A Modification of Teorell's Method for Determining of Small Quantities of Ammonia

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In Teorell's method[1] ammonia first reacts with sodium hypobromite. The excess of the reagent is then used to decolorize the dyestuff, naphtyl-red, by means of which the analysis is made. Krogh[2] applied the method to the analysis of ammonia in water and in the air but the author has encountered difficulties when using it.

In the first instance the oxidation products seem to be adsorbed on the walls of the reaction vessel during the reaction between the hypobromite and naphtylred and are not easily removed by washing with distilled water. Two successive titrations are sufficient to cause serious errors in the later analysis. This source of error can be avoided by rinsing the vessels first with glacial acetic acid, completing the washing with running hot water followed by ammonia free water.

In the second instance if an unexpectedly large quantity of ammonia is present the titration curve may become inflected. Repeated titrations become than necessary to ensure that the correct portion of the curve is used. Teorell has described this peculiarity but has not explained it. Observations made at the Marine Biological Laboratory, Plymouth, have suggested the following explanation.

Normally, with excess of hypobromite in alkaline solution, the overall reaction could be represented as follows:

$$2NH_3 + 3NaBrO = N_2 + 3\,NaBr + 3H_2O \quad . \quad . \quad . \quad . \quad (1)$$

but such a pentamolecular reaction is highly improbable. It should be rather considered as a sequence of three bimolecular reactions. The first step is probably the oxidation of ammonia to hydroxylamine.

$$2NH_3 + 2NaBrO \rightarrow 2NH_2OH + 2NaBr \quad . \quad . \quad . \quad . \quad . \quad (2)$$

Half of the hydroxylamine then reacts with a third molecule of hypobromite to give hyponitrite:

$$NH_2OH + NaBrO \rightarrow HNO + NaBr + H_2O \quad . \quad . \quad . \quad . \quad (3)$$

Finally the hyponitrite reacts with the rest of the hydroxylamine to give molecular nitrogen

$$NH_2OH + HNO \rightarrow N_2 + 2H_2O \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Oxidation of hydroxylamine to nitrite, which requires an additional oxidising agent, is less likely. Qualitatively I could detect no nitrite. It

[1] T. Teorell, Biochem. Z., **248** (1932) 246.
[2] A. Krogh, Biological Bulletin, **67** (1934) 126.

would seem that the reaction (3) must be fast as compared with the reaction (2).

If, on the contrary, there is insufficient hypobromite present to make the molar ratio hypobromite /ammonia = 3/2, than the oxidation proceeds only according the (2) and (3) equations, while forming HNO. In such a way the hyponitrous acid radical formed is accumulated in the solution after the supply of hydroxilamine is stopped.

This hyponitrous acid radical has oxidising properties and decolorises naphtyl-red. It seems that the decoloration takes place only with the HNO and not with dimer $(HNO)_2$ because the decoloration is taking place instantaneously immediately after the formation of HNO, but later becomes very slow. The polymerized form $(HNO)_2$ has no oxidizing properties.

The presence of the hyponitrous acid radical is therefore the reason why in a surplus of NH_3 during the titration more and more naphtyl-red solution is used. If the surplus of NH_3 is very large, as much naphtyl-red is used as would be used in a titration vessel containing only hypobromite without ammonia. The reason is that the »active oxygen« is now present not in the form of hypobromite but of the hyponitrous acid radical.

If we give to such a solution with hypobromite and surplus ammonia before the titration a quantity of hydroxylamine and then start with the titration (using naphtyl-red) we shall find that the decolorisation of the dye does not take place at all, because with the hydroxylamine added we have destroyed all hyponitrous acid [see equation(4)].

In order to avoid these diffuculties we have tried to work with a solution of potassium-indigo-disulphonate instead of the naphtyl-red solution.

We have used this dye for the titration of the surplus of hypobromite both in acid and in alcaline solutions, and have found its use convenient. An important property of this dye is that it is not oxidized by HNO in an a l k a l i n e solution.

Required solutions: (1) K-indigo-disulphonate, a B. D. H. product, 0.063 g in 1000 ml distilled water,

(2) sodium hypobromite n/1000,

(3) hydrobromic acid n/230,

(4) ammonium sulphate solution containing 1 mg NH_3-nitrogen in 1000 ml,

(5) ammonia-free water,

(6) hydrobromic acid 3,9%.

The titration was done in small cylindric flasks with a glass stopper (pure white glass) of 25—30 ml volume. The total volume of liquid before the titration is some 6—8 ml. For the titration in acid medium 1 ml of a weak HBr solution normally needed for catching of NH_3 by distillation was put in the vessel first, then 3—5 ml of distilled water and a known quantity of NH_3-nitrogen [solution instead of the distillate (solution 4)], followed by about 1,30 ml of the hypobromite solution, (with an all glass syringe pipette-always exactly the same quantity). After waiting half a minute 0,2 ml of a 3,9% HBr sol. was added and after another half a minute the content of the

vessel was titrated from a good burette reading to 0,05 ml with K-indigo-disulphonate solution. When conducting the titration in an alkaline medium the addition of 0.2 ml of HBr is omitted.

Typical results obtained are given in Table 1.

Table 1

Micrograms NH_3-nitrogen given in dist. water	ml of K-indigo disulphonate sol used in alkaline	in acid
0	1.97	2.92
	2.01	2.87
1	1.60	2.34
	1.66	2.40
2	1.26	1.81
	1.30	1.78
3	0,87	1.20
	0,92	1.25
4	0.44	0.68
	0.48	0.70
6	0.0 — 0.25	1.05
	0.0 — 0.20	1.00
	0.0 — 0.25	2.85
more than 100	0.0 — 0.25	2.90

The titration in acid medium requires more indigodisulphonate than it is the case with the titration in alkaline medium, but the transient point is sharper in the alkaline titration, and also the decoloration of indigo-disulphonate solution takes place quicker.

An important advantage of titration in the alkaline medium is in the fact the curve does not rise, as the hyponitrous acid radical obviously can not oxidise in such circumstances the indigo-disuphonate.

It is obvious from the data that when titrating in acid solution it is necessary to repeat the titrations with a reduced ammonia content.

By this method one can determine the quantities up to 0.0—4.00 micrograms of NH_3-nitrogen in the sample. Oxydation products of indigo-disuphonate do not affect the following titrations as is the case when using naphtyl-red.

Before starting with a new solution it is neceessary to check the interval of micrograms of NH_3-nitrogen they embrace, by titrating known quantities of ammonium e. g. 0, 2, 4 and 6 micrograms of NH_3-nitrogen in a sample. The straight part of the plotted line only should be used (e. g. in our table only the interval from 0.0—4,0 micrograms NH_3-nitrogen).

If it is desirable to have the possibility of a titration in a larger interval of ammonia-nitrogen concentrations in a sample, it is necessary to increase the quantity of hypobromide solution added. During the titrations it is useful to have another vesel with a liquid having the color of the transient point for the sake of comparison.

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IZVOD

Jedna izmjena Teorell-ove metode za određivanje malih količina amonijaka

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Rad sa Teorell-ovom metodom skopčan je sa nekim poteškoćama. Kod uzastopnih titracija otopine hipobromita otopinom naftilnog crvenila nije lako dobiti jednake rezultate. To se tumači štetnim uplivom oksidacijonih proizvoda naftilnog crvenila, pa je data uputa, kako da se ta teškoća ukloni.

U koliko hipobromit nije bio dodan otopini amonijeve soli u suvišku, to dolazi do daljnje poteškoće u toliko, što titracijona krivulja mijenja svoj smjer, pa potrošak naftilnog crvenila više nije razmjeran stvarno prisutnoj količini amonijaka. Pokušano je dati tumačenje takovog ponašanja hipobromita u suvišku amonijevih soli stvaranjem radikala hipodušičnate kiseline (HNO), koji zatim kod kisele reakcije djeluje oksidativno na otopinu naftilnog crvenila. Predlaže se upotreba otopine kalijevog indigo-disulfonata u svrhu titriranja suviška hipobromita kod lužnate reakcije, čime se uklanjaju obje poteškoće, na koje se nailazi kod originalne metode.

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