the corrections for the electrostatic terms in the previous columns.

For the two most dilute solutions the non-electrostatic residual is very large. This may be due partly to failure of the experimentalists to obtain the limiting slope. At $1 \times 10^{-3}$ and above, the residuals for the isoionic solutions are moderately constant with averages of about 700 for the Yale BMA, 500 for their BSA and 250 for our BMA.

Our results with added acid or base are moderately consistent at 0.15 $m$ salt, but the electrostatic correction is much too large at 0.003 $m$. The discrepancy must be in the theory and it must apply to at least three of the four terms. It may arise from polarization of one protein molecule by the other due to shift of protons, or perhaps of chlorides, as suggested by Kirkwood and Shumaker.[19] Since we are calculating here the interaction of two like charges, polarization will reduce the electrostatic effect. There are no quantitative calculations of such effects. If the ratio $\kappa a/\sqrt{I}$, $\nu_{Cl}$ and the charge fluctuations are all independent of the temperature, the term proportional to $\bar{z}^2$ should have no enthalpy contribution, and the others should have an enthalpy term about $-{}^3/_8$ that of the free energy term. In our measurements in 0.15 $m$ NaCl and at $p$H 5.3 in 0.003 $m$ NaCl the electrostatic enthalpy term should be negligibly small.

It is quite possible that the difference between the enthalpy terms at 0.003 and 0.15 $m$ NaCl are due to the differences in the electrostatic terms and that the variation of the non-electrostatic residuals of both $\beta_{22}$ and its enthalpy counterpart are independent of $p$H and salt concentration in the range of our measurements. This would make the enthalpy contribution about $-600$ and the entropy contribution about $+1100$. The fluctuation theory explains, at least qualitatively, the fact that the slope at the isoelectric point (minimum slope) is less positive for thiocyanate ion than for chloride ion[3] by the fact that the greater binding shifts the isoelectric point nearer the $p$H where half the carboxyls are un-ionized, where the fluctuation is largest.

The greatest difficulty with the interpretation of these measurements is in the correction for excluded volume, which is entirely an entropy contribution. It was noted in ref. 2 that Huggin's extension of the lattice theory for chain molecules[21] gives 160,000 for the excluded volume contribution to $\beta_{22}$. For a sphere of radius 30 Å., the van der Waals theory as used by Zimm[22] and by Mayer[20] gives 204,000. As used by Kirkwood and Shumaker[19] it gives 180,000.

(21) M. L. Huggins, THIS JOURNAL, **64**, 1712 (1942).
(22) B. H. Zimm, *J. Chem. Phys.*, **14**, 164 (1946).

CAMBRIDGE, MASS.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

# Physical Chemistry of Protein Solutions. IX. A Light Scattering Study of the Binding of Trichloroacetate Ion to Serum Albumin

BY G. SCATCHARD AND S. ZAROMB[1]

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The turbidity of bovine serum mercaptalbumin has been measured at 25° in isoionic solutions with 0.01 to 2.5 $m$ sodium trichloroacetate and with addition of acid or of base from 0.05 to 0.25 $m$ salt and corrected for large-particle scattering. The salt causes aggregation so that the binding cannot be calculated from the apparent molecular weight. The second virial coefficient yields values for the binding which agree reasonably with other methods at low salt concentrations, but which exceed the total number of basic groups at high salt concentrations. Over the range studied, the addition of acid or base had very little effect on the apparent net charge.

This work was initiated in an attempt to apply to the binding of small ions to proteins the method used by Ewart, Roe, Debye and McCartney[2] for non-electrolytes. This method uses the change with addition of solute 3 in the apparent light-scattering molecular weight of solute 2 at the limit of zero concentration of 2. We start with equation 10 of the preceding paper.[3]

$$\frac{H'V^0\phi_P^2 w_P}{RT\Delta\tau} = \frac{\frac{1}{p\bar{V}_P} + \left(\frac{z_2^2}{2m_3} + \beta_{22}\right) w_P/\bar{V}_P}{\left(1 + h\psi_{HX}/\bar{V}_P\phi_P + \frac{\nu - h}{2}\psi_3/\bar{V}_P\phi_P + \frac{z_2^2 w_P\psi_3}{2m_3(2+\beta_{33}m_3)\bar{V}_P^2\phi_P}\right)^2} \quad (1)$$

If the degree of polymerization $p$ and the number of bound protons $h$ are known, $\nu$ may be determined from the intercept of $w_P/\Delta\tau$ *vs.* $w_P$, and $\beta_{22}$ may be determined from the slope. If $p$ is unknown we may assume $\beta_{22}$ is known as in the osmotic pressure studies[4] and calculate $\nu$ from the slope at the limit of zero concentration, and determine $p$ from the intercept.

We used sodium trichloroacetate with bovine serum mercaptalbumin because $\nu$ and $\psi_3$ are both larger than for any other anion we have studied. We were unable to use the intercept, however, for the salt caused polymerization of the protein which varied with the details of handling. There were difficulties even with the slope, because of large

(1) Research Department, Philco Corporation, Philadelphia, Pennsylvania. The experimental work reported here was completed in 1955.
(2) R. H. Ewart, C. P. Roe, P. Debye and J. R. McCartney, *J. Chem. Phys.*, **14**, 687 (1946). See also W. H. Stockmayer, *ibid.*, **18**, 58 (1950).
(3) G. Scatchard and J. Bregman, THIS JOURNAL, **81**, 6095 (1959).
(4) G. Scatchard, Y. V. Wu and A. L. Shen, *ibid.*, **81**, 6104 (1959).

particle scattering, probably from a small amount of polymerization to very large molecules.

**Apparatus.**—The modified Aminco light scattering apparatus described in the previous paper[3] was further modified for use with smaller cells. The heater and stirrer were removed. The thermostat cylinder was centered more precisely. With the bath empty the light beam was slightly divergent. A brass rod, 1 mm. in diameter at the beam, was inserted in a hole in the center of the thermostat base. The thermostat was located so that the rod was in the center of the beam. When the thermostat was full of water the light beam was slightly convergent permitting the use of narrow cells.

The cells were constructed by the Pyrocell Manufacturing Company, New York. The inside dimensions were 5 × 15 × 95 mm. The walls were 2 mm. thick with fused edges. The cells were closed with Teflon plugs machined to fit snugly to a depth of $^1/_4$ in. The plugged cells could be inverted without loss of liquid. This greatly facilitated the rinsing and mixing operations.[5]

Before use, all the glassware was first soaked in the proper cleaning solutions (*cf.* below) and then rinsed successively with tap water, distilled water, and Millipore-filtered conductivity water (m.p.w.). Rinsing with m.p.w. was continued until the turbidity reading for the water from the last rinsing was less than $3 \times 10^{-5}$ cm.$^{-1}$ (including stray light).

Type HA filters supplied by the Millipore Filter Corp., Watertown, Mass., were used exclusively. A Lucite filter holder was used for the water, and glassholders of the kind shown in Fig. 1 for the solutions. The latter holders used small volumes of liquid and were especially suitable for concentrated sodium trichloroacetate solutions which dissolve Lucite and many other plastic materials.

The liquid in the glass part above the filter was either delivered directly into the side-arm or else sucked up into a 1-cc. Gilmont micropipet[6] and thence delivered into the light scattering cell.[5] The glass tips for the micropipet were kept in a pipet cleaner from the start of the cleaning process until used. During and after rinsing with m.p.w. the glassware was shielded from dust by plastic and Parafilm covers and usually rinsed again just before use.

The micropipet tips and glass filter holders were soaked for 1 to 2 hr. in a hot nitric–sulfuric acid mixture. The cells were filled with chromic acid for one day. The ruby plunger of the micropipet and the Teflon gaskets and plugs were brushed with soap–water and then rinsed as was the glassware.

**Materials.**—Recrystallized bovine serum albumin mercury dimer was supplied by the Department of Biological Chemistry, the Harvard Medical School, through the courtesy of Professor J. L. Oncley. It was passed through a Dintzis–Oncley ion-exchange column,[7] freeze-dried and stored at −30° until used.

(5) This procedure was partly modeled after that of S. N. Timasheff, H. M. Dintzis, J. G. Kirkwood and B. D. Coleman, *Proc. Natl. Acad. Sci.*, **41**, 710 (1955).

(6) R. Gilmont, *Anal. Chem.*, **20**, 1109 (1948).

(7) H. M. Dintzis, Ph.D. Thesis, Harvard University, 1952.

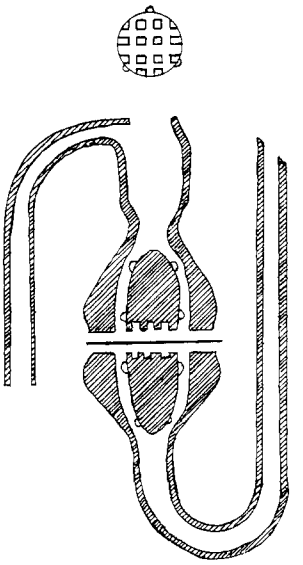

Fig. 1.—Filter holder.

A concentrated solution of trichloroacetic acid (TCA) was frozen and cooled to −30° and partly neutralized with cold 10 *M* NaOH. Freezing was repeated when room temperature was reached. Neutralization was then completed. The resulting NaTCA was freeze-dried and stored at −30° until used.

**Procedure.**—Stock solutions of about 10% protein and of 3 *M* NaTCA were made up prior to the series of experimental runs and stored at −30°. They were used to make up a protein–salt solution (PSS) containing about 1% protein and a salt solution (SS), with equal concentrations of NaTCA. The PSS and SS were stored between runs at about 4° for periods varying from 1 to 30 days. The *p*H of the SS was adjusted approximately to the *p*H of the PSS by adding small amounts of TCA or of NaOH.

A graduated glass Millipore filter holder was rinsed twice with small amounts of the SS. The holder then was filled by alternate inversions and additions of small amounts of SS, with care being taken to expel all air bubbles. In order to rinse out any dust introduced during the rinsing and filling operations, the SS was circulated through the filter for at least 20 minutes. Two light scattering cells were then rinsed with small amounts of the filtered SS and filled with about 3 ml. of SS. The volume of SS in each cell then was determined by weighing.

A second filter holder then was rinsed and filled in the same manner with PSS. The PSS was circulated through the filter for the duration of the experiment, thereby reducing the amount of dust in the reservoir. Parts of the filtered PSS were withdrawn with a micropipet as desired and delivered into a cell in quantities of 0.3 to 1.0 ml. This usually yielded six different protein concentrations in each of the two cells, all at almost constant *p*H and with a constant NaTCA concentration. The scattering at angles of 0, 45, 90 and 135° from the light beam was measured twice for each concentration. The minimum total

TABLE I

SUMMARY OF EXPERIMENTAL RESULTS

| $m_3$ | $p$H | $y'/(x \times y)$ I | II | $A$ | $B'$ | $h$ | $\nu$ | $\nu^*$ | $p\overline{W}_P \times 10^{-4}$ |
|---|---|---|---|---|---|---|---|---|---|
| 0.01 | 5.5–5.6 | 0.00 | 0.01 | 0.174 | 14.2 ± 0.8 | 0 | 9.3 ± 0.3 | 10.0 | 9.1 |
| .02 | 5.55 | .00 | .00 | .120 | 9.8 ± .8 | 0 | 10.8 ± .5 | 11.0 | 13.1 |
| .05 | 5.77 | .07 | .12 | .206 | 10.4 ± .5 | 0 | 17.8 ± .5 | 17.0 | 7.5 |
| .05 | 7.2 | .00 | .00 | .210 | 12.3 ± .3 | −11 | 8.1 ± .1 | 5.8 | 7.7 |
| .05 | 5.15 | .04 | .02 | .160 | 10.5 ± 1.0 | +10 | 28 ± 1 | 25 | 7.7 |
| .1 | 5.79 | .00 | .09 | .196 | 13.5 ± 0.6 | 0 | 29 ± 1 | 27 | 8.0 |
| .1 | 7.15 | .02 | .00 | .221 | 9.9 ± .6 | −10 | 14.3 ± 0.5 | 11.2 | 7.2 |
| .1 | 5.05 | .01 | .01 | .194 | 6.4 ± .3 | +15 | 35 ± 1 | 31 | 7.6 |
| .25 | 5.97 | .12 | .00 | .199 | 6.6 ± .9 | 0 | 32 ± 1 | 26 | 7.6 |
| .25 | 5.93 | .00 | .04 | .213 | 5.2 ± .4 | 0 | 29 ± 1 | 22 | 7.2 |
| .25 | 6.85 | .48 | .32 | .158 | 12.7 ± 4.8 | − 8 | 37 ± 9 | 32 | 9.7 |
| .25 | 5.1 | .13 | .01 | .169 | 5.7 ± 0.5 | +19 | 50 ± 2 | 43 | 8.4 |
| .5 | 6.22 | .08 | .57 | .099 | 13.0 ± 5.6 | 0 | 66 ± 16 | 55 | 14.4 |
| 1.3 | 6.42 | .11 | .00 | .081 | 29 ± 1.0 | 0 | 174 ± 3 | 129 | 14.8 |
| 2.5 | 6.53 | .21 | .00 | .069 | 13.6 ± 1.1 | 0 | 163 ± 8 | 120 | 17.7 |

volume of solution required in a cell was 3.7 cc. Smaller volumes would give high turbidity reading with protein solutions.

A Beckman model G $p$H meter was used to check the $p$H of the PSS, SS, several mixtures of the latter solutions, and of the contents of each cell at the end of the experimental run.

Most of the solutions were isoionic. An excess of NaOH or TCA was added to some solutions so as to raise or lower the $p$H of the PSS by about 2 or 0.5 $p$H units, respectively. The excess of acid or base in the PSS was determined by a subsequent titration with freshly dissolved materials, in which decomposition of NaTCA could be considered negligible.

**Dust and Stray Light Corrections.**—In a direction forming an angle $\theta$ with that of the main beam, the intensity of light scattered from particles whose longest dimension is less than one tenth the wave length of the light used is proportional to the Rayleigh factor $(1 + \cos^2\theta)$. Furthermore, of the volume of solution illuminated by the main beam, the fraction intercepted by the photomultiplier tube is proportional to $1/\sin\theta$. Hence, the instrument readings should be proportional to $(1 + \cos^2\theta)/\sin\theta$. For $\theta = 45°$ and $\theta = 135°$, this proportionality factor is $3/\sqrt{2}$ or 2.12, and for $\theta = 90°$ it is unity.

Usually, however, the 45°/90° ratio was greater than 2.12 and the 135°/90° ratio was smaller. This may be attributed to stray light and/or to the presence of dust or of polymerized protein in the light-scattering cell. The reading at 90°, $R'$, would then be composed of two parts, $x$ and $y$

$$R' = x + y \qquad (2)$$

where $x$ would be the reading resulting from a similar solution in the absence of stray light and of undesirable particles, and $y$ the reading due to the undesirable particles and/or stray light. The readings at 45 and 135° then could be expressed also in terms of $x$ and $y$

$$R_{45} = 2.12x + ay \qquad (3)$$

$$R_{135} = 2.12x + by \qquad (4)$$

where $a$ and $b$ are unknown proportionality factors.

In order to evaluate $x$, it was assumed that during an experimental run the factors $a$ and $b$ did not vary appreciably for measurements made with the same cell and that they may therefore be evaluated as unknown constants by the method of least squares. It was also assumed that equation 1 is valid through the range of our measurements and that the second term in the denominator may be taken as unity, so that

$$w_P/x = A + B'w_P \qquad (5)$$

where $A$ and $B'$ are unknown constants for a given experimental run. These assumptions form the basis of the calculations described in the next section.

**Least Squares Treatment.**—Equations 2 to 4 can be rewritten

$$R'' = R_{45} - 2.12R' = (a - 2.12)y = y/\gamma\phi \qquad (6)$$

$$R''' = 2.12R' - R_{135} = (2.12 - b)y = y/\phi \qquad (7)$$

where the terms $R''$, $R'''$, $\gamma$ and $\phi$ are defined by equations 6 and 7. Equations 5, 6, and 7 give six parameters for each dilution series: $A$ and $B$, which are common to the two cells, $\gamma_I$, $\phi_I$ for the first cell and $\gamma_{II}$, $\phi_{II}$ for the second. These may immediately be reduced to four because, by least squares, $\gamma$ is the average of $R_i'''/R_i''$, or

$$\gamma_I = \sum_{i=1}^{S_I} (R_{i1}'''/R_{iI}''S_1) \qquad (8)$$

in which i refers to the ith set of readings, and $S$ is the number of sets of readings obtained for a given cell in a run, usually twelve. A similar equation holds for $\gamma_{II}$.

Substitution of equations 2, 6 and 7 into 5 yields

$$\begin{aligned} A + B'w_P &= w_P/(R' - y) \\ &= w_P/(R' - \phi R''') \\ &= w_P/(R' - \gamma\phi R'') \end{aligned} \qquad (9)$$

The subscript of $w_P$ is omitted in the subsequent equations. For the individual readings obtained with both cells during a given run, equation 9 may be rewritten

$$\left.\begin{aligned} &(A + B'w_{i1})\left(1 - \frac{\phi_1 R_{i1}'''}{R_{i1}'}\right) - w_{i1}/R_{i1}' = \delta_{i1} \\ &(A + B'w_{iII})\left(1 - \frac{\phi_{II} R_{i}'''}{R_{i1}'}\right) - w_{iII}/R_{iII}' = \delta_{iII} \\ &(A + B'w_{i1})\left(1 - \frac{\gamma_1\phi_1 R_{i1}''}{R_{i1}'}\right) - w_{i1}/R_{i1}' = \delta_{i1}' \\ &\text{and} \\ &(A + B'w_{iII})\left(1 - \frac{\gamma_{II}\phi_{II} R_{iII}''}{R_{iII}}\right) - w_{i1}/R_{iII}' = \delta_{iII}' \end{aligned}\right\} \qquad (10)$$

The method of least squares then requires that

$$\sum_{j=I}^{II}\sum_{i=1}^{S}(\delta_{ij}^2 + \delta'_{ij}^2) = \text{minimum}$$

*i.e.*, that

$$\left.\begin{aligned}
&\sum_{j=I}^{II}\sum_{i=1}^{S}[\delta_{ij}(\partial\delta_{ij}/\partial A) + \delta'_{ij}/\Sigma\partial A)] = 0\\
&\sum_{j=1}^{II}\sum_{i=1}^{S}[\delta_{ij}(\partial\delta_{ij}/\partial B') + \delta_{ij}'(\partial\delta_{ij}'/\partial B')] = 0\\
&\sum_{i=1}^{S}[\delta_{i1}(\partial\delta_{i1}\partial\phi_I) + \delta_{iI}'(\partial\delta_{iI}'/\partial\phi_I)] = 0\\
&\text{and}\\
&\sum_{i=1}^{S}[\delta_{i11}(\partial S_{i11}/\partial\phi_{11}) + \delta_{i1I}'(\partial\delta_{i1I}'/\partial\phi_{11})] = 0
\end{aligned}\right\} \quad (11)$$

The last four simultaneous equations then were solved for $A$, $B'$, $\phi_I$ and $\phi_{II}$ by the method of successive approximation. Most of these calculations were performed with the aid of an IBM 607 computer.

## Results and Discussion

The values of $A$ and $B'$ obtained for various concentrations and acidities are shown in Table I. The first column gives the salt concentration $m_3$, the second gives the $p$H, the third and fourth columns give $y/(x + y)$ for the two cells, the fifth and sixth columns give $A$ and $B'$ from eq. 11, the seventh column gives $h$ calculated from the $p$H and the titrations presented in Table II, the eighth column gives $\nu$ calculated from $B'$ and eq. 13 with $\beta_{22} = 0$, and the ninth gives $\nu^*$ calculated with $\beta_{22} = 500$ plus a small electrostatic term calculable from theory,[3] and the tenth gives $p\bar{W}_P$, calculated from $A$ and eq. 12 and the values of $\nu$ and $h$.

For the titrations, a small amount of 0.7 $M$ NaTCA, 0.02 $M$ NaOH, or 0.035 $M$ HTCA was added from one of three Gilmont micropipets to an initial volume of 2 ml. of 0.785 wt. % isoionic bovine serum albumin. After each addition the $p$H was measured with a Beckman Model G $p$H meter. The solutions were freshly prepared just before the titration in order to minimize any error from a slow decomposition of the NaTCA. There was no sign of denaturation in this $p$H range. The results are presented in Table II. The first column gives the concentration of protein $m_2$, the second gives the mean concentration of sodium and trichloroacetate ions $m_3$, the third gives the $p$H, the fourth gives the number of hydrogens bound per molecule of protein $h$, and the fifth gives the number of bound trichloroacetate ions $\nu$, calculated from the light scattering measurements.

TABLE II

TITRATION OF BOVINE SERUM ALBUMIN IN NaTCA SOLUTIONS

| $m_2 \times 10^5$ | $m_3 \times 10^2$ | $p$H | $h$ | $\nu$ |
|---|---|---|---|---|
| 11.2 | 0 | 5.07 | 0 | 0 |
| 10.4 | 4.5 | 5.70 | 0 | 17 |
| 9.93 | 4.4 | 7.08 | − 9.7 | 7 |
| 9.77 | 4.3 | 7.52 | −12.7 | 5 |
| 9.61 | 4.3 | 7.94 | −15.8 | 2 |
| 9.06 | 4.1 | 5.26 | + 7.05 | 24 |
| 8.93 | 4.2 | 5.45 | + 4.0 | 21 |
| 8.79 | 4.1 | 5.13 | + 9.95 | 27 |
| 7.61 | 12.3 | 5.32 | + 9.95 | 35 |
| 7.54 | 12.2 | 5.07 | +15.6 | 40 |
| 7.44 | 12.1 | 5.20 | +12.5 | 37 |
| 6.85 | 11.2 | 7.12 | − 9.8 | 15 |
| 6.78 | 11.1 | 7.52 | −12.95 | 12 |
| 6.70 | 11.0 | 7.91 | −16.1 | 9 |
| 4.72 | 27.4 | 7.81 | −16.1 | 18 |
| 4.69 | 27.2 | 7.13 | −10.4 | 21 |
| 4.65 | 27.0 | 6.54 | − 4.6 | 26 |
| 4.61 | 26.8 | 6.85 | − 7.7 | 23 |
| 4.48 | 26.0 | 5.28 | +15.4 | 46 |
| 2.59 | 25.9 | 5.07 | +20.95 | 52 |
| 2.57 | 25.7 | 5.18 | +17.8 | 49 |
| 11.2 | 0.0 | 5.14 | 0 | 0 |
| 10.0 | 7.1 | 5.75 | 0 | 21 |
| 9.08 | 6.5 | 8.44 | −19.6 | 1 |
| 8.95 | 6.5 | 7.53 | −13.75 | 7 |
| 8.85 | 6.4 | 6.84 | − 8.75 | 12 |
| 8.72 | 6.3 | 7.28 | −11.9 | 9 |
| 8.25 | 6.1 | 5.98 | +11.4 | 32 |
| 8.04 | 6.0 | 5.40 | + 5.3 | 25 |
| 7.92 | 5.9 | 5.11 | +11.1 | 31 |
| 7.84 | 5.8 | 5.27 | + 8.1 | 28 |
| 7.30 | 9.9 | 5.36 | + 8.1 | 32 |
| 7.22 | 9.8 | 5.08 | +13.75 | 38 |
| 7.15 | 9.7 | 5.22 | +10.7 | 35 |
| 6.69 | 9.2 | 6.88 | − 8.2 | 15 |
| 6.61 | 9.1 | 7.28 | −11.2 | 12 |
| 6.54 | 9.0 | 7.66 | −14.3 | 9 |
| 5.08 | 21.8 | 7.59 | −14.3 | 15 |
| 5.04 | 21.6 | 6.89 | − 8.9 | 20 |
| 5.00 | 21.5 | 6.28 | − 3.1 | 26 |
| 4.96 | 21.4 | 6.61 | − 6.25 | 23 |
| 4.85 | 20.9 | 5.38 | +10.5 | 39 |
| 4.80 | 20.7 | 5.14 | +16.3 | 45 |
| 4.77 | 20.6 | 5.27 | +13.2 | 42 |
| 4.73 | 20.4 | 5.05 | +19.0 | 48 |

For bovine serum albumin we determined $\psi_P = \bar{V}_P\phi_P = 12.6$ at $\lambda = 4360$ Å. in various concentrations of NaTCA, which agrees well with the other values reported in the literature. From the International Critical Tables, we find $\psi_3 = 0.0228$ and $\psi_{HX} = 0.0212$. Therefore the last term in $\psi_3/\bar{V}^2{}_P\phi_P$ in the denominator of eq. 1 is less than 0.001 and may be neglected, but the other terms in the denominator are not negligible. From these values and eq. 1 and 5 we have

$$p\bar{V}_P = 1.61 \times 10^4/[1 + 0.00078h + 0.00091\nu]^2A \quad (12)$$

$$(\nu - h)^2 = [590\ B'(1 + 0.00078h + 0.00091\nu)^2 + 2\beta_{22}]m_3 \quad (13)$$

The factor $1.61 \times 10^4$ includes the calibration constants for our instrument.

We note first that the correction for large particle scattering $y/(x + y)$ is 1% or less in half our measurements and less than 10% in all but four. Large corrections occur with large standard deviations, but there is no other obvious correlation.

We have not tabulated the values of anion-binding from the intercepts and eq. 12. If we take $p\bar{V}_P$ as 71,000, which is the number average for bovine mercaptalbumin determined by Pigliacampi

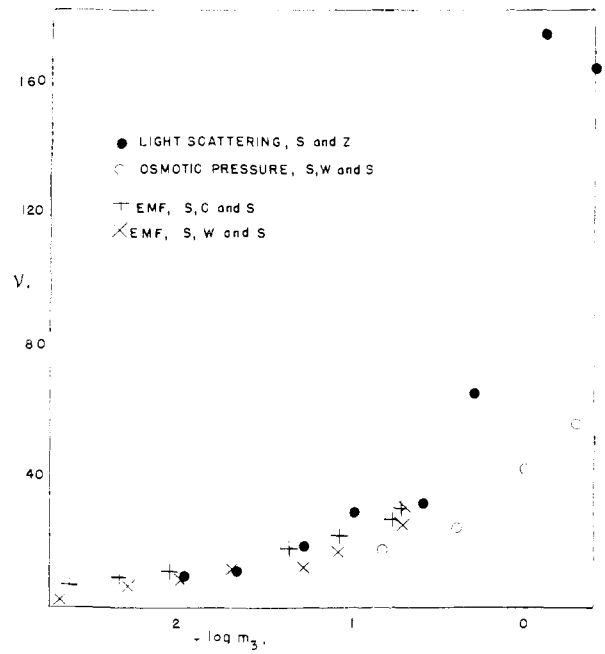


Fig. 2.—Binding of trichloroacetate ion to bovine serum mercaptalbumin.

from osmotic pressures,[8] the values so calculated are absurdly large at the lowest concentrations, and for the others they vary from 1.5 to 8 times those listed in column 8, with an average ratio of 4. It seems much more probable that the variation in the intercept is caused largely by aggregation. Even if the aggregation were constant and known, this method could not yield precise values of $\nu$ because a 1% decrease in the intercept corresponds to five ions bound.

(8) J. Pigliacampi, Ph.D. Thesis, M.I.T., 1957.

The values of $\nu$, in column 8, calculated from the slopes, are compared with the electromotive force results of Scatchard, Coleman and Shen[9] and of Scatchard, Wu and Shen[4] and the osmotic pressure results of the latter in Fig. 2. The values of $\nu^*$ are omitted to prevent the comparison of $\nu^*$ by one method with $\nu$ by another. The qualitative comparison is the same for $\nu^*$'s as for $\nu$'s. The light scattering results agree well with the electromotive force results in the range of overlap 0.01–0.1 $m$ NaTCA, except that the value at 0.1 $M$ is high relative to those at other concentrations. The osmotic pressure measurements lead to smaller binding in the dilute solutions and very much smaller binding in the concentrated solution. It seems almost certain that light scattering results in concentrated salt solutions are too high, but it is not so certain that the osmotic pressure results are not too low. The two sets depend upon the same assumptions: that $\beta_{23}$ is zero, and for $\nu$ that $\beta_{22}$ is zero, or for $\nu^*$ that $\beta_{22}$ is 500 plus an electrostatic part calculable from theory. There is no basis in the present work to choose between the two assumptions.

The results with added acid or base show a surprising tendency for the net charge to remain unchanged at constant salt concentration. This tendency is somewhat obscured by the isoionic result at 0.1 $m$ and the value with added base at 0.25 $m$, which both appear to be high. The latter is one of those with very large correction for large particle scattering. This tendency cannot be maintained for large additions of base, and probably is not maintained for large additions of acid.

(9) G. Scatchard, J. S. Coleman and A. L. Shen, THIS JOURNAL, **79**, 12 (1957).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

# Physical Chemistry of Protein Solutions. X. The Binding of Small Anions by Serum Albumin[1]

BY GEORGE SCATCHARD, YING VICTOR WU AND AMY LIN SHEN

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The binding to bovine serum mercaptalbumin of chloride, fluoride, thiocyanate and trichloroacetate ions from solutions of their sodium salts has been studied by measuring the $p$H and the electrical potentials of anion-exchanger electrodes as in earlier work, and in more concentrated salt solutions by measuring the osmotic pressure. The electromotive force measurements indicate that the first twenty-seven anions are bound at the same sites with one site in the first class, eight sites in the second class and eighteen sites in the third class, and the ratios of contants: $K_{1A}^0 = 24K_{2A}^0 = 720K_{3A}^0$, and $K_{1Cl}^0 = 2400$, $K_{1F}^0 = 3600$, $K_{1SCN}^0 = 24{,}000$, $K_{1TCA}^0 = 120{,}000$. The osmotic pressure results indicate that there are many additional sites, perhaps about seventy, with very little specificity among the anions.

This paper describes the continuation of the work of Scatchard, Coleman and Shen[2] giving more precise values for the binding of thiocyanate and trichloroacetate ions, giving results for fluoride ions, and extending the measurements to much higher bindings through the use of osmotic pressure measurements.

(1) Adapted in large part from the Ph.D. thesis of Ying Victor Wu, M.I.T., 1957. This investigation was supported in part by a grant from the Rockefeller Foundation and by a research grant (H-3249) from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

(2) G. Scatchard, J. S. Coleman and A. L. Shen, THIS JOURNAL, **79**, 12 (1957).

**Apparatus.**—The apparatus for measuring the electrical potentials was modified considerably. It contains no cation-exchange membranes, it can be taken apart to equilibrate the membrane with a new solution or to replace the membrane, and it is particularly designed to permit thorough stirring with a very small amount of solution. The cell, shown diagrammatically in Fig. 1, consists of an anion exchanger membrane held between two blocks of plastic which