curve. The deviation at small H may be due to curvature of the electrode surface, which would have a more pronounced effect as β decreases. The data for lead lie on a different curve of the same general shape, while copper falls far below the calculated line, even at large H. This behavior of the three metals is reflected in terms of the peak potentials in Fig. 3. On the βt scale, 0.1 unit is equivalent to 2.5 mv. for a one-electron process. More detailed comparisons are provided by the complete $\psi'(\beta t)$ curves of Fig. 4. With silver, at $H = 105$, the agreement is remarkably good in all details, and the predicted trends in shape are retained as H diminishes. The deviations of lead and copper appear to be caused by different phenomena.

The contrast between bulk metal films and fractional monolayers is depicted by Fig. 5, which includes the dissolution curve of the silver film with $q^0 = 213$ microcoulombs. The conditions of this measurement, corresponding formally to $H = 8.83$, would give relatively good agreement in the case of a monolayer, according to Fig. 2. The actual film probably has a thickness of several atomic layers, and its transitional character is evident from the shape of the curve.

Any conclusions regarding the distribution of atoms on the surface must be drawn with caution, since the problem doubtless is complicated by many factors not included in the theory presented here. It can be observed, however, that the dissolution patterns of thin silver films are consistent with the assumption of a uniform monolayer, that the curves of lead show some tendency toward macro behavior, even at the lower surface coverages, which may indicate preferential deposition on like atoms, and that the forms of the anodic copper polarograms suggest the possibility of a slow step in the reaction mechanism. Byrne and Rogers[19] eventually concluded that silver atoms deposit first as a monolayer on platinum but probably with a heterogeneous distribution of energies. Haissinsky and Coche[5] arrived at approximately the same result for lead on platinum. Comparable data apparently are not available on the copper-platinum system. Hillson[20] in a study of the copper–cupric ion couple by alternating current electrolysis, reported that the exchange reaction on the bulk metal is rapid but occurs only on a small fraction of the electrode area. Duncan and Oakley[21] found an unexpectedly low rate of isotopic exchange between cupric ion and an oxide-free copper surface.

Although the monolayer theory was derived from a simplified model, it establishes the importance of certain parameters and provides a framework for comparison of results on various chemical systems. Thus it should be helpful in development of analytical methods and in the study of electrodeposition phenomena.

Acknowledgments.—The writer is greatly indebted to Mr. R. E. Tannich for development of the program for machine calculations and to Mr. Carl P. Tyler for aid with the experimental work.

(19) J. T. Byrne and L. B. Rogers, *J. Electrochem. Soc.*, **98**, 457 (1951).

(20) P. J. Hillson, *Trans. Faraday Soc.*, **50**, 385 (1954).

(21) J. F. Duncan and B. W. Oakley, *J. Chem. Soc.*, 1401 (1955).

BAYTOWN, TEXAS

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

Physical Chemistry of Protein Solutions. VII. The Binding of Some Small Anions to Serum Albumin[1]

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A cell is described for the measurement of the change in electromotive force with a change in the concentration or composition of a solution between an anion and a cation exchanger membrane, or between either membrane and a saturated potassium chloride bridge. Measurements are reported for the case of adding bovine serum mercaptalbumin to solutions of sodium chloride, sodium iodide, sodium thiocyanate, sodium trichloroacetate, hydrochloric acid, hydriodic acid, thiocyanic acid or to mixtures of sodium chloride with hydrochloric or with sodium hydroxide. The binding of an anion to the albumin is calculated from the measurements with the neutral salt, and the effect on pH agrees with simple electrostatic theory. This binding corresponds to three groups of sites on the albumin with the ratio of the constants for each group 3.85 for I^-/Cl^- and 19.25 for SCN^-/Cl^- and $Cl_3CCO_2^-/Cl^-$. The first group contains a single site, the second 8 sites and the third 18 sites. The chloride constants are $K_{1Cl} = 2400$, $K_{2Cl} = 100$ and $K_{3Cl} = 3.3$. There are about 70 other groups with smaller constants. The measurements in acid solutions indicate that the affinity for anions becomes smaller than that calculated from simple theory as the affinity for protons becomes larger, though not to the same extent. The changes appear to depend upon the number of protons bound rather than upon the charge.

Introduction

Ion exchangers have advantages in the electrochemical study of protein solutions because their pores are too small to admit protein molecules and because they repel ions of the same sign. Therefore, even in the presence of protein, a cation exchanger membrane behaves as a small-cation electrode and an anion exchanger membrane as a small-anion electrode in the same sense that a glass membrane may behave as a hydrogen electrode.

Some advantages are that there is no oxidation or reduction at these membranes, they are not limited to ions for which there are reversible true electrodes, and the combination of a cation and an anion exchanger permits the measurement of the free energy of transfer of the salt.

We have not used the last two advantages to the utmost in this work because we wished to study

(1) Adapted in large part from the Ph.D. Thesis of James S. Coleman, M.I.T., 1953.

three anions which form insoluble silver salts and because we found in practice that measurements with an anion exchanger and a saturated potassium chloride bridge have advantages over the membrane pair which will be discussed later.

In quite different ways ion exchangers have proven very useful in the deionization of the albumin and in the preparation of hydriodic and thiocyanic acids.

Apparatus.—The special requirements of our apparatus are that it be economical of protein solution and that it be adaptable for use with a wide variety of electrolytes. The electrical conductance of the membranes is large enough so that we have found it advantageous to use them as ribbons about 20 mm. long, 5 mm. wide and 0.5 mm. thick.

The apparatus which is shown diagrammatically in Fig. 1 has the ribbons embedded in polyester resin between two blocks of Lucite. One of the polished faces of a block of Lucite is covered with a thin layer of Selectron 5001 (Pittsburgh Plate Glass Company) to which 1% *t*-butyl hydroperoxide and 0.05% cobalt naphthenate have been added. The ion exchanger ribbons which were surface dried are laid end to end on the block,[2] more Selectron is added to a thickness of 1–1.5 mm. and a second Lucite block is pressed on, with care to avoid entrapment of bubbles. Very small pieces of membrane near the corners of the blocks keep the two blocks parallel. After the Selectron has hardened at room temperature for at least 36 hours, holes are bored along the interface between the blocks cutting away part of each end of each exchanger strip. These holes are drilled slowly with water as lubricant so as to give smooth, regular surfaces. The holes are enlarged at the tops and bottoms to fit the appropriate glass tubes and reservoirs.

In Fig. 1, which is drawn to scale, AA, A and AC are anion exchangers and C is a cation exchanger. In the Y tubes flowing liquid junctions are formed with saturated potassium chloride solutions which lead to saturated calomel electrodes α and γ, which are not shown. At the top of the three-way stopcock under compartment b contact is made with saturated potassium chloride leading to electrode β. A glass electrode, G, may be inserted at the top of vessel b, but most of the pH measurements were made in a separate vessel with a reference electrode β'.

We will call the cell between electrodes α and γ the cell $\alpha\gamma$ with electromotive force $E_{\alpha\gamma}$, etc. Obviously $E_{\alpha\gamma} = E_{\alpha\beta} + E_{\beta\gamma}$.

Some of the measurements were made in a less complicated apparatus in which the exchanger strips AA and AC and the compartments a′ and c′ are omitted and the flowing junction is moved to a and c. There would be some advantages in an apparatus with three-way stopcocks, instead of two-way, under a and c, so that contacts could be made with saturated potassium chloride solutions leading to other calomel electrodes. The advantage of the five-compartment apparatus is that the flowing solutions can be kept at a convenient constant concentration. We have used 0.01 *M* solutions.

Most of the measurements were made with the apparatus, including reservoirs and calomel electrodes, in an insulated box which prevented rapid changes of temperature.

Materials.—The bovine serum mercaptalbumin was furnished by the Harvard University Laboratory of Physical Chemistry Related to Medicine and Public Health through the courtesy of Dr. H. Dintzis, who prepared it by repeated crystallization of the mercury dimer from Bovine Plasma Fraction V donated by Armour and Company, followed by removal of the mercury and deionization by passing through a column containing the following sequence of ion exchangers, ammonium, thioglycolate, acetate, mixed hydrogen and hydroxyl, hydrogen.[3] Stock solutions in conductivity water were prepared and the concentrations determined by dry weight. Other solutions were prepared by dilution of the stock solutions by weight. A molecular weight of 69,000 is assumed in the calculations.

(2) We are indebted to Ionics, Inc., and to Rohm and Haas for supplying us with membranes before they were commercially available. The measurements reported here were made with Nepton CR-51 and ARX-102 from Ionics, Inc.

(3) H. Dintzis, Ph.D. Thesis, Department of Biophysical Chemistry, Harvard, 1952.

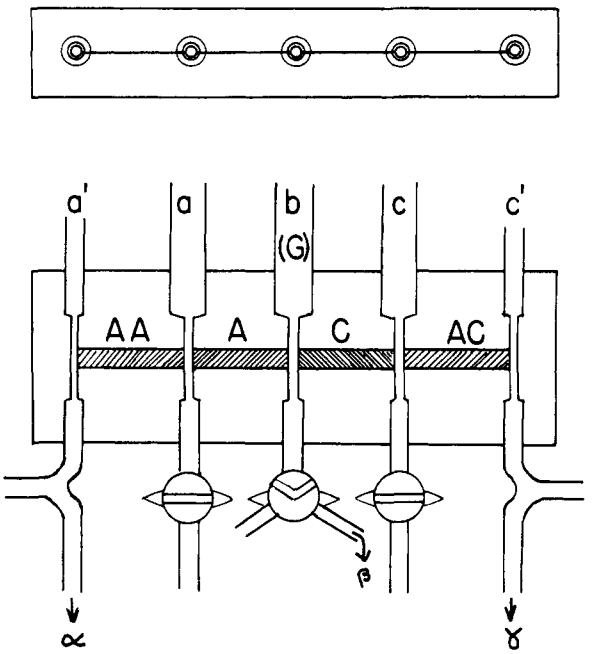

Fig. 1.—Diagram of apparatus: AA, A, C, AC membranes; a′, a, b, c, c′ compartments; (G) glass electrode; α, β, γ saturated calomel electrodes.

The conductivity water was redistilled in a Kraus type still from alkaline permanganate solution and collected and stored under nitrogen. For the preparation of solutions containing hydriodic acid it was freshly boiled to remove oxygen.

Analytical reagent grade (Mallinckrodt) sodium chloride, sodium thiocyanate, sodium iodide and trichloroacetic acid were used without further purification. Sodium trichloroacetate was prepared by neutralizing a solution of the acid with standardized sodium hydroxide. Constant boiling hydrochloric acid was prepared by the usual procedures[4] and used without further analysis.

Hydriodic acid and thiocyanic acid stock solutions were prepared by passing dilute solutions of the corresponding sodium salt through an ion exchanger in the hydrogen form.[5] Iron was removed from Amberlite IR-120 by passing 4 liters of 2 *M* ammonium thiocyanate through 400 ml. of the resin. After thorough rinsing with water, the column was repacked and 11 liters of 5% sulfuric acid were passed through, followed by water until the effluent was neutral to litmus. The resin was further washed by decantation seven times. A hundred milliliters of this resin was used to treat a liter of approximately 0.05 *M* salt. The acids were stored under nitrogen at 5°. The hydriodic acid was originally water white, but it did become visibly yellowish in less than a week and before the measurements were completed.

All solutions were made by diluting by weight stock solutions of these materials. The stock solution of hydrochloric acid was the constant boiling mixture, the concentrations of the other acid stock solutions were determined by titration. The concentration of sodium trichloroacetate was determined in its preparation, the sodium chloride solution was made from the dried salt, the concentrations of the sodium thiocyanate and iodide solutions were determined by weighing the precipitated silver salts.

Procedure and Results.—We will call the water component 1, the isoionic albumin component 2, the salt M^+X^- component 3, the acid H^+X^- component 5, and the base M^+OH^- component 7. The stoichiometric concentrations in moles per kilogram water are m_2, m_3, m_X, etc., and the concentrations of species are (H^+), (X^-), etc. The activity coefficient γ_X is a_X/m_X, and the activity coefficient g_X is $a_X/(X^-)$. We define the activity of any individual ion as the activity of the hydrogen ion is usually defined, that is as

(4) C. W. Foulk and M. Hollingsworth, THIS JOURNAL, **45**, 1220 (1923).

(5) R. Klement, *Z. anorg. Chem.*, **260**, 267 (1949).

the activity determined by an electrode reversible to that ion and a saturated potassium chloride bridge.

We assume that the average number of X^- ions bound to one albumin molecule, $\bar{\nu}_X$, is $[m_X - (X^-)]/m_2$.

Our usual procedure is to have a 0.01 M solution of the salt or acid in compartments a′ and c′, and a solution of concentration m_3 and no protein in compartments a and c. Measurements are made first with this same solution also in compartment b, and then with a solution m_3 in salt and m_2 in protein. The solutions in a′ and c′ flow continuously, the other solutions are flushed from time to time by draining five to ten drops through the stopcocks. The electromotive forces $E_{\alpha\gamma}$ and $E_{\alpha\beta}$ are measured. This gives two separate measures of $\bar{\nu}_X$ if the cation is not bound, but they may not agree exactly.

If the solutions are 0.01 M or more concentrated the electromotive force does not change more than a few millivolts, and is constant after about half an hour. With solutions more dilute than 0.001 M there is a drift after each flushing, and a small over-all drift which may last for days in very dilute solutions. The more dilute the solution, the greater is this difficulty. The value accepted is that immediately after flushing when sufficient time has elapsed so that these readings vary very slowly. The readings are corrected to 25° as though the enthalpy change were zero.

In a few cases the electromotive force G_β was determined in the same cell with a voltage amplifier. Usually, however, the pH is determined in a Beckman meter modified so that the liquid junction with the salt is made at a 5-way stopcock from a MacInnes glass electrode.

We estimate that for the chlorides and iodides the precision of the pH measurements is 0.01 pH unit, and that the uncertainty of $E_{\alpha\beta}$ due to the drift varies from 0.03 mv. at 0.001 M to 0.3 mv. at 0.00003 M, and that the over-all uncertainty in each $E_{\alpha\beta}$ is less than 0.05 mv. greater than this. For $E_{\alpha\gamma}$ each of these uncertainties is about doubled. The measurements with thiocyanate and trichloroacetate are less precise.

The electromotive force of a cell in which there are liquid or membrane junctions was given by Scatchard[6] as

$$E\mathfrak{F}/RT = E_{0\alpha}\mathfrak{F}/RT - \Sigma_i\nu_{i\alpha}\ln a_{i\alpha} - \int_\alpha^\omega \Sigma_i t_i \mathrm{d}\ln a_i - \Sigma_i\nu_{i\omega}\ln a_{i\omega} - E_{0\omega}\mathfrak{F}/RT \quad (1)$$

in which E is the electromotive force, $\mathfrak{F}$ the Faraday, R the gas constant, T the absolute temperature, $E_{0\alpha}$ and $E_{0\omega}$ the normal electrode potentials, $\nu_{i\alpha}$ and $\nu_{i\omega}$ the moles of species i formed at the respective electrodes and t_i the number of moles of species i transferred with the positive current for each Faraday of electricity, and a_i is the activity of species i. For an anion t_i is negative, and for a neutral component it may be either positive or negative. The transport number is $T_i = t_i z_i$, if z_i is the valence of species i. T_i is positive for all ions and zero for a neutral component.

In our procedure the electrodes are symmetrical, or any small asymmetry is compensated by a blank, so we need consider only the transference integral. In measurements of $E_{\alpha\gamma}$ the salt-bridge junctions are symmetrical as are the membranes AA and AC, so that we consider the integral from a to b through A and from b to c through C. In measurements of $E_{\alpha\beta}$ we assume that the salt-bridge junctions are symmetrical by our definition of individual ion activities. It is convenient to consider the cation and anion exchangers separately and to divide the integral into the integral for a perfect membrane and a deviation term.

For the anion membrane and anion X with valence z_X, since $\Sigma_i t_i z_i = 1$

$$\int_a^b \Sigma_i t_i \mathrm{d}\ln a_i = \int_a^b \frac{1}{z_X}\mathrm{d}\ln a_X + \int_a^b \Sigma_i t_i\left(\mathrm{d}\ln a_i - \frac{z_i}{z_X}\mathrm{d}\ln a_X\right) = \frac{1}{z_X}\ln\frac{a_{Xb}}{a_{Xa}} + \int_a^b \Sigma_i t_i\left(\mathrm{d}\ln a_i - \frac{z_i}{z_X}\mathrm{d}\ln a_X\right) \quad (2)$$

and for the cation membrane and cation M with valence z_M

$$\int_b^c \Sigma_i t_i \mathrm{d}\ln a_i = \frac{1}{z_M}\ln\frac{a_{Mc}}{a_{Mb}} + \int_b^c \Sigma_i t_i\left(\mathrm{d}\ln a_i - \frac{z_i}{z_M}\mathrm{d}\ln a_M\right) \quad (3)$$

The results of our measurements of $E_{\alpha\gamma}$ with sodium chloride, hydrochloric acid, calcium chloride and mixtures of sodium chloride with hydrochloric acid and with sodium hydroxide and calcium chloride have been published.[7] The measurements with the other electrolytes show the same effects. Our solutions containing protein are so dilute that the measurements are not affected appreciably by the transfer of water or of the X^- ion through C or the M^+ ion through A, but they are affected by the dilute solution deviations, which are probably caused by part of the change in activity occurring in the transition zones between membrane and solution. We believe that we have taken care of a large part of this difficulty by expressing the relation of log $a_\pm$ to $E_{\alpha\gamma}$ and to $E_{\alpha\beta}$ with appropriate deviation curves. Sometimes we must consider the hydrogen ion or the hydroxyl ion. We assume that

$$\log a_\pm + \frac{1}{2}\log(1 + 4a_{H^+}/a_{M^+})(1 + 0.25\, a_{OH^-}/a_{X^-})$$

is the same function of $E_{\alpha\gamma}$ in the presence of protein as in its absence, or we assume that $\log a_{X^-} + \log(1 + 0.25\, a_{OH^-}/a_{X^-})$ is the same function of $E_{\alpha\beta}$ in the presence of protein as in its absence, and that in the absence of protein $\log a_{X^-} = \log a_\pm$. We found that $u_{H^+}g_{Na^+}/u_{Na^+}g_H = 4$ and that $u_{OH^-}g_{Cl^-}/u_{Cl^-}g_{OH^-} = 0.25$, in which u_i is the mobility of species i, and $t_i = u_i m_i/\Sigma_j z_j u_j m_j$. The assumption of 0.25 for hydroxyl ion and the other anions leads to no appreciable error.

With the $\alpha\gamma$ cell and salt solutions we then calculate the product $(Na^+)(X^-)$, taking $g_\pm$ the same as at the same sodium ion concentration without protein. This gives the concentration of anion (X^-). With the $\alpha\beta$ cell we take g_X the same as $g_\pm$ at the same sodium ion concentration and calculate (X^-) directly. In either case we calculate the bound anion by the relation

$$\bar{\nu}_X = [m_X - (X^-)]/m_2 \quad (4)$$

We designate it as $\bar{\nu}_X^\gamma$ when determined from the $\alpha\gamma$ cell, and as $\bar{\nu}_X$ when determined from the $\alpha\beta$ cell.

In determining $\bar{\nu}_X^\gamma$ we have assumed that the only effect of protein on the mean activity of NaX is due to removal of the bound X^-, and also that the relation of log $a_\pm$ to $E_{\alpha\gamma}$ is the same when the activity of X^- is much smaller than that of Na^+. When the membranes are behaving as perfect elec-

(6) G. Scatchard, THIS JOURNAL, **75**, 2883 (1953).

(7) G. Scatchard, pp. 128-143 in "Ion Transport Across Membranes," H. T. Clarke, Ed., Academic Press, New York, N. Y., 1954; and Chapter 3 in Electrochemistry in Biology and Medicine, T. Shedlovsky, Ed., John Wiley and Sons, Inc., New York, N. Y., 1955.

trodes, this assumption is perfect, but when the deviations are important it may be quite in error.

Our equation for determining $\bar{\nu}_X$ from measurements with the $\alpha\beta$ cell is equivalent to assuming that the only effect of protein on the activity of X^- is due to removal of the bound X^-, and also that the ratio $g_X/g_\pm$ does not change when the concentration of the salt, without protein, changes from m_3 to (X^-). In dilute solutions we have considered these assumptions more probable than those made for the $\alpha\gamma$ cell. Our third reason for believing the $\alpha\beta$ cell measurements more trustworthy is that they were more precisely reproducible and more consistent with each other.

The assumptions made for the $\alpha\beta$ cell are somewhat different from those made by earlier workers. Distribution measurements all assume that $g_\pm$ is the same in the solution with protein as in the solution in equilibrium with it. Scatchard, Scheinberg and Armstrong[8] assumed that g_X is the same in a solution with protein as in one with the same salt concentration but without protein and that the Ag, AgX electrode measures the activity of the X^- ion at all concentrations studied. So they did not need to assume the constancy of the $g_X/g_\pm$ ratio. Carr,[9] who has also used exchanger electrodes, assumed that g_X is the same in the solution with protein as in the solution which gives the same value of $E_{\alpha\beta}$. This differs from our assumption by the ratio of $\gamma_\pm$ in the solutions without protein of concentrations m_3 and (X^-). The difference is not great but we believe that our assumption is more accurate by the amount of the difference. Our assumption is that made by Brønsted[10] and Guggenheim.[11] Scatchard and Breckenridge[12] have shown that it would be more accurate to multiply by the ratio of $(\gamma_{KX}/\gamma_{NaCl})^{1/2}$ at m_3 and at (X^-), if γ_{KX} is $\gamma_\pm$ for a solution of KX, etc.

The mixtures of sodium chloride with sodium hydroxide are treated in the same way as the solutions of isoionic albumin. When the ratio $(H^+)/(Na^+)$ is not very small only the $\alpha\beta$ cell can be used to determine the binding. Mixtures of sodium chloride with hydrochloric acid are treated as the $\alpha\beta$ cell with sodium chloride alone. For the solutions with acids it is assumed that g_X is the same as in the acid solution without protein which gives the same value of $E_{\alpha\beta}$, which is then the solution with the same equivalent concentration of free ions, so that the assumption is equivalent to that made for measurements with salts.

In the solutions with acid and with salt–acid mixtures the $\beta\gamma$ cell gives an independent check of the pH. The agreement with the glass electrode measurements is usually within 0.01 pH unit except when the acid concentration is very low and the potential drop across the membrane very large. We use the exchanger measurements, which require no amplifier, when $E_{\beta\gamma}$ is less than 20 mv. and the glass electrode for larger values. We have calculated $\bar{\nu}_H$, the average number of hydrogen ions bound to each molecule of albumin from the equations

$$\log (H^+) = -pH - \log g_{H^+} \quad (5)$$

$$\bar{\nu}_H = [m_5 - (H^+)]/m_2 \quad (6)$$

We have used the approximation

$$-\log g_{H^+} = 0.5\sqrt{I/2}/(1 + 2\sqrt{I/2}) \quad (7)$$

in which $I/2$ is the ionic strength.

The results are presented in Tables I–IV. The first columns give the concentrations of the components, expressed as $-\log m_3$, etc. Next is the pH, then is w' from eq. 8 and finally the number of ions bound, $\bar{\nu}_X$, $\bar{\nu}_X^\gamma$ and $\bar{\nu}_H$ as defined above. To save space the electromotive forces are not tabulated, but the reversal of our calculations of $\bar{\nu}$ will give the electromotive force which would be measured with perfect exchanger electrodes. The de-

TABLE I

ION BINDING TO ISOIONIC ALBUMIN

$-\log m_3$	$-\log m_2$	pH	w'	$\bar{\nu}_X$	$\bar{\nu}_X^\gamma$
		Albumin (2) and NaCl (3)			
4.070	3.861	5.13	0.0948	0.03	...
3.520	3.830	5.20	.0884	0.60	...
3.031	3.913	5.24	.0800	1.12	0.47
3.000	3.548	5.18	.0794	1.29	...
2.509	3.893	5.29	.0678	2.19	1.57
2.083	3.788	5.33	.0564	3.93	3.85
2.000	3.519	5.26	.0542	4.05	...
1.570	3.338	5.37	.0428	6.25	6.01
1.302	3.468	5.37	.0362	5.37	6.93
1.007	3.111	5.40	.0300	9.16	7.97
		Albumin (2) and NaI (3)			
3.392	3.972	5.19	0.0866	1.32	0.80
2.913	3.946	5.31	.0778	2.32	1.85
2.437	3.889	5.42	.0660	4.33	3.69
2.015	3.832	5.47	.0546	6.26	5.36
1.411	3.451	5.49	.0388	10.2	8.72
0.927	3.413	5.56	.0284	14.7	13.2
0.514	3.180	5.62	.0218	18.3	15.4
		Albumin (2) and NaSCN (3)			
3.570	3.832	5.29	0.0892	1.52	1.38
3.266	3.851	5.35	.0844	3.23	1.60
3.132	3.816	5.38	.0820	2.80	1.86
2.845	3.857	5.42	.0760	4.29	3.09
2.664	3.848	5.46	.0718	5.06	3.85
2.271	3.841	5.51	.0616	8.22	5.87
2.132	3.823	5.54	.0580	7.08	5.83
1.821	3.853	5.58	.0494	9.40	7.98
1.731	3.521	5.59	.0468	11.7	8.88
1.246	3.426	5.65	.0350	15.3	13.6
0.846	3.275	5.68	.0270	27.9	21.1
		Albumin (2) and NaO_2CCCl_3 (3)			
3.058	3.234	5.36	0.0806	1.43	1.38
2.748	3.200	5.41	.0738	2.17	2.67
2.332	3.224	5.49	.0632	6.17	6.00
2.028	3.221	5.53	.0550	8.43	7.98
1.714	3.221	5.60	.0464	10.9	10.4
1.306	3.231	5.73	.0364	12.3	13.3
1.115	3.202	5.80	.0322	16.6	17.2
0.718	3.215	5.97	.0248	25.4	18.9
0.712	3.205	5.98	.0248	30.7	34.7

(8) G. Scatchard, I. H. Scheinberg and S. H. Armstrong, Jr., THIS JOURNAL, **72**, 535, 540 (1950).

(9) C. N. Carr, *Arch. Biochem. Biophys.*, **40**, 286 (1952).

(10) J. N. Brønsted, THIS JOURNAL, **44**, 877, 938 (1922); **45**, 2898 (1923).

(11) E. A. Guggenheim, *Phil. Mag.*, [7] **19**, 588 (1935).

(12) G. Scatchard and R. G. Breckenridge, *J. Phys. Chem.*, **58**, 596 (1954).

TABLE II

ION BINDING TO ACID ALBUMIN

$-\log m_2$	$-\log m_2$	pH	w'	$\bar{\nu}_X$	$\bar{\nu}_H$
		Albumin (2) and HCl (5)			
3.713	4.114	4.83	0.0916	0.28	2.13
3.136	4.077	4.40	.0836	1.59	7.88
2.730	4.096	3.73	.0754	4.18	20.4
2.463	3.906	3.59	.0694	6.07	25.6
2.172	4.093	2.90	.0610	13.3	66.5
1.990	3.906	2.84	.0568	18.1	69.5
1.704	3.929	2.12	.0488	34.6	95.4
		Albumin (2) and HI (5)			
3.638	4.104	4.91	....	...	2.8
3.067	4.142	4.28	0.0822	1.91	11.1
2.554	4.189	3.36	.0716	9.12	36.2
2.043	4.178	2.58	.0582	29.0	91.0
1.555	4.183	1.75	.0438	52.5	116.0
		Albumin (2) and HSCN (5)			
3.515	3.782	4.85	....	...	1.76
2.985	3.795	4.65	0.0860	3.75	6.31
2.499	3.809	3.90	.0732	8.33	19.6
2.057	3.828	3.06	.0604	19.1	53.2
1.711	3.736	2.49	.0504	30.4	88.2

TABLE III

ION BINDING FROM SALT–ACID MIXTURES

$-\log m_3$	$-\log m_5$	$-\log m_2$	pH	w'	$\bar{\nu}_X$	$\bar{\nu}_X^\gamma$	$\bar{\nu}_H$
			Albumin (2), NaCl (3) and HCl (5)				
3.000	...	3.548	5.26	0.0794	1.29	0.72	...
3.051	3.955	3.815	5.07	.0804	1.40	.42	0.67
3.127	3.598	3.860	4.97	.0812	1.42	− .12	1.75
3.314	3.288	3.863	4.75	.0816	1.61	− .96	3.62
2.000	...	3.519	5.26	.0542	4.05	....	...
2.058	2.904	3.818	4.59	.0552	5.64		8.02
2.141	2.558	3.870	3.99	.0556	8.25		19.7
2.338	2.268	3.857	3.52	.0562	11.2		36.5
1.302	...	3.468	5.37	.0362	6.93		..
1.356	2.234	3.760	3.96	.0366	11.9		32.8
1.425	1.907	3.782	3.18	.0370	20.5		70.2
1.600	1.605	3.766	2.18	.0372	26.8		98.0

TABLE IV

ION BINDING FROM SALT-ALKALI MIXTURES

$-\log m_3$	$-\log m_7$	$-\log m_2$	pH	w'	$\bar{\nu}_X$	$\bar{\nu}_X^\gamma$	$\bar{\nu}_H$
			Albumin (2), NaCl (3) and NaOH (7)				
3.056	3.918	3.833	5.51	0.0794	0.96	0.16	− 0.82
3.125	3.604	3.848	5.60	.0794	0.71	−2.75	− 1.75
3.296	3.306	3.845	5.91	.0794	0.44	0.18	− 3.46
2.066	2.852	3.845	7.24	.0542	1.69	2.59	− 9.84
2.127	2.598	3.818	8.08	.0542	0.27	2.44	−16.6
2.307	2.296	3.824	10.31	.0542	−2.71	3.25	−32.2

viations appear to depend somewhat upon the details of construction and so are not of general interest.

The pH of Isoionic Solutions.—Probably the most direct evidence that the anions are bound to the albumin is the effect of neutral salts on the pH of isoionic albumin solutions. The pH of the deionized albumin solutions with $-\log m_2 = 3.85$ is 5.15. This corresponds to an average dissociation of a twentieth of a hydrogen ion from each albumin. When neutral salts are added the pH increases because the dissociation constants of the acid groups of the protein are decreased. The change of $\bar{\nu}_H$ is less than 0.05 and cannot explain the change in constants. The most direct explanation is that the change in charge of the protein molecule due to the combination of anions increases its electrostatic attraction for hydrogen ions.[13–15] For the association constant for the binding of an ion A, with valence z_A, at a site i on a protein is

$$\log K_{Ai} = \log K^\circ_{Ai} - 2\bar{z}_p z_A w/2.303 \qquad (8)$$

in which $\bar{z}_p$ is the average valence of the protein. For a spherical protein molecule with radius 30 Å., which corresponds to the molal volume of the hydrated serum albumin molecule, and small ions with radii 2.5 Å. in water at 25° the Debye theory gives[16,8]

$$w' = 2w/2.303 = 0.1034 - 1.017\sqrt{I/2}/(1 + 10.663\sqrt{I/2}) \qquad (9)$$

Since the hydrogen ion concentration is only 0.06 times that of the protein, the change in binding at any site is negligible and

$$p\mathrm{H} - (p\mathrm{H})_0 = \log K_{Ai} - \log K^\circ_{Ai} = 2\bar{\nu}_X w/2.303 = \bar{\nu}_X w' \qquad (10)$$

Figure 2 shows pH *vs.* $\bar{\nu}_X w'$, with the values of pH and $\bar{\nu}_X$ taken from Table I. The line has unit slope. The agreement is almost within our estimate of the precision. We do not confirm the very large changes in pH for sodium trichloroacetate found by Scatchard and Black.[13] We believe that their salt must have contained some carbonate or bicarbonate.

Tanford, Swanson and Shore[17] take the same values as we do for the parameters of w', but for a molecular weight of 65,000, and introduce an empirical factor of 0.8 to fit their measurements. This gives a net ratio of 0.75. Their evidence from the quinazoline groups is not presented, that from the carboxyls is not clear enough to judge, but their Fig. 5 shows the evidence for phenolic and α-amino groups. It seems to us that much better agreement is obtained by using only the first four points of each curve and omitting the factor 0.8. The only part of their corroborative evidence which has been published is the work of Cannan, Kibrick and Palmer[18] on egg albumin, which is not corrected for the effect of chloride ion binding.[19] The work of these same authors[20] on lactoglobulin lacks this complication and their factor is unity.

It seems to us that the most direct evidence for any protein is that for albumin shown in Fig. 2. In all such calculations $\bar{\nu}$ is proportional to the chosen molecular weight and the calculated w' is approximately proportional to the cube root of its reciprocal, so that $\bar{\nu}w'$(calcd.) is proportional to

(13) G. Scatchard and Elizabeth S. Black, *J. Phys. Colloid Chem.*, **53**, 88 (1949).

(14) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51**, 660 (1949).

(15) Since the publication of these papers, we have been reminded that G. Sandor, *Bull. soc. chim. biol.*, **18**, 877 (1936); **19**, 555 (1937), was the first to measure the effect of neutral salts on the isoionic pH. His explanation is very different from ours.

(16) G. Scatchard, A. C. Batchelder and A. Brown, THIS JOURNAL, **68**, 2320 (1946).

(17) C. Tanford, S. A. Swanson and W. S. Shore, *ibid.*, **77**, 6414 (1955).

(18) R. K. Cannan, A. C. Kibrick and A. H. Palmer, *Ann. N. Y. Acad. Sci.*, **41**, 243 (1941).

(19) G. Scatchard, *American Scientist*, **40**, 61 (1952).

(20) R. K. Cannan, A. H. Palmer and A. C. Kibrick, *J. Biol. Chem.*, **142**, 803 (1942).

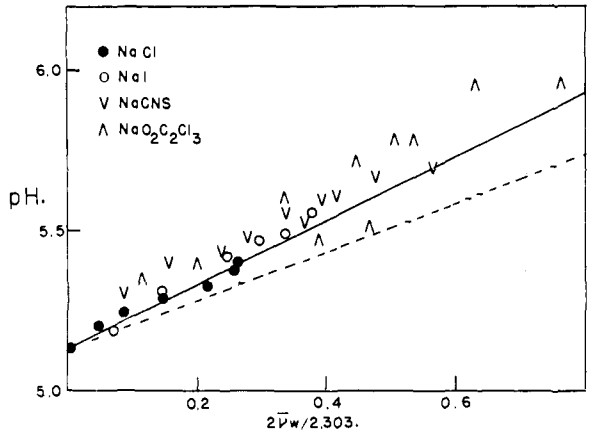

Fig. 2.—Effect of salts on isoionic pH.

the two thirds power of the molecular weight. Aside from that the slope in Fig. 2 depends only upon the assumption that the decrease in anion activity is due to binding. There is no need to assume the available number of sites of any kind. The slope corresponding to that of Tanford, Swanson and Shore is shown by a broken line in Fig. 2.

Anion Binding to Isoionic Albumin.—It appears that at saturation an anion is bound to each of the approximately hundred cationic groups in serum albumin.[21] In the range of our measurements we find no more than 35 bound. These cannot all have the same intrinsic constants. As in titration curves, the sites occur in classes, within each of which the variation in intrinsic constants is small. Unlike the titration curves, however, these classes do not seem to be related to the different kinds of cationic groups. This must mean that the constants depend upon units in the environment of the nitrogens which are larger than the single amino acid residues.

For the binding of an anion, A, to several classes of sites with the constant K°_{iA} for each of the n_i sites in class i, we have[8]

$$\nu_A = \Sigma_i \bar{\nu}_{iA} = \Sigma_i \frac{n_i K^\circ_{iA} a_A e^{-2w\bar{z}_p z_A}}{1 + K^\circ_{iA} a_A e^{-2w\bar{z}_p z_A}} = \Sigma_i \frac{n_i K^\circ_{iA} \alpha_A}{1 + K^\circ_{iA} \alpha_A} \quad (11)$$

This defines α_A as $a_A e^{-2w\bar{z}_p z_A}$.

If there is only one class of sites, a plot of $\bar{\nu}_A/\alpha_A$ *vs.* $\bar{\nu}_A$ is a straight line with intercepts $\bar{\nu}_A/\alpha_A = nK$ and $\bar{\nu}_A = n$,[14] so the slope is $-K^\circ_A$. Figure 3 shows $\bar{\nu}_A/\alpha_A$ *vs.* $\bar{\nu}_A$ for albumin with NaCl and with NaI. The ordinate for NaI is divided by 3.85 to make the plots fall together. The curvature of the line shows clearly that one class of sites is insufficient. If there is more than one class, the curve is concave upwards as in Fig. 3. At $\bar{\nu}_A = 0$ the intercept is $\bar{\nu}_A/\alpha_A = \Sigma_i n_i K^\circ_{iA}$ and the asymptotic slope is $-\Sigma_i n_i K^{\circ 2}_{iA}/\Sigma_i n_i K^\circ_{iA}$. At $\bar{\nu}_A/\alpha = 0$ the intercept is $\bar{\nu}_A = \Sigma_i n_i$ and the asymptotic slope is $-\Sigma_i n_i/(\Sigma_i n_i/K^\circ_{iA})$. The limits at $\bar{\nu}_A/\alpha_A = 0$ are usually indeterminate, but those at $\bar{\nu}_A = 0$ may be determined with moderate precision. The product of the intercept and the asymptotic slope at $\bar{\nu}_A = 0$ is

$$\Sigma_i n_i K^{\circ 2}_{iA} = n_1 K^{\circ 2}_{1A} \left(\Sigma_i \frac{n_i K^{\circ 2}_{iA}}{n_1 K^{\circ 2}_{1A}} \right)$$

(21) G. E. Perlman, *J. Biol. Chem.*, **137**, 707 (1941).

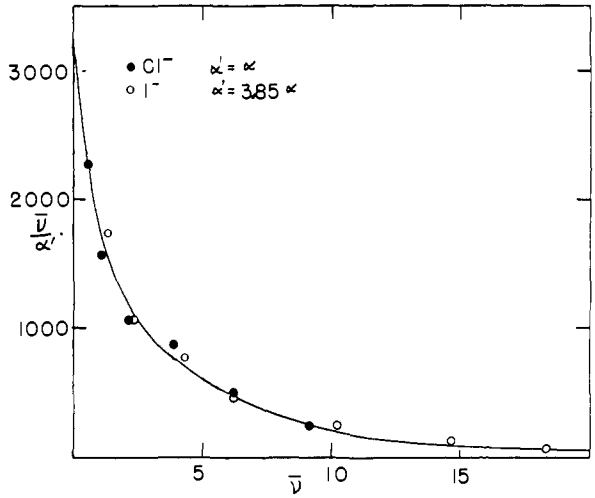

Fig. 3.—Anion binding to isoionic bovine serum mercaptalbumin.

which usually differs but little from $n_1 K^{\circ 2}_{1A}$, and the intercept on the $\bar{\nu}_A$ axis of the asymptotic tangent is

$$(\Sigma_i n_i K^\circ_{iA})^2/\Sigma_i n_i K^{\circ 2}_{iA} = n_1 \left(\frac{\Sigma_i n_i K^\circ_{iA}}{n_1 K^\circ_{1A}} \right)^2 \Big/ \Sigma_i \frac{n_i K^{\circ 2}_{iA}}{n_1 K^{\circ 2}_{1A}}$$

which is somewhat greater than n_1. If n_1 is an integer, it may be determined with some precision, and K°_{1A} may be determined from $n_1 K^{\circ 2}_{1A}$ and n_1. Thus $\bar{\nu}_{1A}$ is determined for each value of $\bar{\nu}_A$.

From the same operation on $(\bar{\nu}_A - \bar{\nu}_{1A})/\alpha_A$ *vs.* $(\bar{\nu}_A - \bar{\nu}_{1A})$, n_2 and K°_{2A} may be determined, and from $(\bar{\nu}_A - \bar{\nu}_{1A} - \bar{\nu}_{2A})/\alpha_A$ *vs.* $(\bar{\nu}_A - \bar{\nu}_{1A} - \bar{\nu}_{2A})$, n_3 and K°_{3A} may be determined, etc., until the last plot gives a straight line within the errors of measurement.

We have preferred to use another method, which depends upon the superposition of a plot of $\log y = \log x/(1 + x)$ *vs.* $\log x$, on translucent paper, upon a plot of $\log \bar{\nu}_A$ *vs.* $\log \alpha_A$. If there is a single class of sites the curves will superpose when $y = \bar{\nu}_A/n$ and $x = K^\circ_A \alpha_A$, or

$$\log n = \log \bar{\nu}_A - \log y \quad (12)$$

and

$$\log K^\circ_A = \log x - \log \alpha_A \quad (13)$$

If there is more than one class of sites, n_1 and K°_{1A} may be determined by superposing the curves at the low values of $\bar{\nu}_A$, making use again of the assumption that each n_1 is an integer. We then repeat using $\bar{\nu}_A - \bar{\nu}_{1A}$ to determine n_2 and K°_{2A}, etc. After a sufficient number of parameters have been obtained, they are improved by working in the reverse direction. The determination of $(\bar{\nu}_A - \bar{\nu}_{1A})$, etc., may be hastened by an auxiliary plot of $\log (\bar{\nu}_A - \bar{\nu}_{1A})/\bar{\nu}_A$ *vs.* $\log \bar{\nu}_{1A}/\bar{\nu}_A$. The latter is read as the distance of the experimental point from the graph on translucent paper. Then the former, determined from the auxiliary graph, is used for the second graph.

Figure 4 shows $\log \bar{\nu}_A$ *vs.* $-\log \alpha_A$ for the results presented in Table I. The curves are for three classes with $n_1 = 1$, $n_2 = 8$, and $n_3 = 18$ and with $K^\circ_{1A} = 24\ K^\circ_{2A}$ and $K^\circ_{3A} = 0.033\ K^\circ_{2A}$; $K^\circ_{2A} = 100$ for the chloride, 385 for the iodide, and 1925

for the thiocyanate and trichloroacetate. The curve in Fig. 3 is for the same values of the parameters.

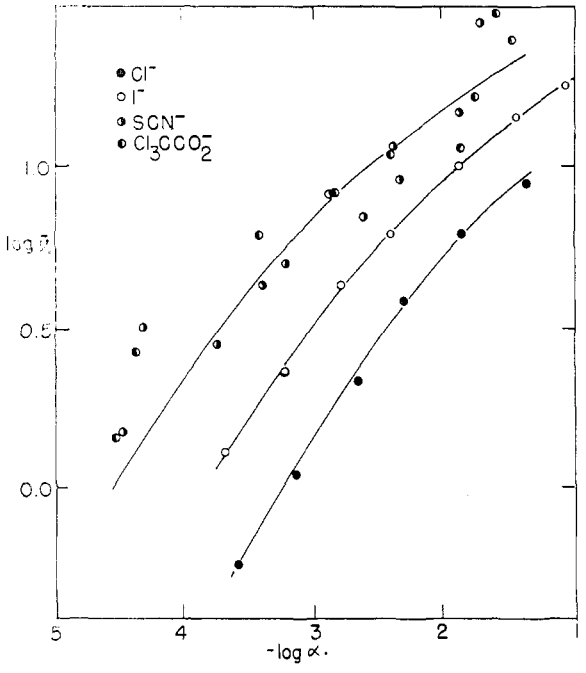

Fig. 4.—Anion binding to isoionic bovine serum mercaptalbumin.

The results with chloride and iodide cannot be fitted with less than three classes and are best fitted by assuming that a single group binds much more tightly than any other. The number of groups in the successive classes are less well determined, but we estimate that $n_2 = 7 \pm 1$, and $n_3 = 18 \pm 4$. Our results indicate clearly that the chloride and iodide ions are bound at the same sites, and that the ratio of the association constants K_{iI}/K_{iCl} is the same for each of these sites. The results are not precise enough to preclude two groups in the most active class. They may also be fitted, though not quite so well, by assuming $n_1 = 2$, $n_2 = 6$, $n_3 = 18$, $K^\circ_{1Cl} = 1000$, $K^\circ_{2Cl} = 100$, $K^\circ_{3Cl} = 5$.

The less precise measurements with thiocyanate and trichloroacetate show no difference between the two. They might be fitted with a large number of sets of n's and K's, but none would be appreciably better than the one we have chosen.

It is certain that the serum albumin is not homogeneous, but that it contains a small amount of a larger molecule which is most probably a polymer and quite probably a dimer. If the protein is homogeneous except for aggregation, we expect an integral number of sites per monomer unit, including the sites which have reacted to form the aggregate. We assume that the mole fraction of dimers is x, that of monomers $(1 - x)$ and that n_{ib} of the n_{im} groups in each monomer are used up in the dimerization. If the molecular weight of the monomer is M_m, the number average molecular weight is $M_N = (1 + x)M_m$. We will call the assumed molecular weight M. Then the number of i sites per weight M is

$$[1 + x(1 - n_{ib}/n_{im})]n_{im} \qquad (14)$$

The term in the brackets may vary from $(1 - x)$ when all the i sites in both molecules are used up in dimerization, through unity, when the sites on only one molecule are used up, to $(1 + x)$ when none of the sites are used up. We chose a molecular weight of 69,000 because that is the number average molecular weight determined in this Laboratory[16] for crystallized bovine serum albumin. Since the calculations were made, the number average molecular weight of the sample we used has been determined in this Laboratory[22] as 71,000. The monomer molecular weight may be as low as the 66,000 found by Low[23] for human serum mercaptalbumin. Even with this large variation, the difference between the number of sites we calculate and the number in a monomer varies less than the uncertainties given above for the numbers, except for variations in the most active class. Although there is no real reason for limiting the values of n to integers when n_{ib}/n_{im} is not zero, there is also no real reason not to do so. The quantity most clearly given by binding measurements is $\Sigma_i \bar{\nu}_i k_i / M$.

The parameters we have obtained for chloride are very different from those of Scatchard, Scheinberg and Armstrong[5] with crystallized human serum albumin, which are 10 groups with $K^\circ_{1Cl} = 44$ and 30 groups with $K^\circ_{2Cl} = 1.1$. The most important difference between their protein and ours is probably that theirs was not deionized, rather than that it was human instead of bovine and was only two-thirds mercaptalbumin. If we assume that two fatty acid groups were bound tightly to their albumin and calculate the binding on the remaining 25 groups, with $-\bar{z}_p = \bar{\nu}_A + 2$, we obtain very good agreement with their binding values up to $\bar{\nu}_A$ about 12. For higher binding, the measurements show too much scatter for confident interpretation.

Our results are at least in qualitative agreement with those of Karush[24] on the competitive binding of *p*-(2-hydroxy-5-methylphenylazo)-benzoate and dodecyl sulfate ions. He found that one site, or possibly two, binds the latter tightly but not the former, and that there are two other groups, one with four or five sites the other with about 17 sites, which bind both ions.

Ion Binding to Acid Albumin.—The binding in acid solutions was studied in the hope that the electrostatic attraction would cause enough more binding that some of the weaker binding constants could be determined and in the hope that the measurements would give some insight into the anomalies of acid albumin solutions. The titration of bovine serum albumin with hydrochloric acid has recently been studied in the presence of various amounts of KCl by Tanford, Swanson and Shore,[17] who have discussed these anomalies and have used the binding of chloride ion presented here in the interpretation of their results. Our measurements have the advantages that the bindings of both proton and anion are measured in the same solution,

(22) J. Pigliacampi, not yet published.
(23) B. W. Low, THIS JOURNAL, **74**, 4830 (1952).
(24) F. Karush, *ibid.*, **72**, 2714 (1950). See also ref. 19.

and that the binding of anions with very different constants are studied. The fact that the anomalies and the increased binding appear together makes the study of either more complicated.

Again the most direct method of correlating our results is to plot the difference of the measured values in Tables II and III from ideal behavior as a function of that calculated for a sphere of given dimensions from the total charge. β_{meas} is this difference for the proton, and minus this difference for the anions, and β_{calcd} is the absolute value of $w'\bar{Z}_p$. In Fig. 2, the ideal behavior is directly measured with the salt free protein solution. In the acid solutions, however, the ideal behavior must be calculated from the dissociation constants. For the anions we have used the constants determined for the isoionic solutions, and for the acids we have used the results of Tanford, Swanson and Shore,[17] one site with pK 3.7, 99 with pK 4.0, and 16 with pK 6.9 in 65,000 g. Figure 5 shows as filled circles minus the differences between the measured pH and that calculated for the same proton binding, and as open circles the difference between minus the logarithm of the anion activity and that calculated for the same anion binding. In each case, the abscissas are calculated from eq. 8 and the measured net charge. A few of the anion points are missing because the measured binding is greater than 27, the maximum for which we have determined constants. However, most of the points are below the line of unit slope, indicating less anion binding than calculated from eq. 7. The proton points are still lower, which indicates more proton binding than calculated from eq. 7.

If the deviations for the proton in Fig. 3 were entirely due to an increase in size of the protein and the resultant decrease in w', this part would be the same for the anion and the differences in Fig. 3 could be used to measure the binding of anion not accounted for by the 27 most active sites. Calculations based on this assumption lead to an imaginary value for the next binding constant ($\bar{\nu}/\alpha$ increases with increasing $\bar{\nu}$). Further evidence that this effect is not electrostatic is the fact that the change in hydrogen binding comes at a higher pH than the expansion determined from viscosities and optical rotation.[17,25]

If the change is considered to be a change in acid dissociation constant without change in electrostatic action, the structural change which makes the proton bound more easily makes anions bound less easily at some of the sites or at all of them. This is an attractive hypothesis for it correlates three of the anomalies of serum albumin: the strong binding of anions, the weak binding of protons to carboxyls near the isoionic point and the much less weak binding at low pH. The same structural peculiarity may account for all.

The abscissas of Fig. 5 are proportional to the electrostatic energies of the protein ion. If the deviations were due to changes in size with constant restoring force, they should be the same function of this change for each series—chloride, iodide, thiocyanate and chloride in mixed acid and salt. If the deviations are functions of some other property, we should compare the deviations from some simple function which represents the trend of the measurements better than β_{calcd}. We have chosen $\beta_{calcd}/(1 + 0.6\ \beta_{calcd})$, represented by the broken line, which gives an approximate average. The agreement is altered but very little if these deviations are plotted against the net charge, or against the pH. The results for chloride in acid–salt mixtures are brought more nearly in line with the other series if these deviations are plotted against the number of bound protons. This would be expected if the change is due to loosening of specific bonds as suggested by both Tanford and Foster. However, the improvement is too small to make very cogent evidence.

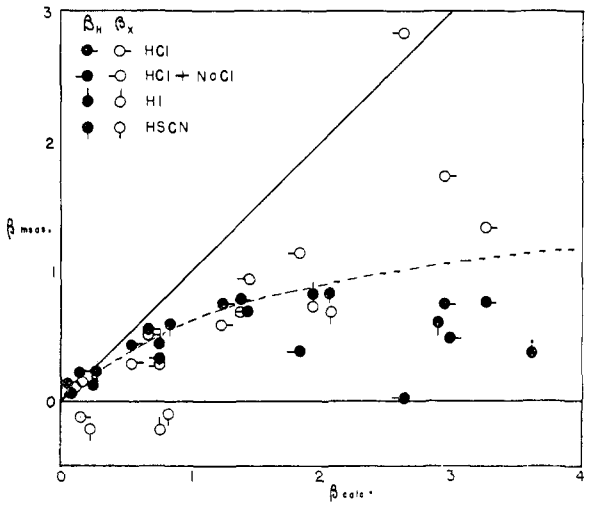

Fig. 5.—Deviations in acid solutions.

General.—A few measurements with sodium hydroxide and sodium chloride at constant total sodium concentration are listed in Table IV. They show the expected decrease in chloride binding due to the increased negative charge, and also show the difficulties with alkaline solutions. These difficulties deterred us, perhaps with insufficient reason, from making measurements at 0.01 M sodium ion and moderately low pH. However, most of the knowledge which could be obtained from these measurements can also be obtained from the measurements with isoionic albumin.

We were unable to obtain the association constants for more than the 27 most active groups because of the anomalies in acid solutions, but these measurements do show that at low pH albumin binds more protons and fewer anions than are calculated from the binding to isoionic albumin, even though some anions are bound at sites at which the constants are too small to give measurable binding with isoionic albumin.[26] The measurements with isoionic albumin together with qualitative results of others at high binding show that there are at least four classes of binding sites for anions in serum albumin, and we have calcu-

(25) J. T. Yang and J. F. Foster, THIS JOURNAL., **76**, 1588 (1954).

(26) The statement that one of us (GS) said that new anion binding sites appear at the point of acid expansion[17] is due to a misunderstanding. We know of no evidence for such a phenomenon.

lated the approximate number and constant for the three most active classes. We have been unable to determine the nature of these sites beyond the indication that some at least are associated with some of the anomalous carboxyl groups.

CAMBRIDGE 39, MASS.

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

Molecular Shape and Rotational Freedom in the Tetrahalogenated Methanes in the Solid State[1]

BY ROBERT C. MILLER[2] AND CHARLES P. SMYTH

RECEIVED JULY 9, 1956

The dielectric constants of ten unsymmetrical tetrahalogenated methanes have been measured in the liquid and solid states at a frequency of 5 kc. over wide temperature ranges extending, in some cases, down to −196°. The freezing points and transition points shown by the dielectric constant–temperature curves are tabulated together with those given in the literature for the four symmetrical tetrahalogenated methanes. When the van der Waals radii along the carbon–halogen axes from the carbon nucleus differ by no more than 9%, molecular rotational freedom is observed in the solid in the temperature region between the freezing point and a rotational transition point. The effect upon the apparent dielectric constant value of shrinkage of the solid between the condenser plates with falling temperature is treated approximately. Specific volume is measured as a function of temperature for dibromodichloromethane and bromotrichloromethane, which have very small dipole moments, and used to calculate approximately the true dielectric constant–temperature curves. For substances in which the orientation polarization is small in comparison with the total polarization, these corrected curves are very different in shape from the uncorrected. The curves for several particularly pure substances indicate the existence of some molecular mobility for several degrees below the freezing point, confirming conclusions based on previous measurements.

Among many more or less symmetrical molecules which have been investigated for possible hindered rotation or order–disorder orientation in the solid state, methane,[3] deuteromethane[3] and the tetrasubstituted methanes[3] have seemed to offer an exceptionally good opportunity for controlled variation in the factors affecting molecular behavior. The critical wave lengths or dielectric relaxation times of a considerable number of tetrasubstituted methanes containing methyl and chloro, bromo, iodo or nitro groups in the pure liquid state or in solution have been measured.[4,5] Six tetrahalogenated methanes have been similarly investigated in the pure liquid state.[6] The present paper reports low frequency dielectric constant measurements upon these six substances in the solid state and similar measurements upon four other tetrahalogenated methanes. It also lists similar rotational transition points for the four symmetrical tetrahalogenated methanes,[7] whose non-polarity makes them less significant for dielectric measurements.

Purification and Properties of Materials

The authors wish to express their gratitude to E. I. du Pont de Nemours and Company for the gift of three of the substances in very pure form and to the Dow Chemical Company for successive samples of the difficultly obtainable tribromochloromethane. The other compounds were procured from Matheson, Coleman and Bell, Inc., and from Halogen Chemicals, Inc., as indicated in Table I. The sharpness of the freezing points on the dielectric constant–temperature curves shows high purity for all except, possibly, the tribromofluoromethane sample. The sources, freezing points and boiling points of the samples are listed in Table I.

TABLE I

SOURCE, PURIFICATION, MELTING POINT AND BOILING POINT OF COMPOUNDS

Compound	Source	M.p., °C. Obsd.	M.p., °C. Lit.	B.p., °C. Obsd.	B.p., °C. Lit.
CF_3Cl[a]	Matheson	−189.0	−181	−81.9	−80
CF_2Cl_2[a]	Matheson	−158.2	−160	−24.9	−28
CCl_3F[b]	Matheson	−109.5	−111	+24.4	24.1
CF_3Br[a]	du Pont	−175.5		−57.2	
CF_2Br_2[a]	du Pont	−110.1		+23.9	
CBr_3F[b]	Halogen	−74.5		+106	
CCl_3Br[c]	Matheson	−5.8	(−21)	103	104.1
CCl_2Br_2[d]	Matheson	+21.8	22	.....	
CBr_3Cl[d]	Dow	+55	55	.....	
CF_2BrCl[a]	du Pont	−159.5		−3.3	

[a] Distilled from cylinder directly into cell. [b] Fractionally distilled. [c] Dried over barium oxide, distilled and fractionally crystallized at the freezing point. [d] Washed with sodium thiosulfate, dried with barium oxide and fractionally crystallized at the freezing point.

Experimental Methods and Results

The dielectric constants ϵ' were measured at a frequency of 5 kilocycles over a wide range of temperature by means of the cell and impedance bridge previously described.[8,9] The dielectric losses at this frequency were negligibly small. The experimental results are shown in the accompanying graphs of dielectric constant plotted against temperature. The actual experimental points are so numerous and lie so well on the curves that they are omitted from the diagrams.

The dipole moments[6] of these molecules are so small that the orientation polarization due to the dipoles may, in some cases, be of the same order of magnitude as the changes of polarization accompanying density changes arising from phase transitions or from considerable changes in temperature. The effect of these density changes has been explored by measuring the densities of dibromodichloromethane and tribromochloromethane over a range of temperature from the melting point of mercury to about 10° above the melting

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(1) This research has been supported in part by the Office of Naval Research. Reproduction, translation, publication, use or disposal in whole or in part by or for the U. S. Government is permitted.

(2) Supported by a grant-in-aid to the Chemistry Department, Princeton University, from E. I. du Pont de Nemours and Company.

(3) C. P. Smyth, "Dielectric Behavior and Structure," McGraw-Hill Book Co., New York, N. Y., 1955, pp. 142, 144, 161–163.

(4) C. P. Smyth, ref. 3, pp. 122–125.

(5) For recent measurements and references to previous work, see R. S. Holland, G. N. Roberts and C. P. Smyth, THIS JOURNAL, **78**, 20 (1956).

(6) R. C. Miller and C. P. Smyth, *J. Chem. Phys.*, **24**, 814 (1956).

(7) L. Deffet, "Composés Organiques Polymorphes," Editions Desoer, Liège, 1942, p. 15.

(8) C. P. Smyth and C. S. Hitchcock, THIS JOURNAL, **54**, 4631 (1932); **55**, 1830 (1933).

(9) J. D. Hoffman and C. P. Smyth, *ibid.*, **71**, 431 (1949).