ESTIMATION OF UREA.

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The quantitative estimation of urea is frequently necessary in biochemical and physiological work in view of the fact that urea plays an important rôle in the nitrogen metabolism of all forms of animal and vegetable life. Among the various methods of estimation that have been proposed, the urease method first suggested by Marshall (1913) is generally employed for the purpose in analytical work. Marshall employed a direct titrimetric method for the estimation of ammonia liberated as a result of the enzymic action; but in view of the low results—from 2 to 20 per cent. in urines (see Van Slyke and Cullen, 1914)—this was given up in favour of the more accurate and reliable aeration-titration method (Marshall, 1913) according to which the digest is made alkaline and the ammonia aspirated into a known volume of standard acid, the excess of the latter being determined by titration against standard alkali. Van Slyke and Cullen (1914) have greatly improved on the original Marshall's method by using (1) highly active enzyme preparations and (2) buffers to control the reaction of the medium during enzyme hydrolysis.

Fiske (1915) observed loss of ammonia in the course of his studies on the estimation of urea, due to incomplete absorption by the standard acid and stressed on the need for extreme care in the initial stages of aeration. Van Slyke and Cullen (1916) in a later publication specified the conditions for complete absorption and recommended slow aeration at the initial stages; during the later stages the aeration should be carefully regulated so as to avoid the risk of spattering of acid; capryl alcohol should be added to prevent foaming in the aerating vessel. They also emphasised the desirability of employing separate sets of tubes for acids and alkalies and applying corrections for ammonia in reagents, particularly potassium carbonate.

The successful application of the above method, therefore, involves a proper control of a variety of factors as well as experience and skill in the manipulation of the apparatus. Besides, the aeration procedure requires time

and for routine analysis as also for work involving periodic examination of the products of hydrolysis of urea, a method combining the simplicity of Marshall's direct titrimetric method and the accuracy of the aeration-titration method is desired.

The applicability of the Linderström-Lang's titrimetric method for amino acids (1927) to the estimation of the products of hydrolysis of urea has been carefully examined and very satisfactory results have been obtained.

From theoretical considerations, Linderström-Lang deduced that "all strong or medium-strong bases of type B (NH_3), $K < 10^{-6}$ corresponding to $K_b = K_w/K > 10^{-14}/10^{-6} = 10^{-8}$ are titrated completely. Guanidine (the one NH_2 group) and ammonia are classed in this group.....". The possibility of direct quantitative titration is thus indicated.

Experimental.

Preliminary trials showed that ammonia in aqueous solution of ammonium carbonate can be quantitatively estimated by titration against standard alcoholic hydrochloric acid, in acetone solutions using naphthyl red as indicator. The procedure adopted was similar to that adopted by Linderström-Lang for the titration of amino acids. In one experiment, direct titration in acetone solution of 10 c.c. aliquot of an ammonium carbonate solution (approximately 1 per cent.) gave a value 21·5 mg. of ammoniacal nitrogen (mean of 2 readings), while a similar aliquot by the Van Slyke's aeration-titration method gave a value of 21·4 mg.

Influence of Buffer Salts.—The common buffer salts do not affect the titrations, as has already been pointed out by Linderström-Lang. As the enzymic hydrolysis of urea has to be carried out under high buffer concentrations, to obviate any change in the reaction, the non-interference of buffer salts with the titration is a matter of considerable significance in the successful application of the method. We have made use of Palitzsch's borax-borate buffers in our experiments, the urea solution being prepared wherever permissible in the buffer mixture.

Urea-Urease Systems.—The acetone titration method was employed for the estimation of ammonia in reaction mixtures of urea-urease. It was observed that while the results obtained for the completely digested urea were strictly comparable with those obtained by the aeration method, those at intermediate stages showed slight discrepancies possibly due to the presence of urea. To ascertain the magnitude of this, varying quantities of urea were added to hydrolysed urea and the mixtures titrated against standard 0·1 N HCl. Table I gives the values obtained.

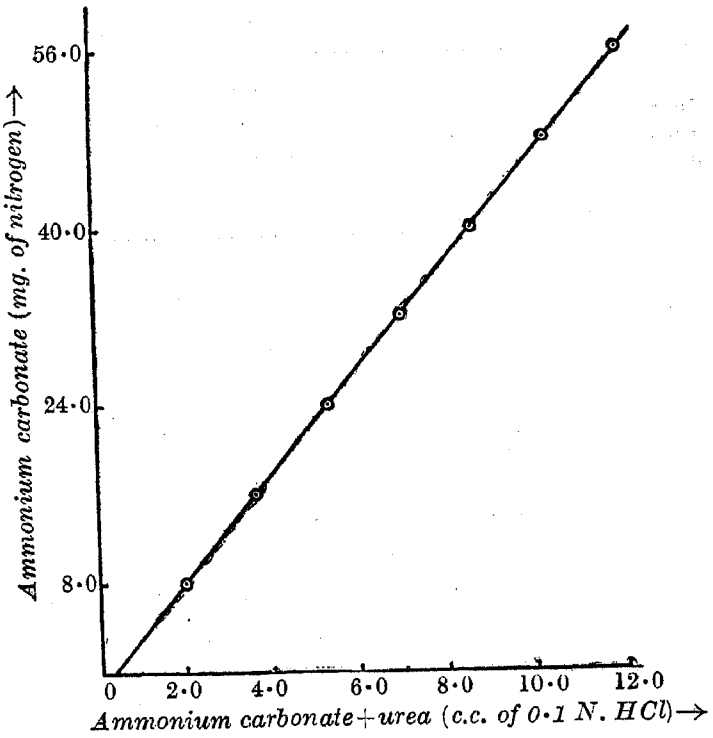
TABLE I.

Urea solution (approximately 1·5 per cent.) added in c.c.	Titration value in c.c. of 0·1 N HCl (mean of duplicates)		
	Exptl.	Control	Difference
Urea digest	14·85	1·35	13·50
5	15·30	1·85	13·45
10	15·80	2·30	13·50
15	16·30	2·85	13·45

The values of controls are obtained by titrating 10 c.c. of water, 10 drops of the indicator solution, 150 c.c. of acetone and the different quantities of urea solution. It is clear that whereas the addition of urea does not affect the corrected values for the urea digests, the values tabulated under "control" vary with the urea added.

Linderström-Lang (1927) carried out titration of urea in acetone solutions and concluded that the urea was indefinitely titrated. A large number of titrations carried out by us under varying buffer and acetone concentrations clearly shows that although urea gives a titre value corresponding to about 2 per cent. of its nitrogen content under given conditions, the titre value is strictly proportional to the urea present. Thus, in the course of the hydrolysis of urea by urease, at the commencement of the reaction, the titre value is due to urea present, and when all the urea has been decomposed the value due to urea is zero and the entire titre value is due to the ammonium carbonate present. In the intermediate stages, the value is due partly to urea and partly to ammonium carbonate, the concentrations of urea and ammonium carbonate being interdependent. For every value of urea, there is a definite calculable value of ammonium carbonate and it is possible to obtain a graph with actual titre value (urea+ammonium carbonate) as abscissa and the nitrogen value corresponding to the ammonium carbonate alone as ordinates. By referring to a graph so constructed, the ammonium carbonate value (in mg. of N) corresponding to a given titre value can be ascertained.

Fig. 1 is a graph constructed according to this method. The initial concentration of urea is 0·10 g. When a one per cent. solution of urea in buffer is hydrolysed by urease, and 10 c.c. aliquots, at noted intervals, are withdrawn and the nitrogen determined by (1) the acetone titration and



(2) aeration-titration method, it is found that the values obtained by (1) are given by the abscissa and those by (2) by the ordinates.

Table II gives a few corrected values at intermediate stages of urea hydrolysis, and the values obtained by the aeration method also being given

TABLE II.

Number	Ammonia Nitrogen in mgs.	
	Acetone-titration	Aeration-titration
1	6·8	6·7
2	6·8	6·9
3	3·6	3·4
4	4·0	3·9
5	2·4	2·4
6	2·4	2·3

for comparison. The reaction mixture consisted of 40 c.c. of 1 per cent. urea solution, 30 c.c. of Palitzsch's borax-boric acid buffer, pH 7·10 and 5 c.c.

of aqueous extracts of different varieties of soya beans (1 gm. of defated powder extracted with 20 c.c. of toluenated water) marked 1, 2, etc., in the table. 10 c.c. of the reaction mixture were pipetted out into a 250 c.c. Erlenmeyer flask after 30 mins. interval, 10 drops of indicator added and titrated to an orange-red colour against standard alcoholic HCl, then 150 c.c. of acetone added in 25 c.c. instalments, the titrations being carried out to an orange-red colour after each addition of acetone. The values by aeration method were obtained by pipetting out 10 c.c. of the reaction mixture after 30 mins. interval and after stopping the action by adding solid potassium carbonate (5 gms.) the ammonia was aspirated into standard acid, the excess of acid being determined by titration against 0·1 N alkali.

These results clearly show that the method can be employed not only for the estimation of urea by the urease method, but also for following the kinetics of urease action.

Estimation of Urea in Urine.—The method has proved very useful for the estimation of urea in biological fluids. The method employed for the hydrolysis of urea is essentially the same as that used by Van Slyke and Cullen except that after hydrolysis acetone is added to the digest which is then titrated against standard 0·1 N HCl. Corrections are applied by running appropriate controls.

Table III gives typical results for urea in 3 samples of urine. The urea values obtained by the Van Slyke method are also given for comparison.

TABLE III.

Urine sample	Urea in mgs. in 1 c.c. of urea	
	Acetone method	Aeration method
1	7·48	7·44
2	10·93	..
3	7·46	7·50

Applications.—The method finds application in all reactions involving the estimation of urea as in the study of arginine-arginase systems. Its utility for the estimation of ammonia in the presence of amides has yet to be investigated.

Summary.

(1) A simple titrimetric method for the estimation of ammonium carbonate in the presence of urea has been worked out and employed for the study of the urea-urease systems.

The method consists in titrating the solution against standard acid after the addition of acetone and is an extension of Linderström-Lang's method for the estimation of amino acids.

(2) The method can be employed for the estimation of urea in urine and possibly in other biological fluids.

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