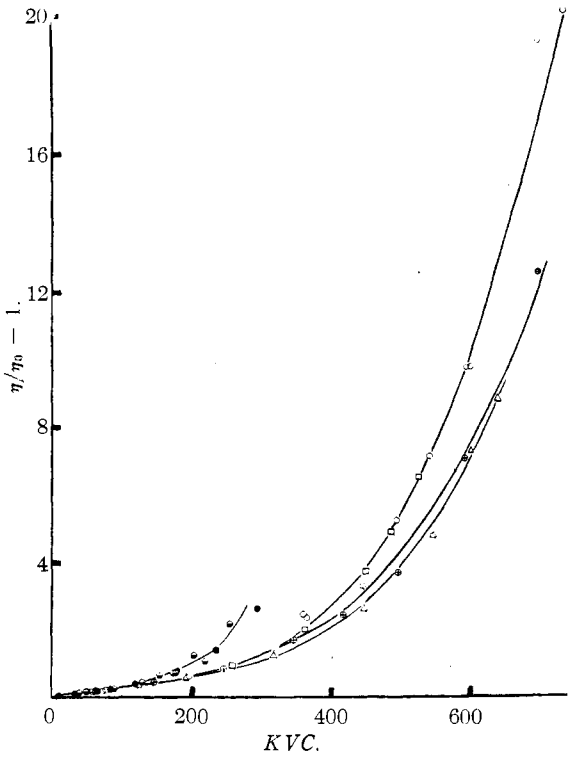

Fig. 3.—Viscosity of more concentrated protein solutions: euglobulin ○; pseudoglobulin ⊕; serum albumin □ and egg albumin △ calculated from Chick's data[4]; PII ●; and PIII ◒.

2. The viscosities of the solutions of three isoelectrically precipitable protein fractions, PI, PII and PIII increase in the order named, whereas that of pseudoglobulin is lower than any of these, and albumin is lowest of all.

3. The viscosity of concentrated protein solutions has been described by the equation

$$\eta/\eta_0 - 1 = \frac{2.5\,KVC}{1000} + Y\left(\frac{2.5\,KVC}{1000}\right)^X$$

4. The viscosities of the various serum proteins differ so greatly as to be essentially characteristic and perhaps are of significance in regard to the shape of the molecules.

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