CCCXXXIV. A SIMPLE PROCESS FOR ESTIMATING AMINO- OR OTHER BASIC GROUPS IN AMINO-ACIDS *ETC.*: THE "GLACIAL ACETIC ACID" METHOD.

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A STUDY of the titration curves of the amino-acids [Harris, 1923, 1] shows that their amino-groups are quantitatively converted into their hydrochlorides (or similar acid salt) when the p_H is reduced to a value approaching 0.5 to 0 (for titration constants of certain individual amino-acids, *cf.* Harris [1923, 2, 3; 1929, 1]; Birch and Harris [1930]). In other words, provided that one starts with the free amino-acid and titrates it to a sufficiently acid end-point the amount of acid entering into combination with it is then stoichiometrically equal to its amino- or other basic groups.

(It does not affect the argument whether the original ampholyte was of the zwitterion or non-zwitterion type—*i.e.* whether any of its acid or basic groups were present in the non-ionised or fully ionised, internal salt, form. For, at the conclusion of the titration all the basic groups are present as hydrochloride, and at the beginning there was no combined HCl.)¹

It becomes apparent at once therefore that a simple method for estimating the amino-groups is to measure the amount of acid needed to titrate them to a highly acid end-point (p_H 0 to 0.5). There is only one disadvantage to this procedure from a practical standpoint, that is that the "blank correction" is very large. That is to say, in addition to the HCl which is needed to bring the amino-acid itself to the desired p_H , a considerable further amount will be needed to bring the solvent (water) to the same highly acid reaction. This is shown in

¹ The equation for an acid ampholyte (*e.g.* glutamic or aspartic acid) is

for a neutral ampholyte (*e.g.* glycine *etc.*),

and for a basic ampholyte (*e.g.* lysine *etc.*),

Fig. 1, from which it will be seen also that the magnitude of the blank correction is increased (and the accuracy of the titration is accordingly diminished) in proportion to the final dilution of the solution.

Fig. 1. Titration curve of glycine with HCl: ideal, and at dilutions of N , $0.1 N$ and $0.01 N$. At any p_H level the difference between the ideal curve and the curves A , B or C represents the blank correction at the given dilution. (In the example represented, the total volume is imagined as kept constant at the dilution shown, for every reading on the curve.)

In short, when the titration is thus carried out in aqueous solution there is no steep change in p_H at the end of the titration. Hence it is impossible to obtain a sufficiently sharp colour change in an indicator to mark a sudden end-point to the titration. As was pointed out in the original paper in this series [Harris, 1923, 1] the difficulty may be obviated in two ways. Either the amino-acid may be titrated in very concentrated solution, in which case the blank is relatively small (if the measurements of p_H are made electrometrically, the blank correction may be worked out theoretically with some degree of accuracy). A more simple procedure, however, is to use in place of the water some alternative solvent which needs only a negligible "blank" to bring it to the required p_H end-point of 0.5 to 0. Then the titration is reduced to the easiest of all chemical methods—*viz.* the simple addition of titrant from a burette until, with the last drop, the indicator abruptly changes its colour.

A number of suitable solvents was tried and eventually glacial acetic acid was chosen as the most satisfactory. It has been known for many years as a convenient solvent for most bases and has already been utilised by Hall and Conant [1927; see also Conant and Hall, 1927; Hall and Werner, 1928] for electrometric measurements on bases in "super-acid" solutions. A suitable indicator was found in brilliant cresyl blue (obtainable from The British Drug

Houses, Ltd.), which is soluble in glacial acetic acid and undergoes a clear colour change from blue to yellow at a p_K value near 0 (yellow with increasing acidity: the p_K value of the indicator in water is given as 1 to -0.2).

Routine tests were accordingly carried out with all the better known amino-acids, and it was found that results of about 99 % accuracy could be obtained without difficulty. This work was concluded in 1929, and a summary of the conclusions was given in that year, when the method was applied to glutathione to determine the number of basic groups and the combining weight [Harris, 1929, 2]. Pressure of other work has since delayed the presentation of the extended figures on the complete series of amino-acids. It is felt however that the method is such a convenient one and capable of such wide application that at the suggestion of other workers the full working directions are now being published.

EXPERIMENTAL.

Preparation of specimen. The principal use of the method in the writer's experience has been to estimate the basic groups in isolated preparations in the solid form, more particularly as an aid in the characterisation of unknown substances. It is suitable for applying directly to a specimen of the free (isoelectric) amino-acid, ampholyte, base *etc.*, rather than to a sodium salt or hydrochloride *etc.* If such added acid or alkali is present it should be neutralised with an equivalent amount of alkali or acid before the titration is begun. It is always preferable and simpler, however, to carry out the estimation on the free substance itself whenever this is available. It is essential that the specimen to be titrated should be dry. If estimations are required on a solution, therefore, an aliquot part of it should be taken and the solvent evaporated.

Standard acid solution. As a titrant possessing more strongly marked acid properties than HCl, and readily miscible with glacial acetic acid, perchloric acid has been used throughout. A stock solution is prepared for the writer by The British Drug Houses at 1.0 *N* dilution,¹ and this is then further diluted with glacial acetic acid to the strength desired for each titration, generally 0.1 *N*.

Standard glycine for back-titration. It is convenient to have a solution of standard base for purposes of back-titration. I have found that at the very acid reactions used, a solution of glycine in glacial acetic acid serves the purpose very well. To prepare a 0.1 *N* solution, dissolve 0.7505 g. of glycine in glacial acetic acid, bringing the final volume to 100 ml.: the glycine may take a few hours to go entirely into solution.

Indicator solution. A suitable final dilution for the indicator, *i.e.* at the end of the titration, is from about 0.0015 to 0.003 %. Stock solutions of 0.01 or 0.1 % should be made up in glacial acetic acid.

Directions for rapid routine procedure—semi-micro-scale. In carrying out a titration, an amount of the dry specimen is weighed out (*e.g.* about 15 mg. in the case of glycine) which would yield about 2 ml. of 0.1 *N* solution. This is placed in a small stoppered weighing bottle (*e.g.* 10 ml. size), and a slight excess of the standard 0.1 *N* perchloric acid solution added from a burette reading to 0.01 or 0.02 ml. (The advantage of adding excess of acid in this way is that the specimen then goes more readily into complete solution.) The stopper is replaced

¹ To prepare *N* perchloric acid in 100 % acetic acid, the following procedure, described by Hall and Werner [1928], may be followed: Add 8 *N* aqueous perchloric acid to the theoretical quantity of chilled acetic anhydride and dilute with glacial acetic acid to *N*. It is important to have no excess of acetic anhydride or water, which can be prevented by adding a few drops of H₂O or Ac₂O, as necessary, until no further temperature rise is shown on a thermometer.

to keep the solution free from moisture, and as soon as the specimen is all dissolved a suitable amount of the indicator solution is added (*e.g.* 1 ml. of 0.01 % solution of brilliant cresyl blue in dry glacial acetic acid).¹ The end-point is then determined by back-titration with 0.1 *N* glycine, a second burette also reading to 0.01 ml. being used. The difference between the acid titre and the back titre with glycine gives the amount of amino-nitrogen, or other basic group, present. For greater accuracy the result should be corrected for a small blank correction. The blank correction is the amount of standard perchloric acid solution needed to bring the solvent and indicator alone, in the absence of the solute, to the same end-point colour change, the volume of solvent being the same as at the end of the actual titration. (Under the conditions described the blank correction is no more than about 1 % of the titre; or less if a more precise technique is adopted; see below.)

Results obtained by the routine, semi-micro-method. In Table I are assembled the results obtained on most of the better known amino-acids, and a number of polypeptides, bases and other substances.

It will be seen that without special precautions having been taken results of about 99 % accuracy were readily obtained.

Table I. *Routine determinations of basic groups in amino-acids etc.*

(1)	(2)	(3)	(4)	(5)	(6)		(7)
No.	Name of specimen	Weight of specimen taken (g.)	ml. of indicator solution (0.01 %) added	Titration data <i>a-b-c</i> *	ml. of 0.1 <i>N</i> acid needed in titration		Error %
					Found	Theoretical	
Amino-acids.							
1	Glycine	0.0173	1.0	2.40-0.09-0.02	2.29	2.31	-0.9
2		0.0167	1.0	2.30-0.04-0.02	2.24	2.23	+0.4
3		0.0167	1.0	2.40-0.18-0.02	2.20	2.23	-1.3
4		0.0177	1.0	2.50-0.10-0.02	2.38	2.38	
5			1.0	2.60-0.20-0.02	2.38		
					2.38	2.36	+0.9
6	<i>d</i> -Alanine	0.0201	1.0	2.50-0.23-0.02	2.25	2.26	-0.4
7		0.0351	1.0	4.00-0.10-0.02	3.88	3.94	-1.5
8	<i>dl</i> -Valine	0.0248	1.0	2.30-0.20-0.01	2.09	2.12	-1.4
9		0.0327	1.0	2.90-0.12-0.02	2.76	2.79	-1.1
10	<i>l</i> -Leucine	0.0227	1.0	1.90-0.15-0.02	1.73	1.73	0
11		0.0320	1.0	2.52-0.08-0.02	2.42	2.44	-0.8
12	Phenylalanine	0.0362	1.0	2.30-0.09-0.02	2.19	2.19	0
13		0.0330	1.0	2.20-0.19-0.02	1.99	2.00	-0.5
14	Tryptophan	0.0194	1.0	1.00-0.03-0.01	0.96	0.95	+1.1
15		0.0443	1.0	2.50-0.30-0.02	2.18	2.17	+0.5
16	Cystine	0.0205	0.5	2.01-0.34-0.01	1.66	1.71	-2.9
17		0.0267	1.0	2.40-0.20-0.01	2.19	2.22	-1.4
18	<i>l</i> -Tyrosine	0.0301	1.0	2.00-0.31-0.02	1.67	1.66	+0.6
19		0.0446	1.0	2.50-0.02-0.02	2.46	2.46	0
20	Aspartic acid	0.0329	1.0	2.50-0.01-0.01	2.48	2.47	+0.4
21		0.0427	1.0	3.30-0.04-0.02	3.24	3.21	+0.9
22	Glutamic acid	0.0394	1.0	2.80-0.12-0.02	2.66	2.68	-0.7
23	Arginine, 2H ₂ O	0.0207	1.0	2.50-0.46-0.02	2.02	1.97	+2.5
24	Arginine, H ₂ O†	0.0241	1.0	2.53-0.04-0.02	2.47	2.50	-1.2

¹ This was the amount taken in nearly all the 51 determinations recorded in Table I. There is some advantage in reducing the volume, *e.g.* to 0.1 ml. of a 0.1 % solution, as the small blank correction then becomes even less (*cf.* Table V).

Table I (cont.).

(1)	(2)	(3)	(4)	(5)	(6)		(7)
No.	Name of specimen	Weight of specimen taken (g.)	ml. of indicator solution (0.01 %) added	Titration data $a-b-c^*$	ml. of 0.1 <i>N</i> acid needed in titration		Error %
			Bases etc.		Found	Theoretical	
25	Sarcosine	0.0250	1.0	2.83-0.00-0.02	2.81	2.81	0
26		0.0234	1.0	2.65-0.00-0.02	2.63	2.63	0
27				2.66-0.02-0.02	2.62		
28				3.01-0.36-0.02	2.63		
29				3.50-0.83-0.03	2.64		
					2.63	2.63	0
30	Creatine, H ₂ O	0.0370	1.0	3.00-0.50-0.02	2.48	2.48	0
31		0.0213	1.0	2.00-0.53-0.02	1.45	1.43	+1.4
32	Creatinine, $\frac{1}{2}$ H ₂ O†	0.0127	1.0	1.05-0.00-0.01	1.04	1.04	0
33		0.0256	1.0	2.15-0.00-0.02	2.13	2.10	+1.4
34		0.0221	1.0	1.85-0.00-0.02	1.83	1.82	+0.5
35	Creatinine + NaCl	0.0221	1.0	1.86-0.00-0.02	1.84	1.82	+1.1
		0.050					
36	Aminosuccinamic acid, H ₂ O	0.0218	1.0	3.00-1.50-0.03	1.47	1.46	+0.6
Peptides and mixtures.§							
37	Glycylglycine	2.50§	1.0	2.61-0.12-0.02	2.47	2.50	-1.2
38		1.13§	1.0	1.20-0.09-0.01	1.10	1.09	-2.7
39			1.0	1.29-0.19-0.01	1.09		
40			1.0	1.36-0.25-0.01	1.10	1.10	-2.7
41	Glutathione, specimen A	1.00§	1.0	1.10-0.13-0.02	0.95	1.00	-5.0
42		1.00§	1.0	1.10-0.10-0.02	0.98	1.00	-2.0
43	specimen B	1.00§	1.0	1.10-0.08-0.02	1.00	1.00	0
44		1.00§	1.0	1.20-0.18-0.02	1.00	1.00	0
45	specimen C	1.12§	1.0	1.31-0.20-0.01	1.10	1.12	-1.8
46		0.91§	1.0	1.10-0.19-0.01	0.90	0.91	-1.1
47	Glycylglycine + glycyltryptophan	2.46§ 1.33§	2.0	4.02-0.19-0.03	3.80	3.79	+0.3
48	Alanylglycylglycine + glycylcysteine	1.21§ 1.13§	2.0	2.77-0.39-0.02	2.36	2.34	+0.9
49	Glycylglycine + glycyltryptophan + alanylglycylglycine + glycylcysteine	1.10§ 0.74§ 1.40§ 1.03§	4.0	6.18-1.87-0.06	4.25	4.27	-0.5
50	Glycine + NaCl	0.0179 0.0223	1.0	2.50-0.02-0.02	2.46	2.39	+2.9
51	Glycine + NaCl	0.0175 0.0415	1.0	2.40-0.05-0.02 2.44-0.09-0.02	2.33 2.33	2.33 2.33	0 0

* a = Volume of 0.1 *N* perchloric acid added, ml.; b = Volume of 0.1 *N* glycine needed in back-titration, ml.; c = Blank correction for given final volume, as ml. of 0.1 *N* perchloric acid.

† Several specimens of arginine examined titrated as though containing varying amounts of water, intermediate between anhydrous, monohydrate or dihydrate.

‡ Results for this specimen agree better with theoretical values calculated for $\frac{1}{2}$ H₂O, rather than 1H₂O or anhydrous.

§ Amounts taken are expressed here in terms of ml. of 0.1 *N* solution.

|| mg.

Additional comments on technique.

Duplicate determinations of end-point. To check the titration reading, whenever desired, a series of further additions of standard acid may be made from the burette and the end-point again determined after each addition, by further

back-titration with standard glycine (see, *e.g.* sarcosine, No. 26, Table I). (If the volume is increased considerably in this way, the "blank" must be corrected in proportion.)

Colour standards. Where great precision is desired, the colour of the indicator at the end-point may be matched between that of control solutions accurately adjusted to two or more given intermediate tints.

Direct titration of bases. Bases in general dissolve more readily than do ampholytes, and therefore the estimation can be completed quickly by direct titration with perchloric acid to a straightforward end-point; little is gained in such cases by back-titration with standard base (see, *e.g.* creatinine, Nos. 32–35, Table I).

Exclusion of moisture. It is essential to keep the burettes and bottles containing the stock solutions well stoppered to prevent access of moisture, which interferes with the sharpness of the end-point. An unsatisfactory reading can in fact generally be attributed to the presence of moisture. The standard glycine and *N* perchloric acid appear to keep almost indefinitely, and the dark brown colour of the latter must not be taken to indicate any deterioration of strength. (A specimen of the *N* perchloric acid was examined after the lapse of 6½ years and found to have retained its strength unimpaired.)

Management of burette. Experience indicates that most accurate results are obtained when the tip of the burette is kept above the surface of the liquid which is being titrated, in order to prevent diffusion. As each fresh reading from the burette is made, the tip is touched on the side of the vessel just above the resting surface of the liquid which is being titrated, so as to remove quantitatively the fraction of a drop expelled. Glacial acetic acid has this advantage as a solvent, that its surface-tension is relatively low and hence it does not "cling" to the walls of the burette or other vessel.

Alternative titrants and indicators. In a series of determinations, aniline in glacial acetic acid was used as an alternative standard base for back-titration. It was found to have the disadvantage of keeping less well than glycine. On the other hand because of its greater solubility it may be made up at greater strengths, and is therefore useful when micro-titrations with *N* reagents are desired (see Table V).

Various other indicators were tried also as alternatives to brilliant cresyl blue, including dianisylacetone and triphenylcarbinol. It was thought that, theoretically, the use of an indicator with a p_K value even lower than that of brilliant cresyl blue might sometimes be useful: but in practice such excellent results were obtained with the latter that there seemed little or no practical advantage in replacing it.

Theoretical considerations. At the p_H value approaching 0, taken as end-point in this method, all the amino-acids, as well as polypeptides and proteins, have their basic groups ionised as acid salts, whilst the ionisation of the acid (carboxyl or hydroxyl *etc.*) groups is repressed. Hence the end-point is adequate for estimation of basic groups without any danger of interference from the acid groups. Or, looking at the question from another angle, we may say that in glacial acetic acid the ampholyte is quantitatively converted from zwitterion into its basic form.

In general, it may be claimed that with the indicator chosen the method is suitable for the estimation of the weakest bases ($K_B \geq 10^{-12}$) even in the presence of relatively "strong" organic acids ($K_A \leq 10^{-2}$). (The figures given in parenthesis are applicable when the amounts of acid present are not grossly in excess: if very large excess of relatively strong acid is present, or the base to be estimated

is very weak, it is always possible to increase the accuracy of the titration by a still further decrease in the p_H value of the end-point.)

Inorganic salts and mixtures. The presence of neutral inorganic salts (*e.g.* NaCl) does not appear to affect appreciably the accuracy of the method (see, *e.g.* Nos. 50 and 51, Table I). Mixtures of amino-acids and bases are estimated as correctly as are single substances (see, *e.g.* Nos. 47 and 49, Table I).

Application to proteins. An effort was made to apply the principle of the method to the estimation of basic groups in proteins. Most of the experiments were carried out with powdered gelatin. The principal difficulties to be overcome were (1) to bring the protein into solution or, at any rate, to get it to react reasonably rapidly with the acid reagent; and (2) to get the protein into a "dry" state. Following a suggestion by Dr David Greenberg, formic acid was also tried as a solvent for proteins; but its use did not entirely obviate the objections referred to. As the technique for proteins needs modifying considerably from that described here, details may be well postponed for a later paper.

Sensitiveness of method.

To determine the sharpness of the end-point the following experiments (Table II) were carried out with 0.1 *N* glycine, and with *N* aniline, each titrated against *N* perchloric acid.

Table II. *Determination of sensitivity of method.*

Exp. no.	Solution taken for estimation	Amount of brilliant cresyl blue indicator added	Amounts of titrants added	Result
1	5.0 ml. of 0.1 <i>N</i> glycine	0.05 ml. of 0.1% solution	Added 0.51 ml. of <i>N</i> perchloric acid. Then back-titrated with 0.1 <i>N</i> glycine	Colour change was sharp to <0.01 ml. of 0.1 <i>N</i> glycine in back-titration
2	1.0 ml. of <i>N</i> perchloric acid	0.2 ml. of 0.1% solution	Titrated with 10 ml. of 0.1 <i>N</i> glycine. As end-point was approached, colour was observed after each addition of 0.01 ml.: 0.01 0.02 0.03 0.04 0.05 0.06	— Olive Olive-green Green Greenish blue Peacock-blue Deep blue
	200 ml. of <i>N</i> aniline in glacial acetic acid	4 ml. of 0.1% solution	Titrated with 200 ml. of <i>N</i> perchloric acid. Then added by 0.02 ml. increments	Good colour change with addition of 0.02 ml.

It will be seen from Exp. 2 (Table II) that when 10 ml. of 0.1 *N* glycine are being titrated against *N* acid there is a well-pronounced colour change with the addition of each 0.01 to 0.02 ml. of the glycine; or, in other words, the method is sensitive to one part in 1000 of the unknown. Now the sensitivity should increase in proportion to the strength of the solutions employed, and this was verified to be so in Exp. 3, in which *N* aniline was titrated against *N* perchloric acid, and it was found that the sensitiveness had increased to about 1 part in 10,000.

Determinations of “blank corrections”.

For most of the determinations recorded an identical amount of indicator solution was taken; the magnitude of the small “blank correction” then depended virtually on the final dilution of the solution. (The indicator solution was so weak that the blank for the indicator itself was almost negligible.) The results in Table III are typical of a number of experimental determinations of the blank correction.

Table III. *Blank corrections—semi-micro-scale.*

Solution taken	Final total volume ml.	Blank correction in ml. of 0.1 <i>N</i> perchloric acid solution
1 ml. of 0.01 % brilliant cresyl blue in glacial acetic acid	1	0.005
1 ml. + 1 ml. of glacial acetic acid solvent	2	0.01
1 ml. + 3 ml. of glacial acetic acid solvent	4	0.02

It will be seen that under the conditions of the “semi-micro-test” the blank correction is about 1 % of the titre.

Uses of method.

As already indicated the method is a convenient one for estimating basic groups in isolated preparations of ampholytes or bases, and so, incidentally, for determining their combining weight. It has already been of use in this way in the characterisation of a number of substances whose structure was uncertain—*e.g.* glutathione [Harris, 1929, 2], vitamin B₁ (unpublished results). A further use is in determining the degree of purity of materials. For example, supposedly pure specimens of the following substances were found on test to give unexpectedly irregular titration results by this method: when their purity was tested by other means it was found that the titration results were correct and that the specimens were in fact impure (Table IV).

Table IV.

No.	Specimen	% deficiency in basic strength	
		As found in titration	As checked by alternative method
1	Glycylcysteine	– 17	– 18 (formaldehyde titration)
2	Glycylcysteine	– 19	– 18 (formaldehyde titration)
3	Glycyltryptophan	– 12.5	– 10 (N by combustion; formaldehyde titration)
4	Glycyltryptophan	– 10.5	– 10 (N by combustion; formaldehyde titration)
5	Arginine	– 7	– 8 (titration curve)

The method seems less suitable for application to crude mixtures, such for example as the unfractionated products derived from hydrolysis of proteins. In the first place the effect of various unspecific buffering substances (*e.g.* phosphate, carbonate) would have to be allowed for; secondly, added mineral acid or alkali, as well as moisture, would also have to be taken into consideration. The method has, in fact, given somewhat irregular results on several salts of amino-acids which have been tested.

Micro-adaptation of method.

It will be evident from the results already cited that the small departures from the theoretical values, as met with in the rather rough, or “routine” semi-micro-method described above, were not so much due to any inherent error

in the method as to such commonplace causes as slight inaccuracies in the burettes or measuring vessels, in the strengths of the solutions, or to minor irregularities in manipulation and other such subjective variations. Theoretically the method is capable of being applied on a highly micro-scale, the only limit to set upon it, practically, being the dimension of the smallest volume of titrant which can be measured by burette and of the smallest volume of solution in which a colour change can be observed. As in all micro-chemical methods, the ideal procedure is to work with the smallest possible volumes of solution rather than to use unduly weak solutions. That is to say, the brunt must be thrown on the fineness of the measuring apparatus employed rather than on dilution of the reagents. The objection to the latter alternative is that each increase in dilution increases the magnitude of the "blank" and so diminishes the sharpness of the end-point.

The micro-estimations referred to below (Table V) were taken to the nearest 0.001 ml. They were carried out by a laboratory assistant who had no previous experience of the method and who received no supervision of any kind in

Table V. *Micro-application of method.*Estimations on 0.025 *N* glycine solution, titrating to nearest 0.001 ml.

ml. of 0.025 <i>N</i> glycine taken (containing 0.0015 % of brilliant cresyl blue)	Vol. of 0.025 <i>N</i> perchloric acid solution required			Strength of glycine solution		
	Uncorr.	Blank correction for given final volume	Corr.	Found	Mean value	% deviation from mean
0.40	0.430					
	0.420					
	0.415					
	0.415					
	0.425					
	0.415					
	0.420					
	0.420					
0.20	0.420	0.013	0.407	0.0254	0.0257	-1.2
	0.218					
	0.221					
	0.206					
	0.217					
0.10	0.216	0.007	0.209	0.0261	0.0257	+1.6
	0.108					
	0.111					
	0.100					
	0.105					
0.05	0.106	0.003	0.103	0.0258	0.0257	+0.4
	0.045					
	0.055					
	0.055					
	0.053					
	0.052	0.001	0.051	0.0255	0.0257	-0.8

carrying them out. They are not intended to show the limits of the micro-method; but they do demonstrate that, even for a person with no special training, there is no difficulty in estimating quantities as small as 0.05 ml. of 0.025 *N* solution, with an error not exceeding 1-2 %. The figures given are typical of many others that were obtained at the same time.

SUMMARY.

1. It is known that the basic groups in amino-acids, or polypeptides *etc.*, and in weak natural bases, cannot be estimated by a simple titration with HCl in water to a sharp end-point with a p_H colour indicator for the reason that the end-point is at a very acid reaction (about p_H 0): a considerable "blank" is needed by the solvent (the water itself) to bring it to so acid a reaction, and there is no sudden fall in p_H to give a sharp colour change with the indicator.

2. Theoretically this difficulty is overcome if the titration is carried out in the entire absence of water. In the method described, glacial acetic acid is used as the solvent, perchloric acid in glacial acetic acid as the titrant, brilliant cresyl blue as the indicator (colour change at about p_H 0 in water); whilst glycine in glacial acetic acid serves as standard base solution for back-titration. The glacial acetic acid solvent itself needs no appreciable amount of titrant (*i.e.* blank correction) to bring it to the desired end-point.

3. The estimation takes only a few moments to carry out. The dry specimen is dissolved in slight excess of the perchloric acid solution, the indicator solution is added, and standard glycine run in from a burette to give a sharp end-point. With the use of ordinary apparatus and without special precautions the error is no greater than 1 to 2% (*e.g.* in estimations on 2 ml. of 0.1 *N* solution). On a micro-scale 0.05 ml. of 0.025 *N* solution may be estimated with no greater percentage error. The sensitiveness of the end-point is such that a definite colour change occurs with the addition of 1 part in 1000 of unknown at 0.1 *N* dilution, or with 1 in 10,000 at *N*, in titrations against *N* standard acid.

4. The method has been successfully applied to all the better known amino-acids, and to a number of polypeptides, natural bases *etc.* and to mixtures of these.

5. The principal uses of the method are (1) in determining the degree of purity of isolated specimens, (2) in characterising new or unknown substances and (3) in determining their equivalent (or molecular) weights. The material to be examined should be free from mineral acid or alkali.

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