# CCCXXI. THE POTENTIOMETRIC DETERMINATION OF POLYPEPTIDES AND AMINO-ACIDS.

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During the course of work on proteolytic hydrolysis the need arose for accurate determinations of small quantities of amino-acids and polypeptides in mixtures. It appeared from the literature that two possible methods were available, firstly, the conductimetric titration of Widmark and Larssen [1923], and secondly, the colorimetric titrations employing aqueous alcohol [Willstätter and Waldschmidt-Leitz, 1921], or acetone [Linderstrøm-Lang, 1928], and the formaldehyde method [Sørensen, 1907].

Willstätter and Waldschmidt-Leitz recommend a first titration with alkali in 40 % alcoholic solution followed by another in 97 % alcohol for the separate determination of peptides and amino-acids in mixtures. It was decided to follow the course of such titrations potentiometrically.

For such a method to be reliable, there should be in the 40 % alcohol titration curve a point of inflection corresponding to the total peptide. For the accurate determination of this point by a colorimetric method, two conditions must be realised. Firstly, the $p_H$ change about this point should be of a high order, and secondly, the point should occur at a fixed $p_H$. It has however been shown in this communication that for mixtures of leucylglycylglycine and glycine in 90 % alcohol, the peptide titrates at $p_H$ 9·25 when 17 % glycine is present, and at $p_H$ 7·5 when 92 % glycine is present. Except in limiting cases, the slope about the peptide inflection in the titration curve is small, and further with such limiting cases, the shapes of the curves show that the constituent present in smaller amount will almost certainly be missed when a colorimetric method is used. These considerations will apply equally well to 40 % alcohol. The accuracy of the second titration (in 97 % alcohol) is not however open to question unless the indicator employed changes at a different $p_H$ from that corresponding to the neutralisation of the weakest acid present.

The degree of separation of the constituents of amino-acid-peptide mixtures attainable by potentiometric titration will be governed by the following factors. Any such mixture when titrated with alkali will show inflections in its titration curve corresponding to the various acids present. In theory the titration curve should contain inflections for every acid present unless the differences between the logarithms of their dissociation constants are less than 1·2 [Auerbach and Smolczyk, 1924].

In practice however, the dissociation constants fall into several main groups each containing acids of very nearly equal dissociation constants. Such groups will appear in the titration curve as a single acid, *i.e.* will give rise to one inflection. Thus glycine, alanine and leucine, having approximately equal dissociation constants ($p_{K_a}$ 9·8 in water), will titrate together as a single acid. Similar considerations apply to simple peptides such as alanylglycine, glycylglycine, and

leucylglycine ($p_{K_a}$ 7·8), so that a mixture containing all the above substances will give two inflections, one corresponding to the total peptide, and the other to the total —COOH of the mixture. Glutamic acid ($p_{K_a}$ 6·0 and 10·3) will give a first inflection before the peptide, and therefore, if also present, will be readily detected. On the other hand, histidine and asparagine, having $p_{K_a}$ 8·8 and 9·0 respectively, will titrate between the peptide and the amino-acids. One could not expect however, on theoretical grounds to obtain inflections for each acid in a mixture of, for example, leucylglycylglycine, glycine and histidine, and this is shown to be the case, the glycine and peptide inflections alone being apparent. The curves of arginine and lysine, as would be expected from their high $K_a$ values, show no inflections whatever and hence cannot be determined by this method.

In practice such points of inflection, the final one excepted, do not appear clearly in the titration curve owing to the buffering of the untitrated acid. In order to determine their exact positions it was found necessary to adopt a differential method of plotting. Curves obtained by this method show peaks corresponding to each inflection. For this method to be successfully applied the titrant must be added in small increments and this necessitates a somewhat high degree of accuracy in the E.M.F. measurement, readings being required to 0·2 mv.

## Experimental.

*The titration vessel* (Fig. 1) consisted of two parts connected by a ground-glass joint. The lower portion held about 12 ml. liquid and the substances to be titrated were weighed directly into it on a micro-balance. The outlet from the cell was connected to a U-tube containing 90 % alcohol.

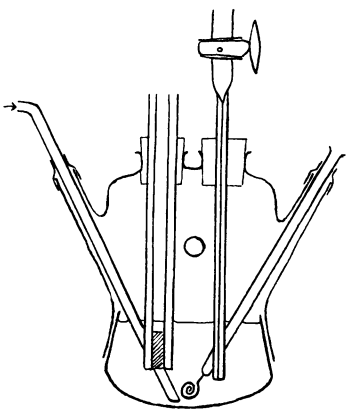

Fig. 1.

The titrations were carried out in 90 % aqueous alcohol at 25°, using a hydrogen electrode, and to obtain satisfactory results it was found necessary to wash the hydrogen with a 90 % alcohol-water mixture before passing it into the titration vessel, and to pass it through the NaOH solution before each titration to remove traces of dissolved oxygen. This second precaution enabled the electrodes to reach equilibrium in 1–2 min. as compared with 5–10 min. with untreated sodium hydroxide. It was found advantageous to employ small electrodes consisting of 1–1·5 cm. of platinum wire as these came to equilibrium more quickly than the larger plate type. Various methods of plating were tried but the electrodes in all cases became rapidly poisoned, becoming useless after about three titrations and sometimes failing after two. Thus to ensure satisfactory results each electrode was used once only. Platinised, gold-plated electrodes were employed as they came to equilibrium rapidly and were easily stripped of black. The electrodes were prepared by gold plating from a solution of gold hydroxide in potassium cyanide (prepared according to the instructions of Clark), using a current of 2 mA. for 5 min., this being followed by platinising from a 3 % solution of chloroplatinic acid containing a trace of lead acetate till the electrode was just covered with black. Electrodes of this type gave E.M.F.'s in water which were usually identical and never more than 0·2 mv. apart, but in alcohol they were

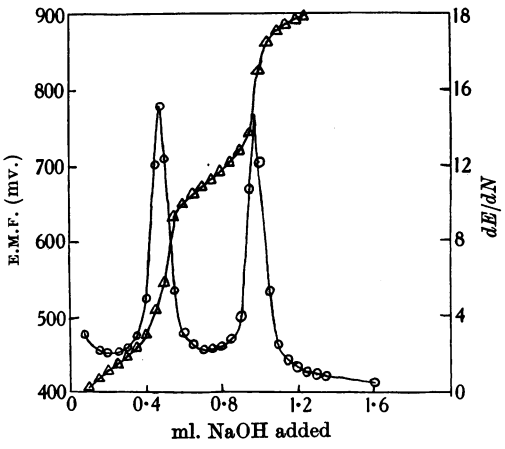

Fig. 2A.

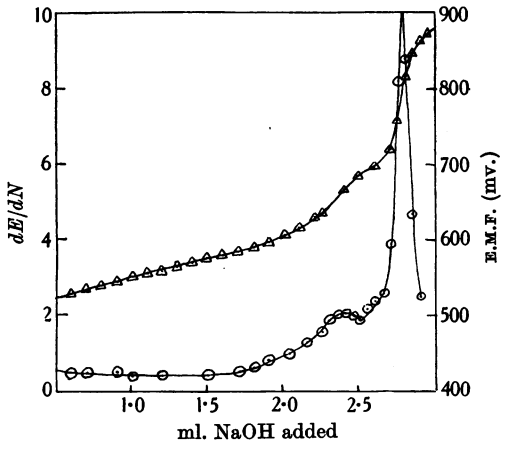

Fig. 2B.

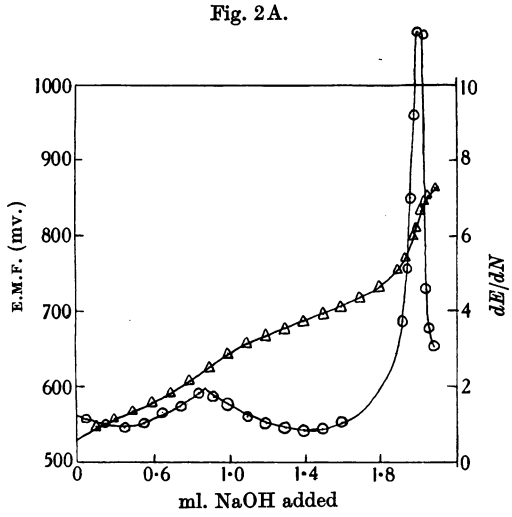

Fig. 2C.

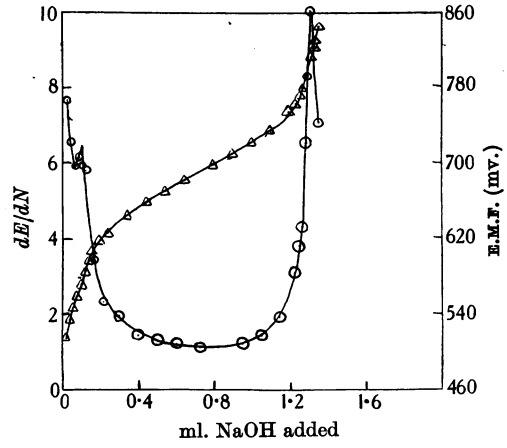

Fig. 2D.

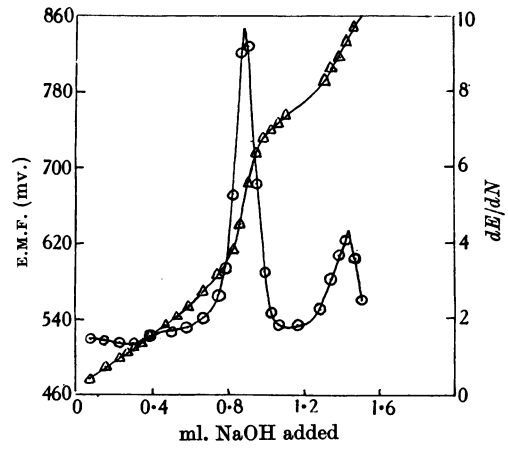

Fig. 2E.

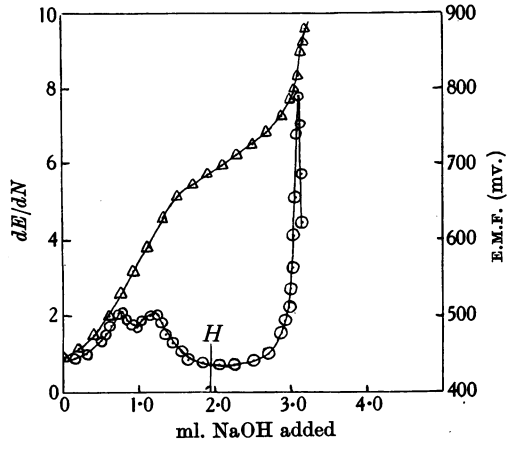

Fig. 2F.

usually 1–2 mv. apart and remained so during the whole of a titration. The contact between the bridge solution (saturated aqueous potassium chloride) and the titration vessel was made in a cotton-wool plug in the side arm of the half cell, which consisted of a silver-silver chloride electrode in saturated potassium chloride solution. The cotton-wool plug was renewed after each titration. The sodium hydroxide was added in increments of 0·02–0·05 ml. depending on the total titre. From the values of E.M.F. and ml. sodium hydroxide added the differential curves were constructed as follows: If the E.M.F. recorded was $E_1$, $E_2$, $E_3$, $E_4$, *etc.* corresponding to $N_1$, $N_2$, $N_3$, $N_4$ ml. of sodium hydroxide added, then the slope of the curve at the point $N_2$ was taken to be $(E_3-E_1)/(N_3-N_1)$, and that at the point $N_3$ as $(E_4-E_2)/(N_4-N_2)$. The potentiometer was a Pye standard slide wire instrument graduated to 0·2 mv. incorporated in a ballistic type of valve potentiometer of similar design to that of Morton [1931], but employing a Marconi receiving valve, type HL2. A Tinsley portable galvanometer of the damped type was employed, the apparatus being sensitive to 0·1 mv.

## RESULTS.

The alcoholic sodium hydroxide was standardised against A.R. glycine in the apparatus.

All amounts are expressed as g. mol. $\times 10^6$.

Titration of histidine hydrochloride.

(Fig. 2A) NaOH, 0·0523$N$; 25·8 histidine hydrochloride present.

| | |
|---|---|
| Found: 1st peak (HCl) ... ... ... ... | 25·1 |
| 2nd peak (HCl + histidine) ... ... | 51·0 |

Mixtures of glycine and leucylglycylglycine.

| | Exp. | Conc. of NaOH | Glycine actual | Leucylglycylglycine Actual | Leucylglycylglycine Found | Total COOH Actual | Total COOH Found |
|---|---|---|---|---|---|---|---|
| (Fig. 2B) | 1 | 0·0420$N$ | 19·8 | 97·1 | 99·6 | 116·9 | 116·6 |
| (Fig. 2C) | 2 | 0·0523$N$ | 59·0 | 45·55 | 46·0 | 104·55 | 105·1 |
| (Fig. 2D) | 3 | 0·0423$N$ | 50·4 | 4·61 | 4·65 | 55·0 | 55·4 |

Mixture of glutamic acid and leucylglycylglycine.

(Fig. 2E) NaOH, 0·0523$N$, actual amounts were: leucylglycylglycine, 20·2; glutamic acid, 27·65.

| | Actual | Found |
|---|---|---|
| Found: 1st peak (glutamic acid) | 27·65 | 26·15 |
| 2nd peak (glutamic acid + peptide) | 47·85 | 47·10 |
| 3rd peak (total —COOH) | 75·50 | 75·90 |

Mixture of histidine hydrochloride, glycine and leucylglycylglycine.

(Fig. 2F) NaOH, 0·0523$N$, actual amounts were: leucylglycylglycine, 25·3; glycine, 58·0; histidine hydrochloride, 38·7.

| | Actual | Found |
|---|---|---|
| Found: 1st peak (HCl) | 38·7 | 38·7 |
| 2nd peak (HCl + peptide) | 64·0 | 62·8 |
| 3rd peak (total —COOH) | 160·7 | 162·0 |

Histidine gave no observable inflection at the theoretical point, $H$, on the curve.

## SUMMARY.

1. The titration of mixtures of amino-acids and polypeptides representative of types to be expected in protein hydrolysates is carried out in 90 % aqueous alcohol.

2. The degree of separation which can be attained in such mixtures is discussed.

3. The $p_H$ of the end-point for such peptides as leucylglycylglycine in mixtures with an amino-acid such as glycine has been shown to vary over a range of nearly two $p_H$ units depending on the proportions of the constituents. Thus any colorimetric titration becomes unreliable and must be replaced by potentiometric titration, for which a special method of differential plotting is described, this method being specially serviceable in the determination of mixtures.

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