AMPHOTERIC BEHAVIOR OF COMPLEX SYSTEMS.

II. Titration of Sulfanilic Acid-Glycine Mixtures.*

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The considerations presented in the preceding paper were developed as an attempt to explain results obtained in studying living cells (1). It is desirable to test them on much simpler systems. Michaelis and Davidsohn (2), in some flocculation experiments, obtained data which support the above ideas in a qualitative way. They state that, if two amphoteric colloids are mixed, a combination may precipitate out whose flocculation optimum lies between their respective isoelectric points. Thus with nucleic acid and denatured serum albumin, with isoelectric points of 2×10^{-1} and 4×10^{-6} respectively, there is a combination with optimum flocculation at a hydrogen ion concentration of 1.6×10^{-4}.

It is the purpose of this and the following paper to test portions of the theory in a more quantitative manner, and in this paper the mutual action of two ampholytes, glycine and sulfanilic acid, is studied by means of titration over a wide pH range. The latter substance was chosen to correspond to the strongly acid nucleic acid in living matter. That it is amphoteric, with a measurable basic ionization constant, is shown in the fourth paper of the series. In fact it has an isoelectric point at a distinctly higher pH, 1.25, than that (0.7) of nucleic acid (2). The glycine used was a preparation from the Eastman Kodak Company; and the sulfanilic acid was a Merck C.P. grade, which was reprecipitated from alkaline solution by hydrochloric acid, washed, and dried.

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Method.

An ordinary potentiometer set up sensitive to 0.25 millivolt, was employed. The hydrogen electrodes were of the type described by Bailey (3). A saturated calomel electrode (4) was used in connection with a saturated KCl bridge.

A series of solutions of hydrochloric acid and carbon dioxide-free sodium hydroxide was made up, each solution of definite known con-

TABLE I.

HCl				NaOH			cOH
N	E.M.F.	pH	cH	N	E.M.F.	pH	
	mv.				mv.		
.001	426.	3.00	.001	.004	924.5	11.425	.00266
.003	399.	2.533	.00293	.009	943.	11.74	.0055
.006	380.	2.217	.00607	.015	958.	12.00	.010
.009	370.	2.050	.00891	.025	968.5	12.175	.015
.012	363.	1.933	.0117	.040	981.7	12.390	.0246
.018	353.	1.760	.0174	.060	990.3	12.540	.0347
.025	345.5	1.625	.0237	.080	997.5	12.660	.0458
.040	333.5	1.425	.0376	.100	1001.5	12.730	.0538
.060	323.	1.250	.0562	.130	1008.5	12.860	.0720
.090	313.	1.083	.0826	.180	1014.8	12.960	.0916
.120	305.	0.950	.1122	.250	1024.	13.117	.1310
.150	300.5	0.875	.1334	.3511	1030.	13.217	.165
.200	292.5	0.742	.181				
.250	287.8	0.663	.217				
.320	282.	0.56	.2754				

centration. Points on the titration curve were obtained by introducing 15 cc. of one of these solutions into a small glass-stoppered bottle with 10 cc. of a standard solution of the substance to be titrated, and the equilibrium hydrogen ion concentration of the resulting mixture determined. The difference between the normality of the acid or base diluted with 10 cc. of water and that diluted with 10 cc. of the glycine or the sulfanilic acid will give the amount neutralized. The original normality, N, is known and the latter can be obtained from the measured cH if we know the degree of ionization, *i.e.* the normality is equal to cH/α, where α is the degree of ionization, which value

must be determined *potentiometrically* (5). Thus the number of mols, n, of the HCl or the NaOH neutralized by the glycine or sulfanilic acid is given respectively by the expressions:

$$n = \text{N} - \frac{\text{cH}}{\alpha} \text{ for HCl; and } n = \text{N} - \frac{\text{cOH}}{\alpha} \text{ for NaOH}$$

The only assumption involved is that at the same normality the acid

TABLE II.

N HCl	E.M.F.	pH	cH	n
	mv.			
.320	285.	0.617	.2415	.041
.250	291.8	0.730	.1862	.0395
.200	298.5	0.842	.1439	.040
.150	307.5	0.992	.1019	.0390
.120	316.	1.133	.0736	.0405
.090	326.5	1.308	.0492	.0375
.060	344.5	1.608	.02466	.0345
.040	365.3	1.970	.01072	.0291
.025	388.	2.350	.00477	.0205
.018	400.5	2.558	.00277	.0152
.012	414.	2.800	.00159	.0104
.009	422.5	2.942	.00114	.00786
.006	434.	3.133	.000736	.00526
.003	455.5	3.492	.000322	.00268
.001	484.	3.98	.000105	.0009
.0000	598.	5.9	—	—
NaOH			cOH	
.004	766.	8.75	.0000056	.004
.009	790.	9.15	.000014	.009
.015	806.5	9.43	.000027	.015
.025	831.5	9.86	.000073	.0249
.0373	871.5	10.53	.00034	.0368
.040	895.	10.933	.00085	.0387
.060	964.	12.10	.01259	.040
.080	981.	12.383	.02415	.0397
.100	990.5	12.542	.03483	.0401
.130	1000.5	12.708	.05105	.0397
.180	1009.5	12.875	.0750	.0398
.250	1019.	13.033	.1079	.0405
.3511	1028.	13.183	.1524	.040

or alkali ionizes to the same extent in the presence of the glycine or sulfanilic acid as it does when these are absent.

Table I gives values of cH and cOH for various normalities of HCl and NaOH at room temperature, 20–22°C. By plotting N

TABLE III.

Sulfanilic acid.				Glycine.			
0.04 N		0.023 N		0.10 N		0.02 N	
pH	n	pH	n	pH	n	pH	n
				1.14	.097	0.913	.020
				1.483	.0864	1.033	.019
				2.26	.0544	1.183	.0194
				2.56	.0372	1.408	.0186
				2.86	.0236	1.638	.016
				3.033	.0178	2.01	.015
				3.233	.0142	2.192	.0115
				3.37	.0086	2.467	.0086
				3.55	.0057	2.622	.0066
				3.85	.00286	2.867	.00464
						3.20	.00237
						3.70	.0008
2.13	—	2.47	—	5.9	—	5.9	—
2.483	.004	3.033	.009			9.533	.00845
2.683	.009	3.50	.015			10.017	.0140
2.917	.015	3.54	.018			10.05	.0164
3.282	.025	11.18	.023			11.45	.0197
4.50	.040	12.03	.0226			12.07	.020
12.084	.0405	12.33	.0234			12.36	.0203
12.367	.0405						
12.553	.040						
12.70	.039						
12.88	.039						
13.033	.0395						
13.183	.04						

against cH or cOH, curves are obtained by means of which the normality of acid or base corresponding to any hydrogen or hydroxyl ion concentration may be read.

Titration of Glycine and of Sulfanilic Acid.—An N/10 solution of

glycine was prepared, and 10 cc. of this added to 15 cc. each of the various solutions of HCl and NaOH. The resulting glycine concentration was thus 0.04. Table II gives the results with glycine. N

TABLE IV.

0.04/0.043		0.02/0.02		0.10/0.04		0.04/0.08	
pH	n	pH	n	pH	n	pH	n
0.617	.041	1.033	.019	1.467	.0841	1.525	.0286
0.73	.0395	1.18	.0194	2.175	.0532	1.825	.0246
0.84	.04	1.41	.0170	2.433	.0363	2.117	.01724
0.99	.039	1.62	.015	2.625	.0226	2.208	.01174
1.125	.039	1.97	.014	2.73	.01614	2.333	.0092
1.31	.0375	2.15	.0108	2.833	.011	2.39	.0049
1.58	.033	2.32	.00715	2.88	.0077	2.48	.0027
1.91	.0273	2.405	.00506	2.97	.0049	2.55	.00017
2.21	.0186	2.525	.00301	3.017	.00204		
2.36	.0136	2.65	.00076				
2.48	.0087						
2.575	.00634						
2.625	.00363						
2.683	.00092						
2.74	—	2.76	—	3.06	—	2.60	—
2.8	.001	2.967	.004				
2.85	.003	3.217	.009				
2.933	.0075	3.583	.015				
3.083	.0133	8.867	.025				
3.483	.0266	10.38	.0397				
4.11	.039	12.067	.041				
4.517	.0412	12.367	.041				
6.14	.0433	12.55	.039				
8.25	.045	12.71	.0397				
8.70	.048	12.883	.039				
9.175	.054	13.03	.0405				
9.45	.060	13.183	.040				
10.15	.0748						
10.92	.082						
12.05	.0829						
12.32	.0824						

is the original normality of the HCl or NaOH after dilution from 15 to 25 cc., and n is the number of mols neutralized by the glycine, per liter of mixture.

Table III gives, in condensed form, results obtained with certain concentrations of sulfanilic acid as well as certain other concentrations of glycine. The solubility of the sulfanilic acid prevented making an N/10 solution at room temperature, so the solution was made up at 40°C. using such a volume that 0.1 mol would occupy a volume of 1 liter at 20°C. A quantity was pipetted from this solution at 40° such that it would occupy a volume of 10 cc. at 20°. As may be expected, acid titration of sulfanilic acid has little meaning, since at the pH where any effect may be expected, a very small

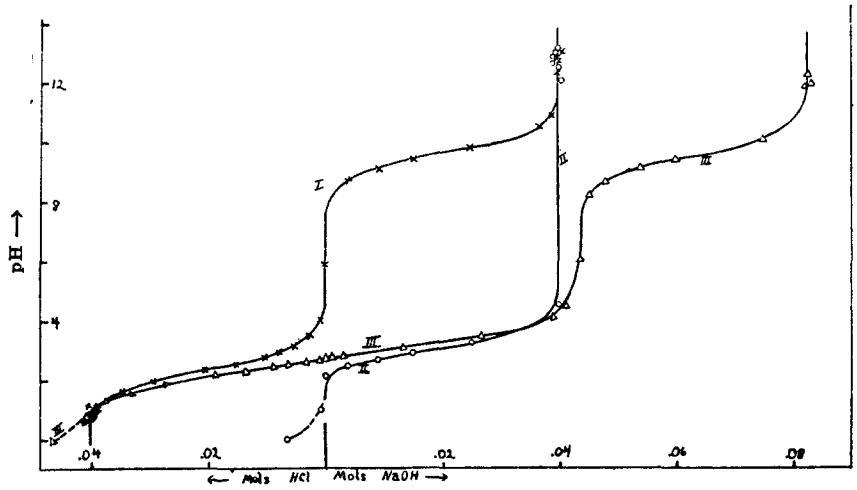

FIG. 1.

change in E.M.F. corresponds to a large quantity of sulfanilic acid neutralized. Thus, except for glycine, only alkaline titrations are included.

In Tables III and IV, n represents mols of HCl neutralized when it is above the center line, and mols of NaOH neutralized when below this line.

Table IV, which is analogous to Table III, gives results obtained from the titration of various mol ratios of glycine and sulfanilic acid. The concentration of glycine is given first in the ratios, *i.e.* the ratio 0.04/0.043 means a mixture containing 0.04 mol glycine and 0.043 mol sulfanilic acid per liter.

The curves in Fig. 1 are plotted from data obtained by titration of the 0.04 N glycine (Curve I), the 0.04 N sulfanilic acid (Curve II), and the 0.04/0.043 mixture of glycine and sulfanilic acid (Curve III). The alkaline titration of the sulfanilic acid (Curve II) is plotted on an abscissæ scale 43/40 that of the other curves to compare its behavior easily with that of the mixture. The broken portions of the curves are drawn, not through directly determined experimental points, but through points calculated from the basic ionization constant of sulfanilic acid (6). Abscissæ give the mols of HCl, measured to the right of the zero line, or of NaOH, measured to the left of this line, neutralized, as calculated by the formula given above, while ordinates give the corresponding pH. Similar curves may be obtained by plotting in a like manner the data from the other titrations.

The striking thing about the curve is the high buffering action of the mixture of glycine and sulfanilic acid about a pH which, it will be shown, corresponds closely with the value that one would calculate for what has been termed the isoelectric point of the system, from the formula developed in the first paper of this series. Moreover, it will be noted that the behavior of the mixture as a seeming individual, at least with a characteristic curve differing from the curves of the components studied alone, is confined to that portion of the pH range between the respective isoelectric points of the components. At a pH above the isoelectric point of glycine the curve representing the behavior of the mixture is an exact duplicate of the glycine curve, displaced to the right, of course, by the amount of the sulfanilic acid concentration in the mixture. In the same way there is no reason to doubt that the curve below the isoelectric point of the sulfanilic acid, at a pH of 1.25 (6), and that of the pure sulfanilic acid would also duplicate each other, though unfortunately, with this substance, that portion of the curve cannot be easily experimentally realized.

Calculation of the Amount of Glycine Neutralized by Sulfanilic Acid.—The total amount of glycine neutralized can be estimated by considering the expression

$$\overset{+}{H} + \text{glycine} = \overset{+}{Gl}\ \text{(glycine ion)} + HOH$$

where

$$\frac{\overset{+}{(Gl)}}{\overset{+}{(H)}\ (glycine)} = K$$

Reading the titration curve at various known ratios of glycine ion concentration to that of unneutralized glycine, K may be evaluated. For example, when the glycine is half neutralized, $K = 1/\overset{+}{(H)} = 223$, since 0.02 mol of glycine is seen to be neutralized at a pH of 2.35 or a cH of 0.0047. In a like manner for the following ratios the corresponding values of K are obtained.

Ratios.	pH	K
0.333	2.8	211.
0.500	2.64	218.
1.000	2.35	223.
2.000	2.05	224.
3.000	1.9	238.
Mean.....		223.

From the value of K the amount of glycine neutralized at any pH can be calculated. In the presence of NaOH this value is, through the pH range included in Table V, also the amount of glycine neutralized by the sulfanilic acid. When HCl is also present one must subtract from the total glycine neutralized the amount neutralized by the HCl. This can be satisfactorily approximated by putting it equal to the sum of the increase in glycine ion and the decrease in sulfonate ion at any pH, referred to their respective concentrations in the absence of the HCl.

Table V gives the results of such calculations for the mixture represented in Fig. 1. Above the center line the mixture contains NaOH, while below, it contains HCl. Glycine ion is represented by $\overset{+}{Gl}$, sulfonate ion by $\bar{S}$, and their respective tabulated increments by $\Delta\overset{+}{Gl}$ and $\Delta\bar{S}$.

The mols of glycine neutralized by the sulfanilic acid are seen to pass through a maximum when there is neither HCl nor NaOH present. Such a mixture has a pH of 2.74. The theoretical value for

the isoelectric point of such a system, it will be remembered from the first paper of this series, is obtained from the expression

$$(H)^+ = \sqrt{\frac{K_a\,(HAOH)}{K_b'\,(HBOH)}\,K_w}$$

where, in the present case, K_a is the acid ionization constant of the sulfanilic acid, 7×10^{-4}, and (HAOH) is its concentration in unionized

TABLE V.

Concentration of HCl or NaOH.	cH	$\dot{G}l$	$\bar{S}$	$\Delta\dot{G}l$	$\Delta\bar{S}$	Glycine neutralized by HCl.	Glycine neutralized by sulfanilic acid.
.0267	.00033	.00273	—	—	—	—	.00273
.0133	.00083	.0062	—	—	—	—	.0062
.0075	.00117	.00825	—	—	—	—	.0083
.0030	.00141	.00954	—	—	—	—	.0095
.00133	.00158	.01040	—	—	—	—	.01040
.0000	.00183	.01155	.0119	—	—	—	.01155
.001	.00186	.0117	.01175	.00015	.00015	.00030	.01140
.002	.00191	.0119	.0115	.00035	.0004	.00075	.01115
.003	.00207	.0126	.01085	.00105	.00105	.0021	.0105
.006	.00237	.0138	.0098	.00225	.0021	.00435	.00945
.009	.00266	.0149	.00895	.00335	.00295	.0063	.0086
.012	.00329	.0169	.00755	.00535	.00435	.0097	.0072
.018	.00439	.0197	.0059	.00815	.0060	.01415	.00555
.025	.0062	.0232	.00436	.01165	.00754	.01919	.0040
.040	.0124	.0294	.0023	.01785	.0096	.02745	.00195
.060	.0261	.0341	.0011	.02255	.0108	.03335	.00075

form; and K_b' is the basic ionization constant of the glycine, 2.2×10^{-12}, and (HBOH) is its concentration in unionized form. Substituting the proper values, one is lead to the theoretical pH value for the isoelectric point of this mixture of 2.73 in place of the observed value of 2.74.

Other mol ratios yield analogous results. Table VI compares the observed pH of the isoelectric points of various mixtures of glycine and sulfanilic acid with the calculated values. These calculated theoretical values, it will be remembered, correspond to the pH at which the negative sulfonate ion concentration is equal to the posi-

tive glycine ion concentration. They are obtained by solving the following two simultaneous equations for y.

$$\frac{y \cdot x}{c - x} = K_a \quad \text{and} \quad \frac{K_w/y \cdot x}{c' - x} = K_b'$$

where y is the hydrogen ion concentration corresponding to the pH of the isoelectric point of the system;

x is the sulfonate (or glycine) ion concentration at this pH, which is the same in case of both ions;

c is the total sulfanilic acid concentration, and K_a its acid ionization constant;

c' is the total glycine concentration, and K_b' its basic ionization constant.

TABLE VI.

Concentration of sulfanilic acid.	Concentration of glycine.	pH observed.	pH calculated.
.043	.040	2.74	2.73
.01	.09	3.40	3.44
.03	.07	2.98	3.01
.04	.10	3.06	3.03
.05	.10	2.98	2.97
.10	.10	2.74	2.75
.02	.02	2.76	2.75
.10	.05	2.58	2.54
.08	.04	2.57	2.54
.07	.03	2.51	2.50

Table VII gives certain results of the same nature as those included in Table VI but for a few other pairs of substances. In connection with the work presented in the following paper a sample of lysine was prepared from hydrolyzed casein. The ionization constants of this substance are evidently not very accurately known, probably since it is difficult to prepare it with a high degree of purity. Scudder (7) gives for K_a about 1×10^{-11}, and for K_b "less than 1×10^{-7}." From Tague's titration of lysine dihydrochloride (8) one may calculate the value of K_a, which is found to be 1.2×10^{-11}, but, though the second basic ionization constant can be obtained from his curve, the first, which is the significant one, cannot. Solutions of the sample prepared for this work gave a pH to water of 8.8, which, using

1.2×10^{-11} for K_a, gives the value 5×10^{-8} for the first K_b. It is almost impossible to be sure that one has not a trace of sulfuric acid in such a preparation, however, and for the calculations of the theoretical pH values given in Table VII the value 7×10^{-8} was used. The agreement between the observed and calculated values of the isoelectric points of these systems, though still quite satisfactory, is not so good as in Table VI. This may be due partly to the problematical value of K_b, for lysine used in calculating the theoretical values, and partly to the fact that small changes in the mol ratio have a much larger effect on the pH than is true for glycine and sulfanilic acid, and thus small errors in the total concentration, which may be the case for the lysine, will contribute largely to the discrepancies. The agreement is, however, considered good.

TABLE VII.

	pH observed.	pH calculated.
0.04 sulfanilic : 0.02 lysin	3.09	3.15
0.02 " : 0.02 "	4.88	5.0
0.02 " : 0.04 "	6.69	6.82
0.02 glycine : 0.01 lysin	8.05	8.09

It will be noted that in the last instance the glycine is playing the rôle of the acid constituent of the mixture, as it is now at a pH above its isoelectric point.

SUMMARY.

Electrometric titrations of glycine, sulfanilic acid, and various mixtures of the two have been made. These mixtures are shown to give a curve which, between their respective isoelectric points, is different from that of either substance. These mixtures have a maximum buffering power at a pH which can be theoretically calculated, and which has the characteristics of an "isoelectric point of the system."

Other pairs of ampholytes are shown to act in an analogous manner.

BIBLIOGRAPHY.

1. Stearn, E. W., and Stearn, A. E., *J. Bact.*, 1923, viii, 567; 1924, ix, 463, 479, 491; 1925, x, 13.
2. Michaelis, L., and Davidsohn, H., *Biochem. Z.*, 1912, xxxix, 496.
3. Bailey, C. H., *J. Am. Chem. Soc.*, 1920, xlii, 45.
4. Fales, H. A., and Mudge, W. A., *J. Am. Chem. Soc.*, 1920, xlii, 2434.
5. Hoffman, W. H., and Gortner, R. A., Colloid symposium monograph, The Chemical Catalog Company, 1925, ii, 209.
6. Stearn, A. E., *J. Gen. Physiol.*, 1926–27, x, Paper IV (in press).
7. Scudder, H., Electrical conductivity and ionization constants of organic compounds, D. Van Nostrand Co., New York, 1914.
8. Tague, E. L., *J. Am. Chem. Soc.*, 1920, xlii, 173.