XCII. AN IODOMETRIC METHOD FOR THE ESTIMATION OF NITROGEN IN OSAZONES.

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It is well known that those compounds containing nitrogen atoms directly linked together do not respond quantitatively to the Kjeldahl process, and for the estimation of nitrogen in such cases resort must be had to Dumas' method. Now although this method leaves nothing to be desired on the score of accuracy, it possesses certain disadvantages. Thus, apart from the question of time and attention required, it not infrequently happens that the quantity of material at disposal does not amount to more than a few decigrams. Among those compounds to which these remarks apply may be mentioned phenylhydrazine and its derivatives, and the method to be described was devised more especially for estimating nitrogen in osazones.

Grimaldi [1903] has suggested a method for the estimation of hydroxylamine in oximes, and phenylhydrazine in hydrazones and osazones by heating these compounds in a flask having a long narrow neck with a known volume of standard hydrochloric acid in excess in a boiling water-bath for one hour. The aldehyde or the ketone is regenerated and the liberated hydroxylamine or phenylhydrazine forms the hydrochloride. The excess of the uncombined acid is then titrated with $N/100$ alkali. From the amount of acid neutralised the hydroxylamine or phenylhydrazine may be calculated. This method appeared to be a convenient one for the estimation of nitrogen in osazones of sugars. The original communication not being available for consultation I am without details of the author's procedure. Some preliminary experiments were therefore carried out to determine the concentration of acid necessary for decomposing the osazones. It was found that $N/10$ – $N/100$ hydrochloric acid did not act on glucosazone under these conditions, but N hydrochloric acid brought about the expected decomposition.

The method entailed the titration of N acid with $N/100$ alkali. It was therefore useless for delicate work and had to be abandoned.

The reaction still seemed interesting and it was investigated in another way. Instead of titrating the excess of the acid after decomposing the osazone under the above conditions it appeared possible to estimate the phenylhydrazine formed during the reaction by the iodometric method described in the

previous paper by Ling and Nanji [1921]. This method of estimating phenylhydrazine depends on the fact that when a solution of phenylhydrazine is added to an excess of a standard solution of iodine it is decomposed according to the equation:

The reaction is an extremely sensitive one and has proved very useful in studying the decomposition of osazones with hydrochloric acid. The method employed was as follows.

A known weight of the osazone was heated with a known volume of *N* hydrochloric acid in a boiling water-bath for one hour. The reaction mixture was then neutralised with an equivalent amount of standard alkali, acidified with a drop of dilute acetic acid, and then made alkaline with an excess of pure sodium bicarbonate solution. The mixture was then washed into an excess of a known volume of standard iodine solution and the excess of iodine titrated with sodium thiosulphate, the difference being equivalent to the phenylhydrazine formed in the reaction mixture.

It was found by studying the above reaction between osazones and hydrochloric acid by this means that the decomposition did not proceed quantitatively, but it proceeded to an extent which was determined by the relative amounts of nitrogen present in the sample and the volume of the hydrochloric acid used. When the amount of hydrochloric acid was kept constant and the amount of nitrogen increased the percentage of the osazone decomposed decreased steadily. As a result of a large number of titrations it was observed that so long as the ratio of the nitrogen to the hydrochloric acid used was fixed, the percentage of the osazone decomposed was constant. Accordingly a curve was obtained by keeping the amount of hydrochloric acid constant and varying the amount of nitrogen present. When this curve is applied to different sugar osazones very concordant results are obtained. This has been made the basis of an empirical method for the estimation of nitrogen in sugar osazones, and it gives trustworthy and accurate results as will be seen by the following experiments, so long as the estimations are carried out under definite conditions.

The method is as follows:

A convenient weight of the osazone which may vary from 5–60 mg. is accurately weighed out in a Freudrich flask. To it is then added 10 cc. of *N* hydrochloric acid, and the mixture heated in a boiling water-bath for one hour. The reaction mixture is washed into a beaker and to it is added 10 cc. of *N* sodium hydroxide. It is then acidified with a drop of dilute acetic acid and the solution made alkaline with an excess of pure sodium bicarbonate solution. The alkaline solution is then washed into an Erlenmeyer flask containing an excess of a known volume of standard *N*/50 iodine solution. The excess of the latter is then determined by titrating with a standard solution of sodium thiosulphate. The number of cc. of *N*/50 iodine used on being

referred to the following table corresponds to the amount of nitrogen present in the sample taken, from which the percentage could be calculated.

cc. of N/50 iodine used	mg. of nitrogen	cc. of N/50 iodine used	mg. of nitrogen	cc. of N/50 iodine used	mg. of nitrogen
4.7	0.782	14.0	3.371	22.0	6.757
5.0	0.839	15.0	3.628	23.0	6.980
6.0	1.029	16.0	3.885	24.0	7.203
7.0	1.219	17.0	4.142	25.0	7.426
8.0	1.409	17.3	4.220	25.7	7.582
9.0	1.599	18.0	4.428	26.0	7.679
9.3	1.656	19.0	4.723	27.0	8.002
10.0	1.958	19.1	4.752	28.0	8.325
11.0	2.389	20.0	5.972	28.6	8.518
12.0	2.820	20.3	6.378	29.0	8.754
12.2	2.906	21.0	6.534	29.5	9.049
13.0	3.114	—	—	—	—

The following are some of the results obtained with different osazones. They represent the mean values of a number of estimations with quantities of the osazone employed varying from 5–60 mg.

Glucosazone	N	Found	15.52 %	Calculated	15.64 %
Maltosazone	N	„	10.56	„	10.76
Isomaltosazone	N	„	10.65	„	10.76
α -Glucosido-isomaltosazone	N	„	8.14	„	8.21

REFERENCES.

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