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A STUDY OF THE DETERMINATION OF AMINO ACIDS BY MEANS OF THE HYDROGEN ELECTRODE.

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Some time ago the writer began a series of investigations on the conditions for the activity of proteoclastic enzymes of wheat and flour.[1] The extent of protein hydrolysis in the auto-digestion experiments was determined by means of the formol titration method of Sörensen. After this had been used for some time an attempt was made to substitute electrometric titration for it. The results obtained were so encouraging that further work was deemed advisable. Before much headway could be made in the study of protein hydrolysis of flour, however, it was necessary to know the behavior of the different degradation products of protein hydrolysis upon the hydrogen electrode. A few of the simpler amino acids were first investigated from this point of view, namely glycocoll, a monoamino monocarboxylic acid; phenylalanin, a monoamino monocarboxylic acid containing the benzene ring; lysin, a diamino monocarboxylic acid; glutamic acid, a monoamino dicarboxylic acid; and tyrosin, a monoamino monocarboxylic acid containing an hydroxyl group in the benzene ring.

Several investigators have determined the hydrogen ion concentration

of aqueous solutions containing glycocoll and sodium hydroxide, and gly-
cocoll and hydrochloric acid in different proportions. These mixtures
are used as standards in the colorimetric determination of the hydrogen
ion. As far as the writer knows, however, none of these investigators
has differentiated between the excess alkali necessary to overcome hydra-
tion and the amount of alkali necessary to neutralize the solute. Strictly
speaking, neutralization consists in bringing the concentration of the hydro-
gen or hydroxyl ions to a concentration of 1×10^{-7} gram ions per liter,
or P_H 7.00. Certain substances, however, such as amino acids, require
the presence of an excess of hydroxyl ions before they will show their
maximum acid characteristics, or an excess of hydrogen ions before they
will show their maximum basic nature. Consequently in titrating an
amino acid with a base it is necessary to have the concentration of the
hydroxyl ion greater than 1×10^{-7} in order to neutralize the acid quan-
titatively. Even with the formol titration method of Sörensen, where
the basic nature of the amino acid is first destroyed by formaldehyde,
it is necessary to have an excess of hydroxyl ions. In such cases a certain
proportion of the added hydroxyl ions are used up in bringing the solvent
itself to the hydroxyl ion concentration required for complete neutraliza-
tion of the solute. It should be possible then to titrate aqueous solutions
of amino acids without the previous addition of formaldehyde provided
proper corrections are made for this factor. The hydrogen electrode
furnishes a means for the accurate determination of this factor.

The ionization equilibria of the amino acids, since they are amphoteric
electrolytes, would be represented by the following equations: (1) by
hydration: $NH_2.R.COOH + H_2O \rightleftharpoons OH.NH_3.R.COOH$; (2) and then
(a) as an acid, $OH.NH_3.R.COOH \rightleftharpoons H^+ + OH.NH_3.R.COO^-$; (b) as a
base, $OH.NH_3.R.COOH \rightleftharpoons N^+H_3.R.COOH + OH^-$; (c) an inner salt,
$OH.NH_3.R.COOH \rightleftharpoons N^+H_3.R.COO + H^+ + OH^-$; (d) $H^+ + OH^- \rightleftharpoons H_2O$.

All these equilibria would exist side by side. The relative values of each
would depend on the magnitude of the ionization constants of the amino
acid as (1) an acid, (k_a); (2) a base (k_b); (3) an inner salt (k_c). The
ionization equilibria of all the amino acids studied in this paper would
reduce finally to the above equations, although in the case of both the
dicarboxylic acids and the diamino acids there would be intermediate
equilibria, depending on the primary and secondary ionizations as an acid,
or as a base, respectively.

Owing to the amphoteric character of the amino acids, their degree of
ionization is comparatively small. For the most part their acid charac-
teristics are stronger than their basic. Consequently their aqueous solu-
tions react slightly acid. When the constants k_a and k_b of an amino acid
are equal, the substance is neutral, i. e., the concentration of the hydrogen

and hydroxyl ions are the same as in pure water. Notwithstanding this neutrality, the solution of the acid would still be an electrolyte because of its ionization as an inner salt, and it would still be capable of acting as a base toward acids and an acid toward bases. In all neutralization phenomena, then, the inner salt would tend to hydrate and subsequently ionize as a base as the hydrogen ion concentration of the solution approached the ionization constant of the acid as a base.

According to Walker,[1] if the concentrations of the different substances taking part in the above equilibrium are represented as follows:

$$\underset{a}{H^+}, \underset{b}{OH^-}, \underset{c}{XOH^-}, \underset{d}{HX^+}, \underset{u}{\frac{HXOH + X}{}},$$
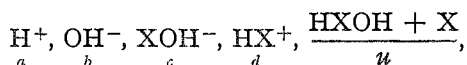
then the equilibrium of the hydrogen ion is represented by the equation

$$a^2 = \frac{K + k_a u}{1 + k_b u}$$

where a is the concentration of the hydrogen ion, K the ionization constant of the water, k_a the ionization constant of the ampholyte as an acid, k_b the constant of the ampholyte as a base, and u the concentration of the undissociated molecules.

The addition of sodium hydroxide to an aqueous solution of an amphoteric electrolyte would decrease a. This decrease in a would have 2 separate effects on the right-hand side of the above equation: (1) u would decrease; (2) k_b of the ampholyte would decrease, since an increase in the hydroxyl ion concentration of the solution would cause a decrease in the basic characteristics of the ampholyte. In other words, the result would be the same as though a series of amphoteric acids with decreasing k_b were dissolved separately in a given volume of water and the above equilibrium formula applied. As the hydrogen ion concentration of the solution approached the value k_b, the value of the magnitude, $\frac{k_b u}{K}$ would decrease and the expression $a^2 = \frac{K + k_a u}{1 + k_b u}$ would become finally $a^2 = K + k_a u$, which is the expression for a simple acid with the same acid constant k_a. The further addition of sodium hydroxide would cause a further decreases in a and thus a corresponding decrease in u. Consequently the expression $a^2 = K + k_a u$ would tend to become $a^2 = K$, i. e., the concentration of the hydrogen and hydroxyl ions as far as the inner salt is concerned would be the same as the pure water. This means that from this point on the solute has no further influence on the equilibrium. Therefore, the amount of hydroxyl ion necessary to bring a solution of an amino acid to equilibrium at any point of less hydrogen ion concentration than the k_a of that acid would be:

[1] Z. physik. Chem., 49, 82 (1904); 51, 706 (1905).

$$OH_{\overline{X}} = OH^-_{\text{neutralization}} + OH^-_{\text{hydration}}$$

where $OH^-_{\text{neutralization}}$ is the amount necessary to neutralize the solute to that point, and $OH^-_{\text{hydration}}$ the amount necessary to overcome hydration at the same point. At all points of greater hydrogen ion concentration than k_b, the amount of base necessary to add would be less than $OH_{\overline{X}}$ because of the ionization of the inner salt as a base. The ratio between $OH^-_{\text{neutralization}}$ and $OH^-_{\text{hydration}}$ would be given by the formula of Walker above mentioned. As soon as the concentration of the hydroxyl ion is sufficient to reduce the concentration of the hydrogen and hydroxyl ions, resulting from the dissociation of the inner salt, to the same value as that of the same ions in pure water, the solute has been quantitatively neutralized, and $OH_{\text{neutralization}}$ becomes a constant.

This investigation of the amino acids then resolves itself into a study of 3 factors: the behavior of (1) the solute plus solvent, (2) the solvent alone, (3) the solute alone. The values of the third can be calculated from those of the first and second.

If to a given volume of the amino acid solution, different portions of a standard alkali are added, and after each addition the P_H value is determined by the hydrogen electrode, a set of values are obtained which show the amount necessary to neutralize the solute and overcome hydration of the solvent plus solute to the same points. Again, if sufficient standard alkali is added to a like volume of the solvent alone so as to bring it to the same P_H values as those obtained with the original solution, care being taken to have the volumes the same at each point measured, respectively, another set of values are obtained which show the amount necessary to overcome the hydration of the solvent alone. Since the P_H values are the same in both cases, subtracting the cc. of alkali necessary to bring the solvent itself to any P_H value, from those used in bringing the solvent plus solute to the same P_H values, will give a set of values which show the course of neutralization of the solute alone. In this way the behavior of the amino acid itself can be determined and its own course of neutralization observed.

Apparatus and Solutions Used.

The apparatus contains the following pieces: a Kohlrausch slide wire bridge; a Type B (No. 2500) Leeds and Northrup galvanometer; a Weston millivoltmeter and multiplier; Edison storage cells; and the hydrogen and normal calomel electrodes made according to the directions of Hildebrand.[1]

Hydrogen made by the electrolytic process was used, and, as a precaution against impurities, the gas was washed in a train of bottles contain-

[1] Joel H. Hildebrand, "Some Applications of the Hydrogen Electrode in Analysis, Research, and Teaching," THIS JOURNAL, **35,** 847 (1913).

ing 2% permanganate solution, alkaline pyrogallic acid, and mercuric chloride. The electrodes as well as the tips of the burets were inserted through a rubber stopper which fitted the electrode vessel. In this way the solution was effectively protected from the carbon dioxide of the air. The electrode vessel was connected to a suitable shaking device run by a small electric motor. The calomel cell, electrode vessel, and connections were placed in a suitable bath and the temperature kept constant at 18°.

The water, distilled, using condensers of block tin, was collected and kept in old glass containers which had been used for this purpose for several years. Carbon dioxide free air was passed through this water until it was practically free from carbon dioxide, and gave a P_H value of 6.0 to 6.6. All solutions used in this experiment were made up from this water and carefully protected at all times from the carbon dioxide of the air.

Tenth molar solutions of the amino acids were used. The requisite amount of each acid was weighed out and dissolved in water, excepting tyrosin, which was dissolved in 30 cc. of 0.1 N hydrochloric acid, and the solutions made up to volume with water. It was desired to compare the results obtained here with those from the work on wheat and flour. Since 100 cc. was the volume used in that investigation, the same volume was adopted here.

The alkali used was 0.1 N sodium hydroxide solution. Two hundred g. of the c. p. salt was dissolved in 300 cc. of carbon dioxide free water. In alkali of this strength the carbonates are insoluble.[1] From this solution portions were pipetted off and used in making up the 0.1 N solution. The alkali was standardized against 0.1 N sulfuric acid, using phenol-phthalein as indicator.

Procedure with the Hydrogen Electrode.

Influence of Solvent on Titration.—Since it was desired to differentiate between the solvent and the solute, the first question investigated was the behavior of water under the conditions of the experiment. The curve obtained experimentally by the addition of different portions of standard alkali to a given volume of water and the determination of the P_H value by the hydrogen electrode after each addition will show what influence the solvent has on investigations of this kind. This curve can be calculated also, and thus it serves as a check on the purity of the water used and on the accuracy of the method.

One hundred cc. of water was pipetted into the electrode vessel and hydrogen gas passed through until equilibrium was attained. This usually required 30 to 60 minutes. During the entire time the electrode vessel was shaken 50 to 60 times per minute. As soon as the potential remained

[1] Michaelis, Leonor, *Wasserstoffionen-Konzentration*, 1914, p. 172.

constant within one millivolt for 15 minutes, 0.1 cc. of 0.1 N alkali was added from a buret. The solution was allowed to come to equilibrium as before. The potential was noted and alkali run in again until 0.2 cc. had been added. The constant potential was determined again and noted. In this way definite portions of alkali, as noted in Table I, were added and after each addition the constant potential was determined and noted. This was continued until 50 cc. altogether had been added. Preliminary experiments showed that it was not necessary to go beyond this value in the present experiment.

In Table I will be found the data thus obtained, together with the theoretical values.

The concentrations of the hydrogen ion were calculated by the following formula:

$$E = 0.0577 \times \log 1/c + 0.283 \tag{1}$$

where E is the potential in volts at equilibrium; C the concentration of the hydrogen ion in gram ions per liter; 0.0577 the value of the constant RT/F at 18° as given by Nernst;[1] and 0.283 the potential difference between the normal potassium chloride electrode and the normal hydrogen electrode as zero.[2] If the ionization constant of water at 18° be taken as 0.64×10^{-14} as given by Nernst,[3] then

$$OH^- = \frac{0.64 \times 10^{-14}}{H+}. \tag{2}$$

The theoretical values were calculated by the following formula:

$$OH = \frac{A \times N}{100 + A} \times I$$

where OH = concentration of OH^- ions in gram ions per liter; A = cc. of alkali added; N = normality of alkali; I = per cent. of ionization.

For example, when 50 cc. of 0.1 N sodium hydroxide is added to 100 cc. of water, the formula becomes

$$OH = \frac{50 \times 0.1}{150} = 0.03333.$$

At this concentration I = 93.5 (%), whence

$$OH = 0.03333 \times 93.5 = 3.12.10^{-2}.$$

The percents ionization were obtained from a graph constructed from the data given by Prideaux.[4]

[1] Nernst, Z. Elektrochem., 12, 1 (1906); 9, 686 (1903); 10, 629 (1904); 11, 537 (1905).
[2] Wilsmore, Z. physik. Chem., 35, 291–332 (1900); Ostwald and Wilsmore, Ibid., 36, 91 (1901).
[3] Nernst, Z. physik. Chem., 14, 155 (1894).
[4] Prideaux, "Theory and Use of Indicators," 1917, p. 28, New York.

TABLE I.

Addition of Standard Alkali to 100 cc. of Water.

Cc. 0.1 M NaOH.	Volt.	P_H.	Gram ions OH (determined).	Gram ions OH (calculated).	Ionization %.
Equi.	0.656	6.462	$1.855.10^{-8}$	.....	..
0.1	0.868	10.136	$8.78.10^{-5}$	$9.99.10^{-5}$	..
0.2	0.884	10.413	$1.67.10^{-4}$	$1.98.10^{-4}$	99.5
0.5	0.910	10.864	$4.70.10^{-4}$	$4.92.10^{-4}$	98.9
1.0	0.928	11.176	$9.62.10^{-4}$	$9.71.10^{-4}$	98.1
5.0	0.967	11.853	$4.57.10^{-3}$	$4.54.10^{-3}$	95.4
10.0	0.983	12.130	$8.64.10^{-2}$	$8.64.10^{-2}$	95.1
20.0	0.998	12.389	$1.57.10^{-2}$	$1.58.10^{-2}$	94.7
30.0	1.006	12.527	$2.17.10^{-2}$	$2.17.10^{-2}$	94.3
40.0	1.011	12.614	$2.63.10^{-2}$	$2.68.10^{-2}$	94.0
50.0	1.015	12.684	$3.09.10^{-2}$	$3.12.10^{-2}$	93.5

Titrating the Amino Acid Solution.—Twenty-five cc. of 0.1 N glycocoll solution and 75 cc. of water were pipetted into the electrode vessel and hydrogen gas passed through until equilibrium was attained. Then different portions of 0.1 N alkali were added from a buret, as indicated in Table II, and after each addition the equilibrium point was noted in exactly the same manner as in the preceding experiment. The addition of alkali was continued until the solution had a P_H value of about 12.5. Preliminary experiments had shown that all the amino acid was neutralized before this value was reached. When the values thus obtained were plotted, as in Fig. 2, a curve was obtained which shows the course of neutralization of the solute and the behavior of the solute plus the solvent under hydration. But since the curve of neutralization of the solute was desired, it became necessary to determine the blank.

Determining the Blank.—By the term "blank" is meant the cc. of alkali necessary to bring the solvent itself to the same P_H values as those obtained by the addition of definite portions of alkali to the solution of the amino acid. Since 100 cc. of the solution of the amino acid was used, 100 cc. of water was taken in determining this blank. To this 0.1 N alkali was added in such portions as were necessary to bring the blank to equilibrium at the same P_H values as those obtained in the solution of the amino acid. But since the solution of the amino acid always required more alkali to bring it to a certain P_H value than the same volume of water alone, sufficient water must be added at each P_H value to bring the blank to the same volume as that of the original. For example, 10 cc. of alkali produced a P_H value of 9.57 in the glycocoll solution. The total volume at this point was 110 cc. It required 0.1 cc. of alkali and 9.9 cc. of water to give the blank a P_H value of 9.57 and a total volume of 110 cc. The water was added to the electrode vessel from a second buret. In practice sufficient alkali was added to the blank to

bring it to equilibrium at approximately P_H 9.57, care being taken not to over run. From this a rough estimate of the amount of water to be added could be made. Somewhat less than the estimated amount was added and the solution brought to equilibrium again. This addition of water or alkali was repeated until the blank came to equilibrium at P_H 9.57 with a total volume of 110 cc. Subtracting the cc. of alkali necessary to bring the blank to the value P_H 9.57 from the cc. which produced the same value in the original solution, gave the cc. of alkali necessary to neutralize the amino acid to the same point. Thus of the entire 10 cc. of alkali added to the glycocoll solution, 0.1 was apportioned to the solvent and 9.9 cc. to the solute. If the blank is determined in this way for all the P_H values obtained in the original solution and the cc. thus obtained subtracted from the cc. obtained in the titration of the glycocoll, a set of values is obtained which represents the course of neutralization of the acid itself. As soon as all of the acid is neutralized this value becomes constant and the curve from that point on runs parallel to the P_H axis. Thus the method becomes a quantitative one. The results are set forth in Table II. Fig. 1 shows this method of differentiating between the solvent and solute.

TABLE II.

Titration of the Solution of Glycocoll.

Volts.	P_H.	Cc. $N/10$ NaOH added to glycocoll solution.	Cc. $N/$ NaOH for blank.	Cc. $N/10$ NaOH used in neutralizing amine acid alone.
0.700	7.22	Equi.	..	..
0.712	7.44	0.1	..	0.1
0.749	8.08	0.5	..	0.5
0.767	8.38	1.0	..	1.0
0.797	8.90	3.0	..	3.0
0.812	9.16	5.0	..	5.0
0.835	9.57	10.0	0.1	9.9
0.855	9.91	15.0	0.2	14.8
0.877	10.30	20.0	0.3	19.7
0.914	10.93	25.0	1.3	23.7
0.940	11.38	28.0	3.2	24.8
0.951	11.58	30.0	5.0	25.0
0.9785	12.05	40.0	15.1	24.9
0.987	12.20	50.0	25.0	25.0
1.008	12.56	100.0	75.0	25.0

Phenylalanin, tyrosin, lysin and glutamic acid were investigated in the same way. The data is set forth in Table III. In the columns headed "Corrected, cc." are found the cc. of alkali necessary to neutralize the amino acid alone to the corresponding P_H value. Fig. 2 shows the curve of neutralization for each amine acid.

TABLE III.

Titration of the Amino Acids.

NaOH added. Cc.	NaOH blank. Cc.	Corrected. Cc	Volt.	P_H.
		Phenylalanin.		
Equi.	..	..	0.518	4.07
0.1	..	0.1	0.527	4.23
0.2	..	0.2	0.531	4.29
0.5	..	0.5	0.546	4.56
1.0	..	1.0	0.573	5.02
5.0	..	5.0	0.769	8.42
10.0	..	10.0	0.800	8.95
15.0	..	15.0	0.822	4.34
20.0	0.2	19.8	0.845	9.74
25.0	0.5	24.5	0.891	10.53
28.0	2.9	25.1	0.938	11.35
30.0	5.0	25.0	0.949	11.58
40.0	15.0	25.0	0.976	12.01
50.0	25.0	25.0	0.987	12.20
		Tyrosin.		
Equi.	..	..	0.437	2.67
1.0	..	1.0	0.497	3.70
5.0	..	5.0	0.724	7.64
10.0	..	10.0	0.802	9.00
20.0	..	20.0	0.846	9.76
30.0	0.2	29.8	0.868	10.14
40.0	0.5	39.5	0.891	10.53
50.0	2.6	47.4	0.931	11.23
60.0	11.5	48.5	0.966	11.84
70.0	20.4	49.6	0.979	12.06
80.0	29.7	50.3	0.987	12.20
90.0	39.3	50.7	0.992	12.29
100.0	50.0	50.0	0.997	12.37
130.0	79.6	50.4	1.005	12.51
		Lysin-Dihydrochloride.		
Equi.	..	..	0.395	1.94
0.3	..	0.3	0.396	1.96
1.5	..	1.5	0.397	1.97
10.0	..	10.0	0.413	2.25
15.0	..	15.0	0.425	2.46
20.0	..	20.0	0.447	2.84
25.0	..	25.0	0.706	7.33
30.0	..	30.0	0.835	9.57
45.0	..	45.0	0.845	9.74
60.0	0.9	59.1	0.902	10.73
75.0	5.1	69.9	0.944	11.45
85.0	12.5	72.5	0.965	11.82
100.0	25.2	74.8	0.980	12.08
120.0	45.1	74.9	0.9925	12.28
150.0	75.0	75.0	1.0013	12.45

TABLE III (continued).

NaOH added. Cc.	NaOH blank. Cc.	Corrected. Cc.	Volt.	P_H.
		Glutamic Acid.		
Equi.	..	..	0.471	3.26
0.2	..	0.2	0.472	3.27
1.0	..	1.0	0.475	3.33
5.0	..	5.0	0.498	3.73
10.0	..	10.0	0.517	4.05
20.0	..	20.0	0.563	4.85
26.0	..	26.0	0.773	8.49
30.0	...	30.0	0.814	9.20
40.0	0.1	39.9	0.856	9.93
50.0	1.0	49.0	0.906	10.79
56.0	6.8	49.2	0.954	11.63
60.0	9.9	50.1	0.961	11.75
80.0	30.0	50.0	0.987	12.20
100.0	50.0	50.0	0.9967	12.37

Discussion of Results.

Differentiation between Solvent and Solute.—The colorimetric method is laborious, and the errors due to the presence of salts, proteins, and other substances render the results in some cases well-nigh worthless. The hydrogen electrode makes possible an exact differentiation between an amount of alkali or acid to be apportioned to the solvent and solute, respectively, at any $P_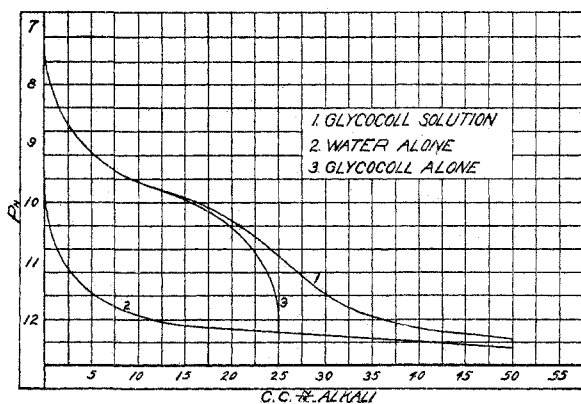H$ value. The results shown in Table II and graphically in Fig. 1 illustrate this method of differentiation. Since at each P_H value the concentration of the OH^- ion both in the blank and in the solution of the amino acid are the same and the volume of the original solution and the blank are equal, there would be no difference in ionization in the observed results for the blank and for the solution of

Fig. 1.—Method of differentiating between the solvent and the solute.

amino acid. In case the blank is calculated, however, a correction must be made for ionization. It will be noted in Fig. 1 that the further addition of alkali beyond the P_H value, 11.58, merely increases the hydroxyl ion concentration of the solvent alone. From this point on the curve for the solute runs parallel to the P_H axis. At this point the solute has been

quantitatively neutralized. The ionization of sodium glycollate as a base would not be appreciable at this hydroxyl ion concentration; hence the method becomes a quantitative one.

Neutralization Curves for the Different Amino Acids.—In Fig. 2 will be found the curves showing the course of neutralization for the substances used in this investigation. The curve for glycocoll is quite symmetrical and shows the influence of the amino group on the neutralization of the carboxyl. The acidic nature of the benzene ring is shown very clearly by the curve for phenylalanin. Not only is the initial P_H value greater than that of glycocoll, but the curve of neutralization lies above that of glycocoll for the entire distance.
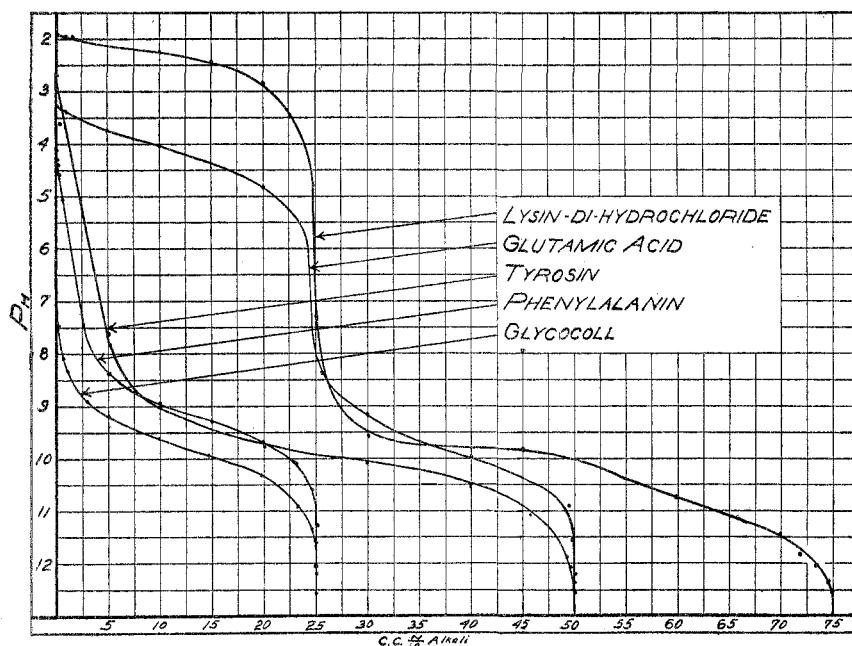

Fig. 2.—Neutralization curves for amino acids alone.

In the curve for glutamic acid the influence of the second carboxyl group can be clearly seen. One carboxyl group is neutralized above the concentration P_H 7; while the other is below this point. The influence of the amino group on the nearest carboxyl makes an enormous difference on the concentration of the hydroxyl ion necessary to neutralize it, as compared with that for the carboxyl further away for the amino group.

Since it was desired to see what influence the combined hydrochloric acid would have on the neutralization curve, no correction was made for the combined acid in lysine dihydrochloride. It will be noticed that

about $^1/_3$ of the acidity is neutralized before the value P_H 7.0 is reached and $^2/_3$ below this point. This would indicate that the strongly negative chlorine influences the molecule to such an extent that lysine dihydro-chloride ionizes as a strong acid. Since both amino groups are combined with hydrogen chloride, the carboxyl will be neutralized above the P_H 7.0 point. An excess of hydroxyl ions are necessary, however, to split off the combined hydrogen chloride groups.

As might be expected, the tyrosin molecule behaves as a dibasic acid. A greater hydroxyl ion concentration, however, is required for complete neutralization than in the case of the simpler amino acids.

A comparison of the neutralization curve of tyrosin with that of phenyl-alanin shows the increased acidic property imparted by the hydroxyl groups in the benzene ring.

Summary.

1. By means of the above adaptation of the electrometric titration method it is possible to obtain the neutralization curves of amino acids alone. In this way the influence of the different groups in the molecule can be seen and certain inaccuracies in the formal titration method explained by definite data.

2. An hydroxyl ion concentration of about 2×10^{-2} (P_H 12.5) will suppress to a negligible quantity the basic ionization of the sodium salts of the amino acids, and thus make possible a more exact quantitative determination of the diamino acids as well as that of others containing strongly negative groups.

3. The quantitative method is carried out as follows: Sufficient standard alkali is added to a definite volume of the aqueous solution of the amino acid under investigation to give it a P_H value of about 12.5. Then to an equal volume of water, the same standard alkali is added in an amount sufficient to give it the same P_H value, care being taken to add sufficient water to give the blank the same volume as that of the original at the P_H value compared. Subtracting the cc. used in the blank from that required in the original gives the cc. of standard alkali necessary to neutralize the amino acid alone.

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