ELECTROMETRIC DETERMINATIONS OF THE DISSOCIATION OF GLYCOCOLL AND SIMPLE PEPTIDES[1]

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Because of their importance in biochemistry and in the theory of amphoteric electrolytes, the acid and basic dissociation constants of amino acids and polypeptides have been measured by numerous investigators. A summary of available data has been published by Kirk and Schmidt (1). The agreement among the results of the several investigators is not very good. This is due to numerous causes, chief among them being the ever present uncertainty of the liquid junction potentials in the potentiometric studies, the difficulty of preparing compounds of sufficient purity and the neglect of certain corrections due to ionic activity.

The first two named difficulties are mechanical in nature. A method of obviating the last was undertaken by Simms (2) in a series of titration experiments wherein he employed the Brönsted and LaMer (3) limiting equation to obtain the activity coefficients of the ionic species involved. He worked with glycocoll and studied its dissociation in the presence of NaCl and $BaCl_2$. The further electrometric titration of glycocoll and gelatin was conducted in the presence of certain antagonistic salt mixtures. The effect on the pH of the solution was complex.

The present paper reports the potentiometric determination of the apparent acidic and basic dissociation constants of glycocoll and several peptides in aqueous solution. Measurements were also made in the presence of KCl or of K_2SO_4 at equal ionic strength. Two methods, hydrolysis and titration, were employed. Hydrolysis afforded a basis of comparison of order of magnitude with the results

[1] This paper reports work done for a thesis submitted by Jesse P. Greenstein in partial fulfillment of the requirements for the degree of Ph.D. at Brown University.

of titration. This was desirable because uncertainties involved in determinations of hydrogen ion activities in a range far from the buffer region are clearly recognized. The hydrolysis method was suggested by the work of Denham (4) on inorganic salts. He pointed out that the potentiometric determination of the degree of hydrolysis constituted the most satisfactory method for this type of study, particularly in solutions of low hydrogen ion activity.

Winkelblech (5), on the other hand, employed the Bredig method of conductance to determine the degree of hydrolysis and studied several of the amino acids in this manner. But difficulties involved in the conductance method make it a rather unsatisfactory means for the study of the dissociation of these substances.

Materials and Apparatus

The following peptides were synthesized according to the general methods of Emil Fischer: glycylglycocoll (6), alanylglycocoll (7), leucylglycocoll (8), methylleucylglycocoll (9), valylglycocoll (10), phenylalanylglycocoll (11) and glycylglycylglycocoll (12). All compounds were carefully boiled with absolute alcohol until no trace of chloride remained. They were then crystallized twice from pure distilled water in some cases under addition of alcohol and were dried at 100°C. Most of the intermediates were likewise crystallized from the appropriate solvents or else fractionally distilled. Previous to use in the experiments the peptides were kept for several hours over phosphorus pentoxide. No specific tests for purity could be made inasmuch as the compounds exhibit a wide range of fusion with decomposition within the fusion interval.

The usual scheme of measuring hydrogen electrode potentials was employed with a Type K potentiometer, a carefully calibrated Eppley standard cell and a reflecting type of galvanometer with lamp and scale. A saturated calomel electrode was used. All materials for its preparation were carefully purified. Its constant, determined by checking against a tenth normal calomel electrode, was 0.2454.

Hydrochloric acid was prepared by distilling a high grade commercial product and taking the middle fraction. It was standardized against carefully dried, pure sodium carbonate using methyl orange as indicator. Sodium hydroxide was prepared according to the method of Cornog (13) and was standardized against the standard hydrochloric acid. The KCl and K_2SO_4 were from LaMotte and Kahlbaum stock respectively. The water was distilled from a Barnstead still and had a specific conductance of 1×10^{-6} mho. The salt bridge was the saturated KCl model recommended by Michaelis (14). This seems eminently satisfactory in comparison with other types in common use. The recent work of Guggenheim may be referred to in this connection (15).

All measurements were made in a thermostat at 25°C. ± 0.1°. Readings were taken until potentials checked to 0.2 mv. The value of pK_w at 25°C. was taken to be 13.895.

Hydrolysis Studies

The method of hydrolysis involves, essentially, the potentiometric determination of the hydrogen ion activity (a_{H^+} or pH) in equimolar mixtures of the peptide and either HCl or NaOH. Lùden (16) has given a theoretical treatment of this type of reaction but his formulations were made some time before the advent of the modern theories of solution.

The following method of formulating the reactions involved was suggested by the work of Fenwick and Gilman (17) on the dissociation of the salts of certain complex organic compounds.

It is assumed that the acid reacts with the compound to form the hydrochloride whereas the base reacts to form the sodium salt. Complete dissociation of the salt in either case is taken for granted.

Letting R represent the general molecular grouping of the ampholyte, the reactions occurring in acid solution may be given thus:

$$R^+ + Cl^- + HOH \rightleftarrows ROH + Cl^- + H^+$$

$$\frac{a_{R^+} \times a_{HOH}}{a_{ROH} \times a_{H^+}} = \frac{k_b}{K_W} = \frac{a_{R^+}}{a_{ROH} \times a_{H^+}} = \frac{1}{K_a}$$

It is assumed that water possesses unit activity although Harned (18) in a series of calculations on the vapor pressure of water, showed that its activity at ordinary temperatures was slightly less than this value.

The degree of hydrolysis of the salt, represented by X, will be:

$$X = \frac{a_{H^+}}{\gamma_{H^+} \cdot C},$$

where C is the total concentration of ampholyte, reckoned on a molal basis, and γ_{H^+} is the activity coefficient of hydrogen ion concentration. Substituting in the mass action equation:

$$\frac{\lambda_{R^+}(1 - X)C}{\gamma_{ROH} \cdot XC\, a_{H^+}} = \frac{k_b}{K_W} = \frac{1}{K_a},$$

$$\frac{\gamma_{R^+}(\gamma_{H^+}C - a_{H^+})}{\gamma_{ROH}\, a^2_{H^+}} = \frac{1}{K_a}$$

Converting the expression into logarithmic form,

$$\log [\gamma_{H^+}C - a_{H^+}] + \log \gamma_R - \log \gamma_{ROH} + 2pH = pK_a$$

The values of the mean activity coefficient of hydrogen ion, γ_{H^+}, were taken from Schatchard's data (19) on solutions of HCl. It is assumed that, within the experimental error, the activity coefficient of hydrogen ion in the presence of ampholyte is approximately the same as in pure acid solution.

In order to determine the activity coefficient of the ampholyte ion, the ionic strength of the solution must be taken into account. Debye and Hückel (20), employing the assumption of complete dissociation of strong electrolytes, elaborated a theory which accounts for the deviations from the ideal state by taking into consideration the electrical forces among the ions. Their mathematical treatment was further simplified by Brönsted and LaMer (21) who found that the activity coefficient was related to the ionic strength, μ, of the solution by the equation: $-\log \gamma = Az^2\sqrt{\mu}$, where A is a coefficient depending upon the dielectric constant of the medium and the absolute temperature and z refers to the valence. This equation is employed in the following formulation. The coefficient, A, at 25°C., is very nearly 0.5, so that, $\log \gamma_{R^+} = -0.5z^2\sqrt{\mu} = -0.5\sqrt{C}$, and assuming that the activity coefficient of the undissociated portion of a weak electrolyte is very nearly unity, the working equation is obtained:

$$\log [\gamma_{H^+}\, C - a_{H^+}] - 0.5\sqrt{C} + 2pH - pK_a$$

A similar equation, based on reactions in alkaline solutions, is:

$$\log [\gamma_{OH^-}C - a_{OH^-}] - 0.5\sqrt{C} + 2pOH = pK_b$$

Values of γ_{OH^-} are the mean values taken from Harned's data on NaOH solutions.

The following compounds were studied by the hydrolysis method: glycocoll, glycylglycocoll, alanylglycocoll, leucylglycocoll, methylleucylglycocoll, valylglycocoll, phenylalanylglycocoll, and glycylglycylglycocoll.

The experimental procedure consisted in making up a normal solution of either HCl or NaOH in a volumetric flask and adding an equivalent normal weight of ampholyte. In some cases the initial solution was 0.1N, in others, particularly among the less soluble variety, the normality was lower. 50 cc. of the original solution were removed, transferred to another flask (100 cc.) and made up to the mark with distilled water. Several successive dilutions were made in this manner whereby the equivalence of peptide to reagent was kept constant and the concentration halved at each successive dilution. The pH of each dilution was determined at 25°C. and the dissociation constants calculated.

The potentials obtained in the case of phenylalanylglycocoll were erratic and considerable time elapsed before equilibrium was reached. Believing this to be an effect of the platinum black, we employed a quinhydrone electrode arranged according to Biilmann (22) in the hope of attaining equilibria more rapidly. This electrode yielded no better results, however, and it was discarded. Abderhalden and Suzuki (23) have shown that peptides containing aromatic radicals are rapidly split at the imide linkage in the presence of dilute acid and alkali even at ordinary temperatures.

The measurements by the hydrolysis method and the computed constants, pK_a and pK_b, are presented in Tables I and II. The results for glycocoll are less consistent than those for the peptides. This is probably due to impurity of the glycocoll.

A consideration of the deviations of the dissociation constants with variation in ionic strength, indicates in general a decided shift toward higher values of K_a and lower values of K_b as the ionic concentration is increased. These constants should give, if the formulations were adequate, a uniform value in the case of each compound. The mass action law has been stated in the requisite terms of activities; the failure to yield constants of more uniform character merits a critical analysis of the method of formulation.

It has been assumed that the activity coefficient of the ampholytic ion R, may be expressed in terms of the limiting law:

$$-\log \gamma_R = 0.5\, z^2 \sqrt{\mu}$$

whereby the activity coefficient is solely a function of the valence and the ionic strength. Such a relationship will hold only in extremely dilute solutions ($\mu < .01$) where the ionic diameters may be neglected. In the case of long ions, the fundamental equations of Debye and Hückel must be modified although as yet no theoretical treatment of the spatial distribution of charges has been attempted.

TABLE I

Measurements by the Hydrolysis Method in Acid Solution

c	$0.5\sqrt{c}$	Glycocoll		Glycylglycocoll		Valylglycocoll		Alanylglycocol.	
		pH	pK_a	pH	pK_a	pH	pK_a	pH	pK_a
0.100	0.157	1.778	2.238	2.116	2.958	.....	.....	2.159	3.048
0.050	0.110	1.949	2.293	2.277	3.023	.....	.....	2.328	3.129
0.025	0.080	2.138	(2.372)	2.450	3.087	2.478	3.148	2.477	3.145
0.012	0.055	2.304	2.331	2.637	3.158	2.656	3.201	2.637	3.158
0.006	0.0385	2.490	2.322	2.785	3.134	2.810	3.193	2.795	3.154
0.003	0.0275	2.702	2.331	2.974	3.175	3.021	3.292	2.967	3.161

c	$0.5\sqrt{c}$	Leucylglycocoll		Methylleucylglycocoll		Phenylalanylglycocoll		Glycylglycylglycocoll	
		pH	pK_a	pH	pK_a	pH	pK_a	pH	pK_a
0.100	0.157	2.165	3.060	.....	.....	.....	.....	2.191	3.114
0.050	0.110	2.324	3.121	2.465	3.398	.....	.....	2.355	3.186
0.025	0.080	2.467	3.123	2.587	3.384	2.042	2.118	2.524	3.249
0.012	0.055	2.632	3.148	2.715	3.334	2.204	2.034	2.666	3.226
0.006	0.0385	2.796	3.156	2.869	3.332	2.416	2.048	2.832	3.248
0.003	0.0275	2.959	3.146	3.022	3.294	2.648	2.046	3.003	3.256

TABLE II

Measurements by the Hydrolysis Method in Basic Solution

c	$0.5\sqrt{c}$	Glycocoll		Glycylglycocoll		Valylglycocoll		Alanylglycocoll	
		pOH	pK_b	pOH	pK_b	pOH	pK_b	pOH	pK_b
0.050	0.110	2.879	4.293	3.687	5.920	.....	.....	3.870	6.287
0.025	0.080	3.019	4.309	3.824	5.933	4.048	6.383	3.933	6.157
0.012	0.055	3.159	4.296	3.987	5.976	4.175	6.355	4.071	6.147
0.006	0.0385	3.312	4.317	4.166	6.056	4.312	6.348	4.193	6.111
0.003	0.0275	3.432	4.251	4.301	6.036	4.479	6.393	4.323	6.081

c	$0.5\sqrt{c}$	Leucylglycocoll		Methylleucylglycocoll		Phenylalanylglycocoll		Glycylglycylglycocoll	
		pOH	pK_b	pOH	pK_b	pOH	pK_b	pOH	pK_b
0.050	0.110	3.907	6.361	3.811	6.169	.....	.....	3.912	6.371
0.025	0.080	3.975	6.235	3.895	6.075	3.202	4.680	4.026	6.339
0.012	0.055	4.100	6.205	4.019	6.043	3.542	5.982	4.165	6.335
0.006	0.0385	4.251	6.227	4.175	6.075	3.894	5.505	4.313	6.351
0.003	0.0275	4.405	6.245	4.320	6.075	4.215	5.865	4.461	6.357

Simms has suggested an empirical formula to apply to certain large organic ions. His equations however are confined to those concentrations where μ varies from 0.01 to 0.1.

In the application of the fundamental equation, it has also been assumed that the peptide ion is univalent. Such a conception, in view of the several reactions described in the Introduction, seems somewhat doubtful. While the monovalent hydrochlorides of the peptides may be easily isolated, it is uncertain that such combinations are strictly stoichiometric. However, the question of extra valencies within the peptide molecule is so far from being settled that it need not be further considered here.

This leaves the problem of the ionic diameters to be considered. Probably this is where the present calculations are most in error. The simple limiting equation holds in concentrations where $\gamma < 0.01$. Above this limit, the activity coefficient of an ion must be a function of some value characteristic of all the ionic diameters in the solution. Where this factor is considered, the activity coefficient is defined by Hückel (24) as follows:

$$-\log \gamma = \frac{0.5\, z^2 \sqrt{\mu}}{1 + 3.3 \times 10^7\, r \sqrt{\mu}} + B\,(2\mu)$$

Here r may be interpreted as the average effective diameter of all the ions. Data relating to this variable are meager in the case of inorganic ions and are practically non-existent in the case of organic ions. The second term on the right hand side of the above equation represents the effect of the solute concentration on the dielectric constant. The zwitter ion with its dual charge and permanent moment might be expected to influence the dielectric constant considerably, and the experiments of Blüh (25) indicate that its value increases progressively with the concentration. Beyond the fact that at higher concentrations the dielectric constant of the solution is no longer the same as that of the pure solvent, little hope of arriving at the nature of the solute from capacity measurements can at present be held. It may be that the zwitter ion is an association complex in the sense of Bjerrum (26). Recent very accurate measurements (27) indicate that aqueous solutions of strong electrolytes slightly depress the dielectric constant of the solvent.

In view of the paucity of our knowledge concerning this problem, it seems best to leave the dissociation constants in their present form, referring to them as "apparent" constants since they are functions of several variables.

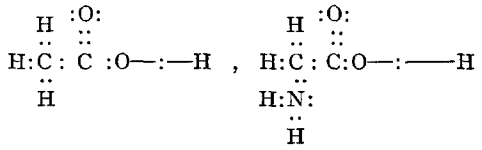
Turning our attention to the relative order of magnitude of the constants of the various compounds, we see a partial proof of the conceptions of Bjerrum and Adams.

Considering acid constants alone, their relative order of magnitude may be represented as follows:

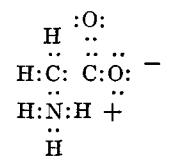
Phenylalanylgycocoll > glycocoll > glycylglycocoll > (alanylglycocoll, leucylglycocoll, valylglycocoll) > glycylglycylglycocoll > methylleucylglycocoll.

Bjerrum and Adams postulated a high degree of acidity for the glycocoll molecule in consequence of the presence of the highly electronegative group. The latter will further the dissociation of the hydrogen ion from the carboxyl group, such influence decreasing as the amino group is removed further from the carboxyl.

Representing the electronic structure of acetic acid and glycocoll thus:



it is apparent why glycocoll is a far stronger acid than acetic (some 100 times) and yet is neutral in pure water solution. The highly electronegative amino group will effect a deformation in the electronic nucleus, causing a shift in the position of the electron pairs about the carbon and oxygen atoms of the carboxyl group. This shift is toward the amino group. In the case of the oxygen-hydrogen linkage of the carboxyl group, the electron pair is drawn closer to the oxygen and farther away from the hydrogen. This amounts to a decrease in the strength of the bond, and an increase in the degree of dissociation of the acid. The proton thus set free combines immediately with the two unshared electrons of the nitrogen, forming the dipolar molecule, or zwitter ion.



It is apparent therefore that the farther away the amino group is from the carboxyl (the greater the number of carbon atoms intervening), the smaller will be the deformation of the electronic nucleus and the slighter the dissociation. Hence the observed decrease in the relative magnitude of the dissociation constants.

Two apparently significant exceptions occur to this rule, namely, in the case of phenylalanylglycocoll and of methylleucylglycocoll. In the former compound it is to be expected that the very strongly electronegative character of the phenyl group would be sufficient to make the compound a stronger acid than glycocoll. In the latter, introducing a methyl radical into the amino group decreases its electronegativity. This lowering of hydrogen-accepting capacity is apparently decreased as the dilution increases, and the compound forms an apparent exception to the rule of increased value of acid dissociation constant with increasing hydrogen ion activity.

In alkaline solution, the basic dissociation constants in general show a decided increase in value as the ionic strength decreases.

As a result of these particular studies, the generalization may be advanced that the dissociation constants are functions of the hydrogen ion activity and the ionic strength.

Titration Studies

The following methods of formulating the reactions involved in titration measurements were suggested in part by the work of Simms and the discussions of Kolthoff (28), but several modifications have been introduced. If we consider the reaction in acid solution, we may formulate the equilibrium thus:

$$^{+}X^{-} + H^{+} = H^{+}X$$

where $^{+}X^{-}$ refers to the zwitter ion, $NH_3^{+}RCOO^{-}$. In consequence of the small ionic product of water, the concentration of ampholytic

anions in the presence of acid will be negligible. In mass action form, the equation is:

$$\frac{H^+ \times {}^+X^-}{H^+X} = K'_a$$

If α represents the apparent "degree of dissociation" or the mol fraction of ampholytic cations ($\alpha = \frac{H^+X}{C}$, where C is the total concentration of ampholyte) then the mass action equation may be written thus:

$$K'_a = a_{H^+} \frac{(1 - \alpha)}{\alpha}$$

and in logarithmic form:

$$pK'_a = pH - \log \frac{(1 - \alpha)}{\alpha}$$

For electroneutrality in the solution the sum of the concentrations of cations must equal that of the anions or:

$$H^+ + H^+X = OH^- + Cl^-$$

and, assuming complete dissociation of HCl and negligible concentration of hydroxyl ions,

$$H^+ + H^+X = A$$

where A is the total concentration of acid.

It follows that:

$$H^+ + \alpha C = A \text{ and } \alpha = \frac{A - H^+}{C}$$

but, inasmuch as α is to be expressed as concentration, the H^+ values, observed as activities, must be converted to concentrations by means of the equations:

$$H^+ = \frac{a_{H^+}}{\gamma_{H^+}} \text{ and } \log \gamma_{H^+} = -0.5 \sqrt{\mu}$$

In basic solution, the reactions may be formulated thus:

$$^{+}X^{-} + OH^{-} = XOH^{-} \quad \text{and} \quad \frac{^{+}X^{-} \times OH^{-}}{XOH^{-}} = K_b'$$

Representing α as the mol fraction of ampholytic anion, $\alpha = \frac{XOH^{-}}{C}$,

$$K_b' = a_{OH^{-}} \frac{(1 - \alpha)}{\alpha} \quad \text{and} \quad pK_b' = pOH - \log \frac{(1 - \alpha)}{\alpha}$$

For electroneutrality:

$$H^{+} + Na^{+} = OH^{-} + XOH^{-}$$

and assuming the sodium hydroxide, B, as completely dissociated:

$$H^{+} + B = OH^{-} + \alpha C$$

whence

$$\alpha = \frac{B + H^{+} - OH^{-}}{C}$$

Hydroxyl ion concentrations were calculated from the concentrations of hydrogen ions by means of the ionic product of water at 25°, $K_w = 1.27 \times 10^{-14}$.

The dissociation constants so calculated are of a hybrid or "apparent" character, inasmuch as the expression $\log \frac{1 - \alpha}{\alpha}$ is expressed as concentration while pH is the negative logarithm of the hydrogen ion activity.

In order to obtain the "true" dissociation constants which shall be independent of the ionic strength of the solution, it is necessary to have an accurate knowledge of the activity coefficients of the ampholyte ions. Any attempt to derive a value for these coefficients on the basis of the Debye-Hückel theory involves certain *a priori* assumptions which have been considered in the previous section on hydrolysis.

The experimental technique consisted in adding acid or base to a constant quantity of ampholyte alone in solution, and in the presence of salt. A 0.002 N solution of either HCl or NaOH was made up in a 200 cc. volumetric flask. To

this was added a weighed amount of ampholyte so that its concentration would be 0.02 M. 25 cc. samples were placed in 50 cc. volumetric flasks with the desired amounts of 0.1 N HCl or NaOH, and with 5 cc. of 0.5 M KCl or 0.166 M K_2SO_4, or with no salt. The aliquots were then made up to the mark with distilled water. The ionic strength, then, of each of the salts was the same (0.05μ). The final concentration of total ampholyte in each sample was 0.01 M.

Potentiometric data were obtained on the following compounds: glycocoll, glycylglycocoll, alanylglycocoll, leucylglycocoll, methylleucylglycocoll, and glycylglycylglycocoll. The dissociation constants of each ampholyte were calculated by means of the above formulae. The results are reported in Tables III–VIII. The lack of a sufficient supply of glycylglycylglycocoll was the cause of the paucity of data on this compound.

TABLE III

Electrometric Titration of Glycocoll (0.01 M)
With HCl

A	HCl without salt		HCl with KCl = 0.05μ		HCl with K_2SO_4 = 0.05μ	
	pH	pK'_a	pH	pK'_a	pH	pK'_a
0.001	3.419	2.232	3.495	2.292	3.614	2.484
0.002	3.123	2.262	3.182	2.295	3.279	2.461
0.003	2.973	2.335	2.979	2.271	3.093	2.480
0.004	2.818	2.311	2.849	2.289	2.962	2.506
0.005	2.707	2.314	2.725	2.257	2.852	2.516
0.006	2.612	2.311	2.629	2.244	2.744	2.502
0.007	2.511	2.264	2.539	2.209	2.668	2.526
0.008	2.450	2.287	2.467	2.200	2.597	2.540

Titration with NaOH

B	NaOH without salt		NaOH with KCl = 0.05μ		NaOH with K_2SO_4 = 0.05μ	
	pH	pK'_b	pH	pK'_b	pH	pK'_b
0.001	8.715	4.226	8.656	4.285	8.697	4.244
0.002	9.116	4.174	9.033	4.257	9.059	4.231
0.003	9.319	4.204	9.262	4.271	9.267	4.256
0.004	9.526	4.185	9.456	4.257	9.462	4.251
0.005	9.678	4.208	9.621	4.267	9.631	4.257
0.006	9.842	4.215	9.773	4.289	9.792	4.268
0.007	10.003	4.238	9.942	4.305	9.974	4.271
0.008	10.184	4.268	10.133	4.330	10.135	4.328

TABLE IV

Electrometric Titration of Glycylglycocoll (0.01 M)
With HCl

A	HCl without salt		HCl with KCl = 0.05μ		HCl with K_2SO_4 = 0.05μ	
	pH	pK'_a	pH	pK'_a	pH	pK'_a
0.001	4.110	3.115	4.145	3.146	4.128	3.129
0.002	3.790	3.140	3.790	3.128	3.829	3.173
0.003	3.541	3.109	3.578	3.137	3.622	3.188
0.004	3.362	3.101	3.392	3.119	3.458	3.199
0.005	3.218	3.104	3.248	3.119	3.309	3.197
0.006	3.077	3.092	3.108	3.100	3.179	3.203
0.007	2.944	3.075	2.969	3.084	3.049	3.194
0.008	2.810	3.041	2.849	3.049	2.945	3.216

Titration with NaOH

B	NaOH without salt		NaOH with KCl = 0.05μ		NaOH with K_2SO_4 = 0.05μ	
	pH	pK'_b	pH	pK'_b	pH	pK'_b
0.001	7.304	5.637	7.047	(5.894)	7.267	5.674
0.002	7.676	5.617	7.590	5.703	7.607	5.686
0.003	7.854	5.673	7.792	5.735	7.818	5.709
0.004	8.059	5.660	7.993	5.726	8.003	5.716
0.005	8.225	5.670	8.162	5.733	8.169	5.726
0.006	8.387	5.684	8.338	5.733	8.356	5.715
0.007	8.594	5.669	8.533	5.730	8.539	5.724
0.008	8.803	5.694	8.758	5.739	8.769	5.728

TABLE V

Electrometric Titration of Alanylglycocoll (0.01 M)
With HCl

A	HCl without salt		HCl with KCl = 0.05μ		HCl with K_2SO_4 = 0.05μ	
	pH	pK'_a	pH	pK'_a	pH	pK'_a
0.001	.....	.....	4.164	3.169	4.206	3.211
0.002	3.773	3.123	3.813	3.155	3.857	3.205
0.003	3.571	3.143	3.610	3.176	3.649	3.219
0.004	3.399	3.143	3.423	3.156	3.473	3.217
0.005	3.248	3.141	3.275	3.154	3.326	3.220
0.006	3.106	3.132	3.126	3.129	3.204	3.237
0.007	2.967	3.110	2.976	3.084	3.064	3.217
0.008	2.839	3.085	2.866	3.058	2.964	3.246

Titration with NaOH

B	NaOH without salt		NaOH with KCl = 0.05μ		NaOH with K_2SO_4 = 0.05μ	
	pH	pK'_b	pH	pK'_b	pH	pK'_b
0.001	7.331	5.610	7.260	5.681	7.277	5.664
0.002	7.688	5.605	7.619	5.674	7.629	5.664
0.003	7.886	5.641	7.839	5.688	7.844	5.683
0.004	8.071	5.648	8.011	5.708	8.033	5.686
0.005	8.248	5.647	8.186	5.709	8.211	5.684
0.006	8.423	5.648	8.377	5.694	8.380	5.691
0.007	8.631	5.632	8.570	5.693	8.588	5.675
0.008	8.837	5.660	8.778	5.719	8.792	5.705

TABLE VI

Electrometric Titration of Leucylglycocoll (0.01 M)
With HCl

A	HCl without salt		HCl with KCl = 0.05μ		HCl with K_2SO_4 = 0.05μ	
	pH	pK'_a	pH	pK'_a	pH	pK'_a
0.001	4.152	3.163	4.176	3.181	4.194	3.199
0.002	3.798	3.151	3.808	3.151	3.849	3.197
0.003	3.577	3.149	3.609	3.173	3.649	3.219
0.004	3.392	3.136	3.434	3.171	3.477	3.221
0.005	3.252	3.147	3.275	3.154	3.328	3.222
0.006	3.103	3.127	3.132	3.139	3.191	3.219
0.007	2.978	3.127	2.988	3.103	3.072	3.230
0.008	2.840	3.090	2.874	3.094	2.969	3.255

Titration with NaOH

B	NaOH without salt		NaOH with KCl = 0.05μ		NaOH with K_2SO_4 = 0.05μ	
	pH	pK'_b	pH	pK'_b	pH	pK'_b
0.001	7.209	5.733	7.154	5.787	7.164	5.777
0.002	7.577	5.716	7.499	5.794	7.506	5.787
0.003	7.768	5.759	7.709	5.818	7.712	5.815
0.004	7.968	5.751	7.905	5.814	7.910	5.809
0.005	8.133	5.762	8.081	5.814	8.071	5.824
0.006	8.321	5.750	8.253	5.818	8.260	5.811
0.007	8.511	5.752	8.448	5.815	8.451	5.812
0.008	8.725	5.772	8.653	5.844	8.663	5.834

TABLE VII

Electrometric Titration of Methylleucylglycocoll (0.01 M)
With HCl

A	HCl without salt		HCl with KCl = 0.05μ		HCl with K_2SO_4 = 0.05μ	
	pH	pK'_a	pH	pK'_a	pH	pK'_a
0.001	4.216	3.232	4.226	3.237	4.184	3.189
0.002	3.846	3.205	3.890	3.243	3.913	3.269
0.003	3.631	3.212	3.665	3.237	3.698	3.275
0.004	3.453	3.208	3.490	3.236	3.521	3.273
0.005	3.304	3.212	3.331	3.225	3.377	3.281
0.006	3.154	3.196	3.184	3.210	3.233	3.275
0.007	3.011	3.175	3.040	3.182	3.109	3.283
0.008	2.879	3.155	2.912	3.162	2.996	3.298

Titration with NaOH

B	NaOH without salt		NaOH with KCl = 0.05μ		NaOH with K_2SO_4 = 0.05μ	
	pH	pK'_b	pH	pK'_b	pH	pK'_b
0.001	7.340	5.601	7.289	5.652	.....	.....
0.002	7.680	5.613	7.636	5.657	7.648	5.645
0.003	7.891	5.636	7.849	5.678	7.851	5.676
0.004	8.089	5.630	8.032	5.687	8.037	5.682
0.005	8.257	5.638	8.208	5.687	8.213	5.682
0.006	8.434	5.637	8.396	5.675	8.387	5.684
0.007	8.629	5.634	8.575	5.688	8.587	5.676
0.008	8.840	5.657	8.797	5.700	8.800	5.697

TABLE VIII

Electrometric Titration of Glycylglycylglycocoll (0.01 M)
With HCl

A	HCl without salt		HCl with KCl = 0.05μ	
	pH	pK'_a	pH	pK'_a
0.001	4.209	3.225	4.236	3.247
0.003	3.648	3.231	3.671	3.246
0.005	3.318	3.229	3.353	3.252
0.007	3.032	3.204	3.052	3.199

Titration with NaOH

B	NaOH without salt		NaOH with KCl = 0.05μ	
	pH	pK'_b	pH	pK'_b
0.001	7.003	(5.938)	7.032	5.909
0.003	7.656	5.871	7.615	5.912
0.005	8.035	5.860	7.978	5.917
0.007	8.390	5.873	8.372	(5.891)

DISCUSSION

On an examination of the data, the following facts are brought to light. The first is that in general the dissociation constants are a function of the hydrogen ion activity. They show definite trends: the values of K_a and K_b of each compound increasing with increasing hydrogen ion activity in solution without the presence of salt and in the presence of KCl; while K_a in the presence of K_2SO_4 decreases with hydrogen ion activity whereas K_b in the presence of K_2SO_4 increases. At equal ionic strengths the acid constants in the presence of KCl are greater than in the presence of K_2SO_4, the reverse holding true of the basic constants in alkaline solution.

The following chart indicates the trend of the apparent dissociation constants in each titration series with H^+ ion activity and ionic strength: the arrows pointing upwards indicate increasing values, those downward, decreasing values.

Without salt	$K'_a \uparrow$	$a_{H^+} \uparrow$	$\mu \uparrow$
	$\vee$	$\vee$	$\wedge$
With KCl	$K'_a \uparrow$	$a_{H^+} \uparrow$	$\mu \uparrow$
	$\vee$	$\vee$	$\|\|$
With K_2SO_4	$K'_a \downarrow$	$a_{H^+} \uparrow$	$\mu \uparrow$
Without salt	$K'_b \uparrow$	$a_{H^+} \uparrow$	$\mu \downarrow$
	$\vee$	$\wedge$	$\wedge$
With K_2SO_4	$K'_b \uparrow$	$a_{H^+} \uparrow$	$\mu \downarrow$
	$\vee$	$\wedge$	$\|\|$
With KCl	$K'_b \uparrow$	$a_{H^+} \uparrow$	$\mu \downarrow$

A satisfactory explanation of the deviations of these constants is difficult to give on purely theoretical grounds. It is known that the apparent dissociation constant of a weak electrolyte is markedly dependent upon the ionic strength of the solution, and in the case of the ampholyte should decrease in absolute value on the addition of other electrolytes.

Stated in terms of the simplified Debye-Hückel theory:

$$-\log \gamma = 0.5\, z^2 \sqrt{\mu},$$

if the equilibrium of the ampholyte be represented as above,

$$K'_a = a_{H^+} \frac{(1 - \alpha)}{\alpha}$$

where K'_a is the apparent dissociation constant, then the true dissociation constant based on activities will be $K_a = K'_a \frac{1}{\gamma}$, assuming that the activity coefficient of the undissociated ampholyte is equal to unity.

Therefore

$$\log K'_a = \log K_a - 0.5\, z^2 \sqrt{\mu}$$

Inasmuch as K_a is a constant, the apparent dissociation constant should decrease as the ionic strength increases. The same relation should hold for K'_b.

On the contrary, in acid solution in each titration series with the exception of those involving K_2SO_4, we observe an increase of the apparent constant with increasing ionic strength. Furthermore, according to the principle of ionic strength, the constants in the presence of the salts should be equal at equal ionic strengths. It must be remembered, however, that this law is of limited validity, holding only in such very dilute solutions that specific ion effects are negligible. In the present studies, the difference in the specific effects of the sulfate and chloride ions is clearly evidenced. The work of Harned (29) on the activity coefficients of sulfuric acid in the presence of sulfate ion is illuminating in this regard. He found a decreased activity of the acid in the presence of this ion as the latter's concentration increased. It seems probable that the decrease is due to the formation of HSO_4 ions. Kraus (30), listing the activity coefficients of K_2SO_4 solutions, indicated the very low activity coefficient of this salt as compared with that of KCl. The latter, for example, at 0.01 M has a value of 0.922, the former at the same concentration a value of 0.687. Sulfuric acid yields a coefficient of 0.617. The effect of the sulfate ion will be to lower the ionic activity of the medium; increase in ionic strength will then so favor the formation of ampholyte ions that their excess will outweigh the effect of reduced hydrogen ion activity on the magnitude of the apparent constant. This is indicated by a comparison of α values and H^+ ion activities in acid-ampholyte, KCl-ampholyte, and K_2SO_4-ampholyte solutions:

$$\alpha_{K_2SO_4} > \alpha_{\text{no salt}} > \alpha_{KCl}$$

$$a_{H^+\text{no salt}} > a_{H^+KCl} > a_{H^+\,K_2SO_4}$$

It may be that the lowering of the degree of dissociation in the presence of KCl is due to a common ion effect. The apparent disagreement of K'_a with the Debye-Hückel equation confirms the work of Simms on glycocoll.

The agreement of the equation with theory in the case of the apparent basic constants is substantially good. Here the constants in general decrease as the ionic strength is increased.

It would be unwise to speculate further on specific ion effects in view of the slight amount of data now available in the literature. The present paper is simply a contribution to the study of ampholytes in salt solutions, and a more nearly complete elucidation must await further theoretical advances.

SUMMARY

1. The apparent acid and basic dissociation constants were determined potentiometrically by the methods of hydrolysis and of titration for the following ampholytes: Glycocoll, glycylglycocoll, alanylglycocoll, valylglycocoll, leucylglycocoll, methylleucylglycocoll, phenylalanylglycocoll and glycylglycylglycocoll. The constants were also determined in the presence of KCl and of K_2SO_4 at equal ionic strength.

2. In general, the relative order of magnitude of the constants decreased as the number of carbon atoms between amino and carboxyl groups increased. An explanation of this is offered on the basis of theories of electronic structure.

3. The application of the modern concepts of solutions to the case of the ampholytic ions is discussed. The inadequacy of the present theories is pointed out.

4. The constants were found, in general, to be functions of the hydrogen ion activity and the ionic strength of the solutions. Apparent contradictions to the Debye-Hückel theory are pointed out and partially explained on the basis of specific ion effects.

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