

Deriving Equations to Account Quantitatively for the Effect of Neutral Salts on the pH of Isoionic Bovine Serum Mercapt-albumin without Assuming Anion Binding

B. N. GHOSH

University College of Science, Department of Chemistry, Calcutta-700009

Manuscript received 29 July 1975 ; accepted 22 September 1975

As protein molecules at a concentration of about 0.2 per cent can cover the surface of particles of glass, quartz etc. so it has been assumed that the surface of a glass electrode, placed in a solution of concentration above 0.7 per cent of bovine serum mercapt-albumin (BSA) at isoionic pH or above it, is almost completely covered by the protein molecules. On the basis of this assumption and the theory of colloidal electrolytes proposed by the author equations have been deduced which can quantitatively account for the rise in pH of isoionic (BSA) caused by the addition of neutral salts, without assuming anion binding.

It has also been shown that the number of proton donors per mole of (BSA) (molecular weight 69,000), dissociating in the pH range 5.1 to about 7.5, is 12.6 and their pK is 6.18.

SCATCHARD, Coleman and Shen¹ have measured the pH of isoionic (BSA) solution and its mixtures with neutral salts like NaCl using glass electrode and the anion activity of the mixtures by using resin membrane electrode. They have noticed that the p^H of the (BSA) solution increases with the concentration of the neutral salt added. To account for this observation, they have assumed that there are groups of sites on the surface of the spherical (BSA) macromolecule which can bind the anions of the added salt. Owing to such anion binding the electrostatic attraction of the (BSA) macromolecules for the H^+ ions increases and they are withdrawn from the bulk of the solution. On the basis of this theory they have deduced the following equation which agrees fairly with their experimental data,

$$pH = pH_0 + \nu_x W' \quad \dots \quad (1)$$

where $pH_0 = 5.13$ a constant, ν_x the number of anions bound by a mole of (BSA) and W' has been calculated from the ionic strength of the solution and some constants, for the details of which their paper may be consulted.

According to Nagasawa and Holtzer² the rise of pH of isoionic (BSA) on addition of neutral salts may be due to specific conformation of discrete charges rather than by ion binding. The observations of Scatchard, Coleman and Shen¹, it will be shown here, can be accounted for on the basis of the theory of colloidal electrolytes proposed by the author³⁻⁸.

It is well-known⁹⁻¹³ that protein molecules at a concentration of about 0.2 per cent can cover almost completely the surface of particles of glass, quartz and other indifferent materials. It is, therefore, reasonable to assume that when the concentration used is above

0.7 per cent, the (BSA) molecules will almost completely cover the surface of the electrodes immersed in their solution. Therefore, the glass electrode and the resin membrane electrode will indicate the H^+ ion activity and the anion activity respectively of the diffuse double layer of the (BSA) molecules. Since the (BSA) molecules are negatively charged at the isoionic pH and above it, so according to Boltzmann's law* the activity of the anions in the diffuse double layer will be lower than that in the intermicellar solution and also lower than that in the salt solution initially taken. Thus on the indications of the resin membrane electrode no definite conclusion on anion binding can be drawn.

Derivation of the equations : It has been shown in previous papers^{5,8} that the neutralisation of a colloidal acid by an alkali can be represented by the following equation

$$pH = p(\theta K_a) + n \log \alpha / (1 - \alpha) \quad \dots \quad (2)$$

where K_a is the dissociation constant of the acid, θ , the fraction per unit area of the electrode surface covered by the diffuse double layers of the colloidal particles. $\theta = C / (K + C)$ where C is the concentration (unit arbitrary) of the colloidal particles and K , a constant.

When the surface of the electrode is almost entirely covered by the (BSA) molecules, θ becomes nearly equal to unity and under this condition it has been found¹⁴ that n also becomes unity. Therefore, equation (2) may be written as follows :

$$pH = pK + \log \alpha / (1 - \alpha) \quad \dots \quad (2a)$$

At and above the isoionic pH the (BSA) molecules are negatively charged. Therefore, the H^+ ion activity

*When Boltzmann's distribution law written as $n = n_0 \exp(-eE/kT)$ is applied to a (uni-univalent) electrolyte, n and n_0 should be expressed as activities and not number of ions per unit volume.

in their diffuse double layer must be greater than that in the intermicellar solution. When NaCl is added to the (BSA) solution, some Na⁺ ions enter the diffuse double layer and displace an equal number of H⁺ ions which enter the intermicellar solution. This disturbs the equilibrium between the H⁺ ions in the diffuse double layer and the proton donors in the Stern¹⁵ Mukherjee¹⁶ layer and to restore it a few proton donors dissociate. Therefore, the net effect of addition of a neutral salt like NaCl is that the degree of dissociation, α of the proton donors increases and the activity of the H⁺ ions in the diffuse double layer decreases. Let y be the number of proton donors in a mole of (BSA) (molecular weight 69,000) ionising in the pH range 5.1 to about 7.5 and x of them dissociate at a given pH, so that $\alpha = x/y$. Substituting x/y for α in equation (2a) it may be written as follows ;

$$pH = pK + \log x/(y - x) \quad \dots \quad (3)$$

To ascertain how x increases with the concentration of the added salt we may apply virial expansion and express x as follows

$$x = x_0 + A\sqrt{R} + A'R + \text{etc.} \quad \dots \quad (4)$$

where R is the ratio of the stoichiometric concentration of salt to that of (BSA) and A and A' are constants. It has been found that A' is very small compared to A and

negative in sign. Therefore, neglecting higher order terms equation (4) is written as follows

$$x = x_0 + A\sqrt{R}(1 - \frac{A'}{A}\sqrt{R}) \quad \dots \quad (4a)$$

$$= x_0 + \frac{A\sqrt{R}}{1 + B\sqrt{R}} \quad \dots \quad (5)$$

where $B = A'/A$.

The values of the constants pK; y and x₀ have been found by trial as explained in the section entitled "Discussion" and are 6.18, 12.6 and 1.02 respectively. Using these values of the constants, A and B have been found from two known values of pH and the corresponding \sqrt{R} with the help of the equations (3) and (5). Throughout this paper the values of pK=6.18, y=12.6 and x₀=1.02 have been used and x has been calculated by using equation (5).

Experimental results of Scatchard, Coleman and Shen with explanatory notes : In Table 1 are recorded the values of pH, -log m₁ and -log m₂ taken from Table 1 of the paper of Scatchard, Coleman and Shen¹, m₁ and m₂ represent respectively the stoichiometric concentrations of (BSA) and the neutral salt per kg. of water. It may be mentioned that A, B, R, x and log x/(y - x) have the same significance as stated already.

TABLE 1—EFFECT OF NEUTRAL SALTS ON THE pH OF ISOIONIC (BSA) SOL.

Values of A and B	-log m ₁	-log m ₂	\sqrt{R}	x	$\log \frac{x}{y-x}$	pH observed	pH calculated from Eqn (3)	pH calculated from Eqn.(1)
For NaCl	3.861	4.070	0.79	1.10	-1.02	5.13	5.16	5.13
A=0.1	3.830	3.520	1.43	1.16	-0.99	5.20	5.19	5.18
B=0.033	3.913	3.031	2.77	1.27	-0.95	5.24	5.23	5.22
	3.348	3.000	1.88	1.20	-0.98	5.18	5.20	5.23
	3.893	2.509	4.92	1.44	-0.89	5.29	5.29	5.28
	3.788	2.083	7.12	1.60	-0.84	5.33	5.34	5.35
	3.519	2.000	5.74	1.50	-0.87	5.26	5.31	5.35
	3.338	1.570	7.66	1.63	-0.83	5.37	5.35	5.40
	3.468	1.302	12.10	1.88	-0.76	5.37	5.42	5.32
	3.111	1.007	11.30	1.84	-0.77	5.40	5.41	5.40
For NaI								
A=0.16								
B=0.05	3.972	3.392	1.95	1.31	-0.94	5.19	5.24	5.24
	3.946	2.913	3.29	1.47	-0.88	5.31	5.30	5.31
	3.889	2.437	5.32	1.69	-0.81	5.42	5.37	5.42
	3.832	2.015	8.10	1.95	-0.74	5.47	5.44	5.47
	3.451	1.411	10.47	2.12	-0.69	5.49	5.49	5.53
	3.413	0.927	17.50	2.51	-0.61	5.56	5.57	5.55
	3.180	0.514	21.50	2.68	-0.57	5.62	5.61	5.53
For NaCNS								
A=0.25								
B=0.05	3.832	3.570	1.35	1.34	-0.92	5.29	5.26	5.27
	3.851	3.266	1.96	1.47	-0.88	5.35	5.30	5.40
	3.816	3.132	2.20	1.52	-0.86	5.38	5.32	5.36
	3.857	2.845	3.20	1.71	-0.80	5.42	5.38	5.46
	3.848	2.664	3.87	1.83	-0.77	5.46	5.41	5.49
	3.841	2.271	6.10	2.19	-0.68	5.51	5.50	5.64
	3.823	2.132	7.01	2.32	-0.65	5.54	5.53	5.54
	3.853	1.821	10.37	2.73	-0.56	5.58	5.62	5.57
	3.521	1.731	7.85	2.43	-0.62	5.59	5.56	5.68
	3.426	1.246	12.30	2.92	-0.52	5.65	5.66	5.67
	3.275	0.846	16.40	3.27	-0.46	5.68	5.72	5.88
For CCl ₃ COONa								
A=0.40								
B=0.05	3.234	3.058	1.22	1.48	-0.88	5.36	5.30	5.25
	3.200	2.748	1.68	1.64	-0.82	5.41	5.36	5.29
	3.224	2.332	2.79	2.00	-0.72	5.49	5.46	5.51
	3.221	2.028	3.95	2.34	-0.64	5.53	5.54	5.59
	3.221	1.714	5.67	2.79	-0.55	5.60	5.63	5.64
	3.231	1.306	9.17	3.53	-0.41	5.73	5.77	5.58
	3.202	1.115	11.05	3.87	-0.35	5.80	5.83	5.66
	3.215	0.718	17.78	4.78	-0.21	5.97	5.97	5.76
	3.205	0.712	17.64	4.77	-0.22	5.98	5.96	5.90

TABLE 2—pK OF THE GROUP OF PROTON DONORS IONISING BETWEEN pH 5.1 AND 7.5

$-\log m_1$	$-\log m_2$	$-\log m_3$	\sqrt{R}	x	$(\alpha)y$	$\log \frac{\alpha}{1-\alpha}$	pH observed	pK
3.833	3.056	3.918	2.45	1.25	2.07	-0.71	5.51	6.22
3.848	3.125	3.604	2.30	1.23	2.98	-0.51	5.60	6.11
3.845	3.296	3.306	1.88	1.20	4.66	-0.23	5.91	6.14
3.845	2.006	2.852	7.75	1.64	11.48	+1.01	7.24	6.23
							Average 6.18	

In Table 2 are recorded the values of $-\log m_1$, $-\log m_2$, $-\log m_3$ and pH taken from Table 4 of the paper of the aforesaid authors¹. m_3 is the concentration of NaOH in moles per kg. of water and the other terms have the same significances as stated already. The value of the degree of dissociation α of the proton donors y in number, has been calculated from the following expression,

$$\alpha = [(x/y) + (m_3/ym_1)] \quad \dots \quad (6)$$

where x/y and m_3/ym_1 represent the contributions of the added NaCl and NaOH respectively. In calculating x , the values of A and B used are the same as those used for NaCl in Table 1 and pK has been calculated using equation (2a).

Discussion

The values of pK, y and x_0 have been found in the following way. Using the data in Table 4 of Scatchard, Coleman and Shen's¹ paper when pH is plotted against NaOH added per mole of (BSA) an abrupt rise in the curve begins near pH 7.24 indicating that the end point of titration is being approached and that it lies between 12 and 13 moles of NaOH per mole of (BSA). The pH corresponding to the mid-point of titration is about 6.3 but the correct value should be lower than this, because a considerable amount of NaCl is present in the system. Furthermore, the pH of the isoionic (BSA), in the presence of the lowest concentration $10^{-4.07}$ mole of NaCl per kg. of water, has been found to be 5.13. Therefore, in the absence of NaCl the pH of the isoionic (BSA) sol should be slightly lower than 5.13. Bearing these points in mind different probable values have been tried and it has been found that pK=6.18, $y=12.6$ and $x_0=1.02$ fairly satisfy the aforesaid requirements. Using these values of pK, y and x_0 the values of A and B in equation (5) have been found in the way discussed already.

It will be noticed from the data recorded in Table 1 that the rise of pH of the (BSA) sol. with the increasing concentration of NaCl or NaI can be accounted for

fairly satisfactorily by both the equations (3) and (1) deduced by the author and Scatchard, Coleman and Shen respectively. The data obtained by using NaCNS and CCl_3COOH however, agree somewhat better with equation (3).

The data recorded in Table 2 show that the average value of pK of the group of proton donors ionising between pH 5.1 and 7.5 is 6.18. Fresh determination of pK without adding any salt to (BSA) and titrating it with NaOH in the pH range 5.0 to 8.0 appears desirable.

For interpreting the data on activity measurements of small ions correctly, in the presence of protein macroions, the fraction of the electrode surface covered by the protein ions should be taken into consideration.

References

- G. SCATCHARD, J. S. COLEMAN and A. L. SHEN, *J. Amer. Chem. Soc.*, 1957, 79, 12.
- M. NAGASAWA and A. HOLTZER, *J. Amer. Chem. Soc.*, 1964, 86, 531.
- B. N. GHOSH, *J. Indian Chem. Soc.*, 1971, 48, 185.
- B. N. GHOSH, *J. Indian Chem. Soc.*, 1972, 49, 359.
- B. N. GHOSH, *J. Indian Chem. Soc.*, 1972, 49, 847.
- B. N. GHOSH, *J. Indian Chem. Soc.*, 1973, 50, 114.
- B. N. GHOSH, *J. Indian Chem. Soc.*, 1973, 50, 463.
- B. N. GHOSH, *J. Indian Chem. Soc.*, 1974, 51, 57.
- H. FREUNDLICH, *Kapillarchemie* 11, Leipzig 1932, p. 449.
- A. SILBERBERG, *J. Chem. Phys.*, 1968, 48, 2835.
- F. TH. HESSELINK, *J. Phys. Chem.*, 1971, 75, 65.
- H. A. ABRAMSON, L. S. MAYER and M. H. GORIN, "Electrophoresis of Proteins." New York, 1942, p. 152, et. Seq.
- D. K. CHATTORAJ and H. B. BULL, *J. Amer. Chem. Soc.*, 1959, 81, 5128.
- B. N. GHOSH, *J. Indian Chem. Soc.*, 1975, 52, 567.
- O. STERN, *Z. Electrochem.*, 1924, 30, 50.
- J. N. MUKHERJEE, *Trans. Faraday Soc.*, 1920-21, 16, 103.