

A NOTE ON THE DETERMINATION OF VERY SMALL QUANTITIES OF HYDROXYLAMINE BY BLOM'S METHOD

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In a study on the possible occurrence of hydroxylamine as an intermediate compound in the oxidation of ammonium salts to nitrites in sea water (see Cooper, 1937), the method of Blom was tried in order to estimate the hydroxylamine. The method consists in the oxidation of the hydroxylamine by a solution of iodine in acetic acid, removal of the excess of iodine with $N/10$ thiosulphate, and estimation of the nitrite so produced by the Griess method; the method has been used by several workers for the estimation of small amounts of hydroxylamine in biological material.

The method had not been used for such small amounts of hydroxylamine as would be expected in sea water, and it was therefore tested at high dilutions. It was found that nitrite itself, however, could not be recovered after treatment with the above reagents, and it was noticed that in many cases a precipitate (probably sulphur) was produced, and a distinct smell of hydrogen sulphide was often apparent. It was thought probable therefore that the loss was due to the interaction of the nitrite or the red dye with the thiosulphate used for removing the excess of iodine. This view was confirmed by noting the removal of the red colour, the appearance of a precipitate, and the smell of hydrogen sulphide when a solution of thiosulphate was added to a solution of the dye.

Experiments were therefore set up to determine the amount of thiosulphate that could be present in excess without interfering with the estimation. Varying amounts of thiosulphate were added to a known concentration of nitrite (30 mg. per $m.^3$), and the colour produced on adding the Griess reagents was then compared with that obtained without the addition of thiosulphate. The solutions were placed in 100 ml. cylinders and matched in the usual manner. The results are shown in Table I.

It is clear from the Table that when the concentration of the thiosulphate is greater than about 1 mg. per litre the nitrite estimation is inaccurate, and that when the concentration is about 6 mg. per litre only 50 % of the nitrite is found. Taking this latter figure for illustration, although it represents a very considerable excess when compared with nitrite present, it is very small in terms of $N/10$ thiosulphate used in the removal of iodine in the normal process. According to these results, if in estimating the strength of solution of hydroxylamine, equivalent to a nitrite production of 30 mg. per $m.^3$,

$\frac{3}{500}$ of a ml. of thiosulphate more than that required to remove the excess iodine were added, only half the nitrite actually produced would be found. It is clearly very difficult to add the thiosulphate within these limits.

Table I

Conc. of thiosulphate (mg. per litre)	Reading of control	Conc. of thiosulphate (mg. per litre)	Reading of control
0.025	100	1.5	82
0.05	100	2.0	71
0.15	100	3.0	66
0.2	100	4.0	62
0.25	100	5.0	56
0.3	100	6.0	53
0.35	100	7.0	50
0.4	95	8.0	51
0.45	99	9.0	42
0.5	100	11.0	41
0.75	100	14.0	32
1.00	100		

Conc. of nitrite 30 mg. per m.³

I should like to thank Dr Cooper for suggesting this work and for his continued interest during its progress.

REFERENCES

- BLOM, J., 1928. Zum Nachweis von Hydroxylamin. *Bioch. Zeit.*, Vol. CXCIV, p. 385.
 COOPER, L. N. C., 1937. The nitrogen cycle in the sea. *Journ. Mar. Biol. Assoc.*, Vol. XXII, pp. 183-204.