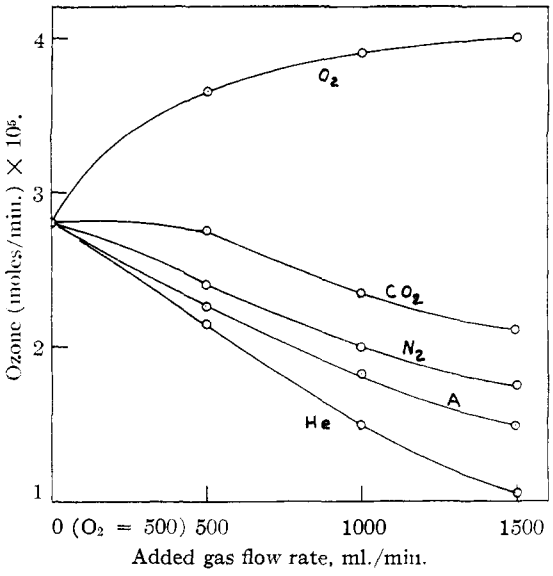

Fig. 2.—Unsensitized reaction; effects of added gases on ozone yield.

by the effect of the foreign gases on reaction (5) common to both the sensitized and unsensitized mechanism. In this competition, the effects of the foreign gases on reaction (7) more than overcome the effects on reaction (5) for the sensitized reaction. This result would be expected as a consequence of the greater probability of two body over three body collisions apart from any other consideration.

If one assumes that the entire effect of the foreign gas for the sensitized reaction is on reaction (7), the relative values of rate constants for the various gases in this reaction is approximately: CO_2, 1; A, 0.6; N_2, 0.6; He, 0.05. These values are in good agreement with those which may be calculated from the data of Holmes and Daniels,[15] for foreign gas effects on the photodecomposition of NO_2 where the postulated foreign gas effect is likewise the deactivation of an excited molecule, in this case NO_2. The order observed is also in agreement with that expected from the discussion of Russell and Simons[16] indicated that the effects are primarily determined by the magnitudes of the intermolecular force fields and therefore parallel the magnitudes of the van der Waals forces, the boiling points and the critical temperatures.

(15) H. H. Holmes and F. Daniels, THIS JOURNAL, **56**, 630 (1934).

(16) K. E. Russell and J. Simons, *Proc. Roy. Soc. (London)*, **A217**, 271 (1953).

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Light Scattering of Isoionic Bovine Albumin[1]

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The interpretation of light scattering measurements from two-component systems, *e.g.*, a salt-free protein and water, is simpler than if supporting electrolytes are added. A comparative study of bovine plasma albumin in water and in 0.15 *M* sodium chloride is presented. For this protein in the absence of salt, the extrapolation of intensities for molecular weight (M) determination appears to be straightforward and yields values of M in close agreement with other methods. The data suggest that values of M obtained in sodium chloride solutions may occasionally be elevated by an impurity which aggregates in the absence of salts and can be removed by high speed centrifugation or suitable filtration in water alone. The techniques described here can quite probably be used advantageously for studying salt-free solutions of other proteins which can be deionized by ion exchange or electrodialysis.

Light scattering measurements for the determination of the molecular weights of proteins are usually carried out in the presence of electrolytes added to suppress the effects of electrostatic charge. The addition of supporting electrolyte to a protein solution results in at least a three-component system, or if a buffer is used, in a four-component system. Equations to describe the small particle scattering from multi-component systems have been derived by several groups of workers[2-4] and have been applied to proteins by Edsall, *et al.*,[5] in approximately the following form

$$2R_{90,u} = \frac{K''\sum_i\sum_j \psi_i\psi_j A_{ij}}{|a_{ij}|} \qquad (1)$$

In eq. 1, $R_{90,u}$ is the excess scattered intensity observed at 90° with unpolarized incident light and $K'' = 4000\ \pi^2 n^2/\lambda_0^4 N_0$ where n is the refractive index, λ_0 the wave length *in vacuo* and N_0, Avogadro's number; ψ stands for molar refractive increment. The terms a_{ij} in the determinant $|a_{ij}|$ are the derivatives $\partial \ln a_i/\partial m_j$ where a is the activity and m the molar concentration. The term A_{ij} in the double summation is the cofactor of the term a_{ij}, *i.e.*, the determinant obtained from $|a_{ij}|$ by striking out the row and column in which a_{ij} occurs and multiplying the resulting determinant by $+1$ if $i + j$ is even and by -1 if $i + j$ is odd.

(1) The results presented here have been previously reported in part at the 38th annual meeting of the American Society of Biological Chemists, April, 1954; W. B. Dandliker, *Federation Proc.*, **13**, 196 (1954). After these results were substantially in their present form, a report of very similar experiments appeared from Professor Kirkwood's laboratory.[12] In addition, the author learned that Professor J. T. Edsall and Dr. R. H. Maybury had carried out some unpublished work along these lines in the summer of 1953. This work has been supported by funds from Initiative 171, State of Washington. Invaluable technical assistance was given by Mr. Nozar Pirzadeh.

(2) H. C. Brinkman and J. J. Hermans, *J. Chem. Phys.*, **17**, 574 (1949).

(3) J. G. Kirkwood and R. J. Goldberg, *ibid.*, **18**, 54 (1950).

(4) W. H. Stockmayer, *ibid.*, **18**, 58 (1950).

(5) J. T. Edsall, H. Edelhoch, R. Lontie and P. R. Morrison, THIS JOURNAL, **72**, 4641 (1950).

For a two-component system, eq. 1 reduces to the well-known form

$$\frac{K''\left(\frac{dn}{dc}\right)^2 c}{2000R_{90,u}} = \frac{1}{M_2} + \frac{1000\beta_{22}c_2}{M_2} \qquad (2)$$

where dn/dc is the refractive index increment, M_2 is the molecular weight of solute present at concentration c_2 and $\beta_{22} = \partial \ln \gamma_2/\partial m_2$ where γ is the activity coefficient. For a two-component system an extrapolation to $c = 0$ gives the molecular weight unambiguously.

Equation 1 reduces for a three-component system (*e.g.*, protein, water and sodium chloride) to

$$2R_{90,u} = \frac{K''[\psi_2^2 a_{33} - 2\psi_2\psi_3 a_{23} + \psi_3^2 a_{22}]}{a_{22}a_{23} - a_{23}^2} \qquad (3)$$

In general, for this case, the interpretation of scattering measurements requires information on a_{23}, *i.e.*, on the protein–salt interaction which might be obtained, for example, by equilibrium dialysis experiments.

The system, bovine plasma albumin, water and sodium chloride has been thoroughly studied by Edsall, *et al.*,[5] who concluded that under certain limiting conditions of low charge and sufficiently high salt concentration the extrapolated values of $c/R_{90,u}$ give very nearly the true value for the protein molecular weight when eq. 2 is applied. For proteins which interact strongly, or to an unknown extent with the added electrolytes, the same approximations may not be satisfactory.

As we shall show here, it is possible, at least in some cases, to study the scattering in a two-component system and to use the simple form of eq. 2 without any assumptions concerning the importance of the protein–salt interaction. Such a system can be realized for bovine plasma albumin by removing the salts on mixed-bed ion-exchange columns according to the procedure of Oncley and Dintzis.[6]

Experimental

Preparation of Protein Solutions.—In a typical experiment 10 g. of crystalline bovine plasma albumin[7] was dissolved in 50 ml. of water and passed at 0° through a column of 2.5 cm. inside diameter in about 7 hr. The topmost bed was 46 cm. of mixed IR-120 and IRA-400 on the ammonium acetate cycle; the second was 36 cm. of mixed IR-120 on the hydrogen cycle and IRA-400 on the hydroxyl cycle and the last was 17 cm. of IR-120 on the hydrogen cycle.

The specific conductivity of the water from this column was 1.3×10^{-7} mho cm.$^{-1}$ (at 0° and 1000∼). After deionization the protein solution was either used the same day for scattering measurements or dried from the frozen state and redissolved before use. Some of the light scattering experiments were carried out in the absence of carbon dioxide. On these occasions all vessels were protected from the atmosphere with soda lime tubes and transfers were made inside a glove box kept under slight pressure by a current of carbon dioxide-free air.

Dilutions were made with water from an all-Pyrex still. This water when boiled and cooled in the glove box had a specific conductivity of 1.4×10^{-7} mho cm.$^{-1}$ (0°) and a *p*H of 7.05 (glass electrode at 25°).

To remove dust for light scattering measurements, the solutions were centrifuged for three hours either at 20,000 or at about 60,000 times gravity, both speeds giving the same results. Dilutions usually were made in the light scattering cell by adding protein solution to clean water (or 0.15 *M* sodium chloride) weighing and mixing with a Lucite rod.

Protein concentrations were determined by the light absorption at 280 mμ using the relation $(1/lc)(\log I_0/I) = 6.66$ with the path length l in cm. and c in g./100 ml. The factor was determined on a deionized solution by dry weight at 100° over phosphorus pentoxide *in vacuo*.

Light Scattering Measurements.—The scattered intensities were measured with an instrument described by Brice, *et al.*,[8] modified in minor details.

Results were reduced to an absolute scale with sols of colloidal silica (du Pont Ludox). After dilution in water the sols were filtered on a Corning Ultra-fine sintered glass filter and the transmissions measured in 10-cm. cells in the Beckman DU spectrophotometer using a mercury arc source. Appropriate corrections were made for the limited angular resolving power of the Beckman photometer. Both the angular dependence and polarization of these sols are very small so that reduced intensities are readily calculated from the measured turbidities (on the order of 0.055 cm.$^{-1}$). A measurement on the sol in the light scattering apparatus then suffices to establish the instrument constant. This method of calibration has been studied by Edelhoch, *et al.*,[9] by Mommaerts[10] and by Oth, *et al.*[11]

Results and Discussion

In the two-component system, protein plus water, the *p*H is dependent upon protein concentration; at concentrations on the order of 1%, the *p*H approaches some limiting value characteristic of the protein. As the concentration of protein decreases toward zero, the *p*H must approach that of water. Similar remarks apply to the specific electrical conductivity (κ). The data in Fig. 1 show that the regions of concentration in which the *p*H and κ undergo rapid variations overlap with those which can be studied by light scattering. The results are largely self-explanatory and indicate that the *p*H will change from about 5.4 at 1% protein to 6.2 at 0.004%, which represents about the range of concentrations studied here.

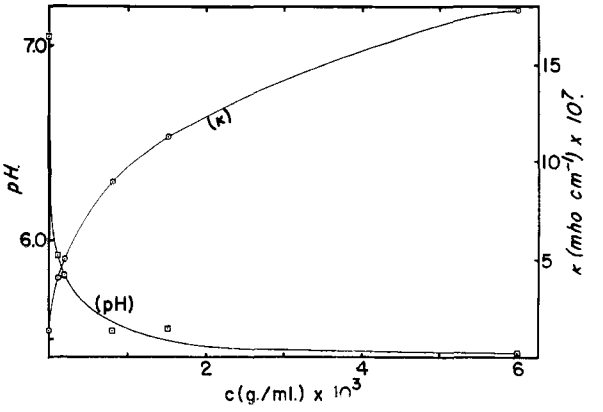

Fig. 1.—The dependence of *p*H (25°) and specific conductance (κ at 0°) on protein concentration for bovine plasma albumin in water.

For all the conductivities shown, a major portion of the total κ can be accounted for by the conductance of hydrogen ion alone, the contribution in the region of 0.1% protein being about 77%. It is not certain at present whether the remainder is chiefly due to the protein ion itself or to ionic impurities.

(6) J. L. Oncley and H. Dintzis, 122nd Meeting, American Chemical Society, Sept., 1952.

(7) Obtained from Armour and Company and from Pentex Incorporated.

(8) B. A. Brice, M. Halwer and R. Speiser, *J. Opt. Soc. Amer.*, **40**, 768 (1950).

(9) H. Edelhoch, E. Katchalski, R. H. Maybury, W. L. Hughes, Jr., and J. T. Edsall, THIS JOURNAL, **75**, 5058 (1953).

(10) W. F. H. M. Mommaerts, *J. Colloid Sci.*, **7**, 71 (1952).

(11) A. Oth, J. Oth and V. Desreux, *J. Polymer Sci.*, **10**, 551 (1953).

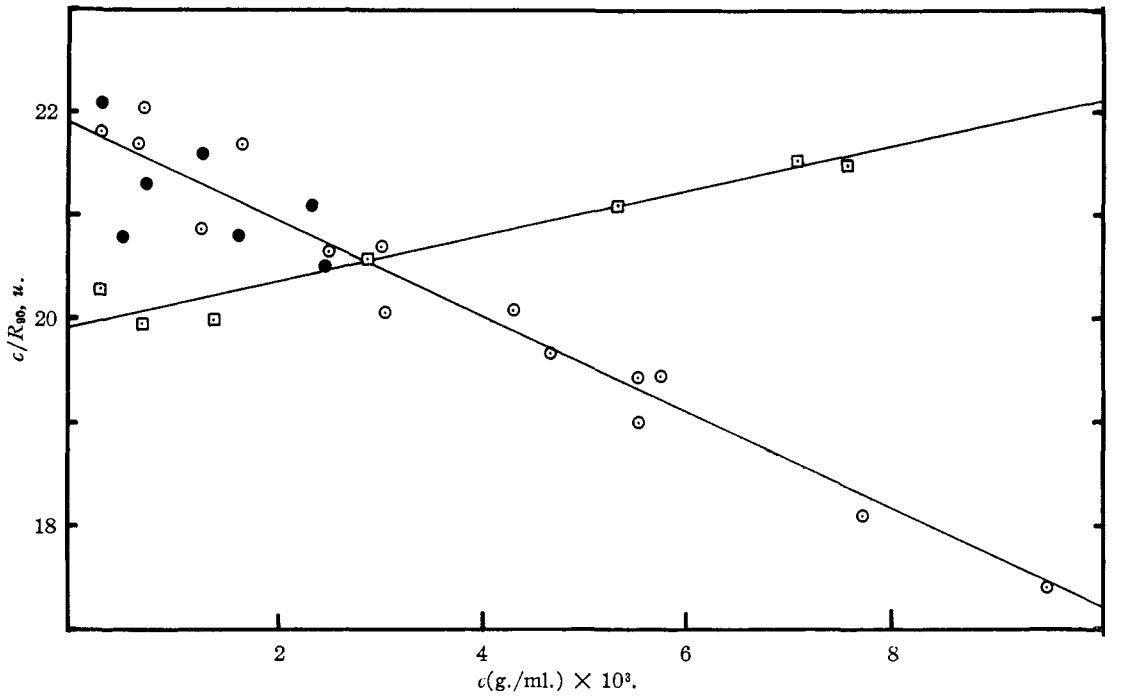

Fig. 2.—Light scattering of bovine albumin (sample A) at 4358 Å.: O, in water without precautions to exclude carbon dioxide; ●, in water with carbon dioxide excluded; □, in 0.15 *M* sodium chloride; —, obtained by least squares.

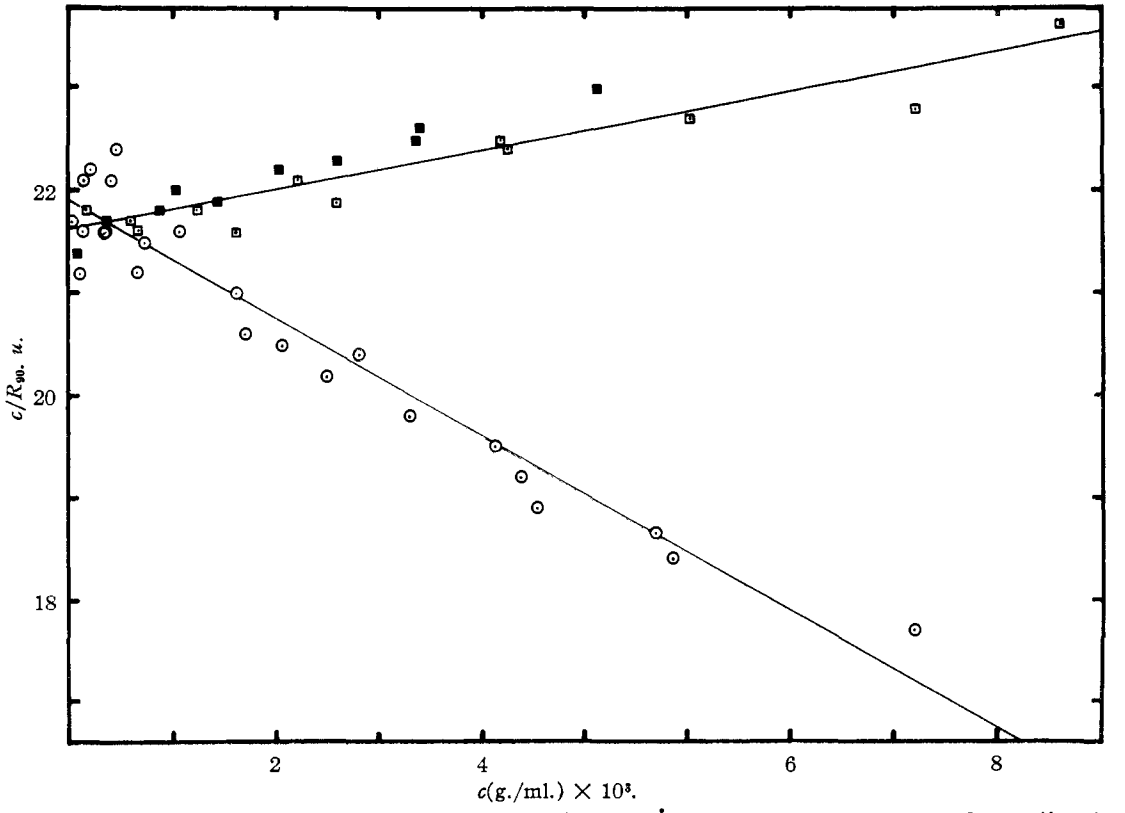

Fig. 3.—Light scattering of bovine albumin (sample B) at 4358 Å., coördinates same as Fig. 2: O, centrifuged and measured in water; □, centrifuged and measured in 0.15 *M* sodium chloride; ■, centrifuged in water and measured in 0.15 *M* sodium chloride.

Light scattering results for two different lots of bovine albumin are shown in Figs. 2 and 3. All the data have been treated by the method of least squares and the resulting straight lines shown. It

may be noted that the data obtained in the absence of carbon dioxide (filled circles) are, within experimental error, the same as those obtained when no precautions were taken to exclude carbon dioxide; this may not be the case for proteins with very high isoionic points. In both cases the curves in sodium chloride show the well-known small positive slopes[5] while for those in water somewhat larger negative slopes are found. An interpretation of the latter has been given by Timasheff, *et al.*,[12] using the theory of Kirkwood and Shumaker[13] which predicts a long range, intermolecular, attractive force for the isoionic protein in water resulting in a negative excess chemical potential. The theory also predicts that $c/R_{90,u}$ should be linear in $\sqrt{c}$ instead of c. In Fig. 4 the data of Fig. 3 (in water) are plotted against $\sqrt{c}$ together with the least squares line. The average deviation from the line for the two types of plots is nearly identical and we cannot conclude from these data which plot is preferable. In any case, the slopes are sufficiently small so that an extrapolation to $c = 0$ apparently can be made readily.

In Fig. 2 we note that the intercepts in water and in sodium chloride differ by about 10%. This behavior seems to be due to the presence of a contaminant in bovine albumin which aggregates in pure water and which is readily removed from salt-free solutions by the high speed centrifugation preliminary to light scattering measurements. Visual evidence of such a contaminant in certain preparations may be obtained by examining salt-free solutions of albumin which occasionally show a great deal of very large particle scattering. If a trace of sodium chloride be added to such a solution, the large aggregates rapidly dissolve leaving only Rayleigh scattering. The curves in Fig. 2 were obtained on solutions which had been clarified separately in water or in 0.15 M sodium chloride. A lot of mercaptalbumin[14] (sample C), which showed a difference of 14% in the water and sodium chloride intercepts when centrifuged in the two different solvents before scattering measurements, gave intercepts within 1% of each other when centrifuged in the absence of salt followed by dilution in water or sodium chloride for the scattering measurements.

In an attempt to remove this contaminant, the preparation in Fig. 3 (sample B) was filtered through a Corning Ultra-fine filter immediately after deionization. The curve in sodium chloride includes data both from solutions clarified in sodium chloride or clarified in water, the salt then being added subsequently. The two sets of data in this case cannot be distinguished. Moreover, the molecular weight from the intercept in sodium chloride is the same as that in water, within experimental error. However, when the data in water

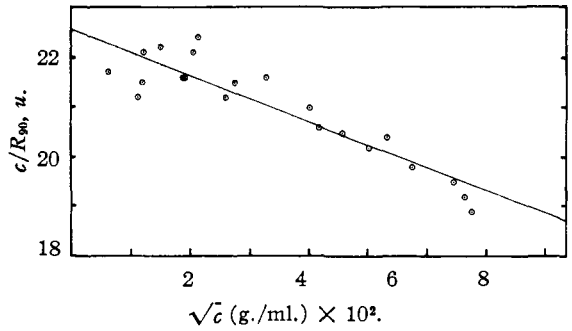

Fig. 4.—Light scattering of bovine albumin in water; same data as in Fig. 3 least squared against the square root of the concentration.

are plotted as in Fig. 4 the resulting intercept seems to be significantly higher than the sodium chloride data of Fig. 3.

On the basis of the work of Edsall, *et al.*,[5] we should expect the limiting values of $c/R_{90,u}$ to be the same, in either water or 0.15 M sodium chloride. Inasmuch as the theoretical interpretation of the two-component system appears to be subject to fewer assumptions and approximations than that of the three-component system, probably the greatest reliability for molecular weight determinations should be placed on results obtained in salt-free solutions.

Utilizing the absolute calibration with Ludox silica sol, values of M_2 can be calculated by eq. 2 (allowing for a depolarization of 0.02) from the values of $c/R_{90,u}$ obtained in various ways. The results are summarized in Table I. For this purpose the value of dn/dc for bovine serum albumin was taken to be 0.197 at 4358 Å. in 0.15 M sodium chloride and 0.195 in salt-free solution.[15,16] Although the absolute values of M are in some doubt, it seems quite certain that under proper conditions the intercepts in water and in 0.15 M sodium chloride are within a few per cent. of each other, the resulting M in water being perhaps a few per cent. lower.

TABLE I

MOLECULAR WEIGHT OF BOVINE PLASMA ALBUMIN (SEE TEXT)

Protein preparation	Clarifying centrifugation	Scattering measurements	$c/R_{90,u}$ ($c = 0$)	$M_2 \times 10^{-5}$
A (Fig. 2)	H_2O	H_2O	21.9	70.4
		0.15 M NaCl	19.9	76.0
B (Figs. 3 and 4)	H_2O	H_2O	21.9 (*vs.* c)	70.4
			22.6 (*vs.* $\sqrt{c}$)	68.5
	H_2O	0.15 M NaCl	21.6	70.0

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(12) S. N. Timasheff, B. D. Coleman and J. G. Kirkwood, 125th Meeting American Chemical Society, March, 1954.

(13) J. G. Kirkwood and J. B. Shumaker, *Proc. Nat. Acad. Sci.*, **38**, 863 (1952).

(14) Prepared in this Laboratory from Armour bovine Fraction V by the method of H. M. Dintzis, Thesis, Harvard University, 1952. See also footnote 6 in reference 9.

(15) G. E. Perlmann and L. G. Longsworth, THIS JOURNAL, **70**, 2719 (1948).

(16) M. Halwer, G. C. Nutting and B. A. Brice, *ibid.*, **73**, 2786 (1951).