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14. Makoto Hayashi, Tsutomu Unemoto, and Komei Miyaki: Effect of Volatile Amines on the Colorimetric Determination of Ammonia.

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For the determination of a small amount of ammonia, the Conway microdiffusion method is suitable for use. Employing this procedure, separated ammonia is usually estimated by titration or colorimetry.

Since Van Slyke and Hiller[1] published such a colorimetric method, several modifications have been reported by many authors, i.e. Borsook,[1] Crismer,[1] Russel,[1] and Akamatsu and Katsumata,[2] which are similar to or varied only slightly from each other. These colorimetric methods are based upon the formation of blue indophenol dye from ammonia and phenol as a result of oxidation with alkaline hypochlorite solution.

Accordingly it is easily supposed that coexistence of any volatile amine would affect the formation of the blue dye, and then the colorimetric determination of ammonia would become inaccurate as in the case of titration.

This paper deals with the effect of methylamine and trimethylamine, which are likely to be formed with ammonia in various biological materials, in comparison with other amines listed in Tables I and II, using the Van Slyke-Hiller and Akamatsu-Katsumata methods.

It was found that methylamine produces a blue color which shows the same absorption spectrum and has a peak at 625 mμ, as that produced from ammonia, and in the case of the Van Slyke-Hiller method color development proceeds more rapidly than that of ammonia even at room temperature. Table I shows the percentage color intensity produced from 1

TABLE I. Substances which show the Color Reaction

	Colorimetric Method	
	Van Slyke-Hiller	Akamatsu-Katsumata
Ammonia	100 (100)	100 (100)
Methylamine	71 (68)	22 (18)
Ethylamine	40 (38)	16 (14)
Ethanolamine	16 (2.7)	10 (1.3)
Ethylenediamine	134 (130)	149 (147)
Trimethylenediamine	46 (8.8)	30 (6.7)
Putrescine	29 (8.2)	* (6.1)
Cadaverine	25 (2.7)	* (2.0)
Dimethylamine	0	0
Trimethylamine	0	0

* The solution became turbid on addition of hypochlorite reagent and its optical density was not measured.

μmole of the amine as compared to ammonia, measured at 625 mμ using the Coleman spectrophotometer. Numbers in parentheses are values estimated after treatment of microdiffusion. It is concluded that some primary amines exhibit the same color reaction as ammonia and when the determination of ammonia is performed by this method, the effect of methylamine, ethylamine, and ethylenediamine, if present, cannot be eliminated. The effect of other amines can be ignored in microdiffusion.

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1) E. J. Conway: "Microdiffusion Analysis and Volumetric Error," 2nd ed., Crosby Lockwood, London, 1947.

2) S. Akamatsu: J. Biochem.(Tokyo), **39**, 203(1952).

TABLE II. Substances which inhibit the Color Reaction of Ammonia

	Colorimetric method	
	Van Slyke-Hiller	Akamatsu-Katsumata
Trimethylamine	77	73
Methylguanidine	39	10
Agmatine	21	*

* The solution became turbid on addition of hypochlorite reagent and its optical density was not measured.

In addition, there are other amines which interfere in the color reaction (see Table II). Trimethylamine, methylguanidine, and agmatine inhibit the color development of ammonia. Among these amines, the latter two do not diffuse and are easily separable from ammonia. However, the difficulty is encountered when the determination should be carried out in the presence of trimethylamine, which behaves like ammonia. In practice, the inhibition of trimethylamine is remarkable as indicated in Figs. 1 and 2 which show the relationship between the degree of inhibition and concentration of trimethylamine per 1 μmole of ammonia.

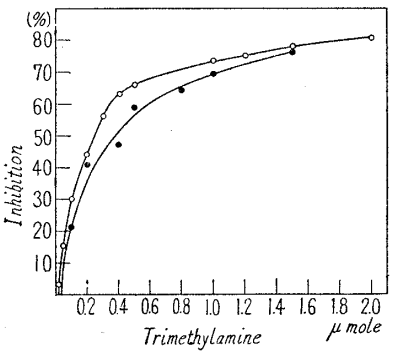

Fig. 1. Relationship between Amount of Trimethylamine and its Inhibition of Color Development

1 μmole of ammonia by the Akamatsu-Katsumata method
○——○ Before diffusion ●——● After diffusion

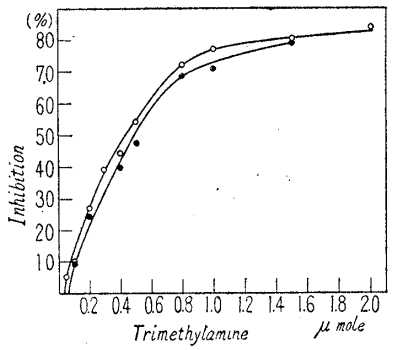

Fig. 2. Relationship between Amount of Trimethylamine and its Inhibition of Color Development

1 μmole of ammonia by the Van Slyke-Hiller meth
○——○ Before diffusion, ●——● After diffusion

The reason why trimethylamine interferes in the formation of indophenol dye from ammonia during its determination is not clear, because it was shown by titration that the consumption of hypochlorite by trimethylamine does dot occur, and the mechanism of inhibition seems to be not due to the reducing effect of trimethylamine.

It is interesting to find that dimethylamine does not cause any inhibition.

Experimental

Reagents—Hypochlorite Solution: Commercially available hypochlorite solution was diluted to a suitable concentration with distilled water. The color intensity produced in the reaction mixture may change with the concentration of hypochlorite to be added as shown in Fig. 3. Therefore, 2% solution of hypochlorite, which is optimal for analytical purpose, was usual in the case of Akamatsu-Katsumata method.

Colorimetry—Van Slyke-Hiller Method: To 5 cc. of the test solution, 1 cc. of phenol solution (25% phenol in 20% NaOH) and 0.5 cc. of hypochlorite reagent (1% as available chlorine) were added, the

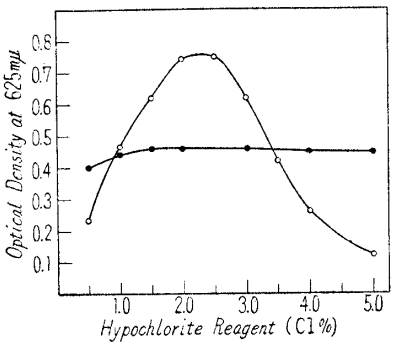

Fig. 3. Effect of the Concentration of Hypochlorite Reagent on the Color Development

1 μmole of ammonia, ○——○ the Akamatsu-Katsumata method, ●——● the Van Slyke-Hiller method

solution was mixed well, and instantly immersed in a boiling water for 3 mins. The solution was cooled to room temperature and its optical density was measured at 625 mμ against the control. The final volume was 6.5 cc.

Akamatsu-Katsumata Method: 2 cc. of 5% phenol solution, 1 cc. of satd. $NaHCO_3$ solution, and 3 cc. of hypochlorite reagent (2% in 3.5% Na_2CO_3) were successively added to 1 cc. of the sample and the whole solution (7 cc.) was mixed thoroughly. After 20 mins. at room temperature, the color intensity was measured at 625 mμ against the control.

Microdiffusion—Exactly 1 cc. of 0.02*N* H_2SO_4 solution was pipetted into the center well and 1 cc. of sample into the outer well of a Conway No. 1 microdiffusion unit. The lid was greased with liquid paraffin and placed lightly in position, leaving an opening at one side. 1 cc. of satd. Na_2CO_3 solution was added rapidly to the outer well, and the unit immediately sealed. Diffusion was allowed to proceed for 2 hrs. at 28~30°. Within this period ammonia diffuses completely and then ammonia was determined.

Summary

Effect of volatile amines such as methylamine, dimethylamine, and trimethylamine on the determination of ammonia with the Conway microdiffusion method using colorimetry by the so-called Van Slyke-Hiller and Akamatsu-Katsumata methods was examined.

Among these amines, methylamine shows the same color reaction as ammonia, trimethylamine inhibits the color development, and dimethylamine does not show any effect.

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