

LVIII. THE DETERMINATION OF SMALL AMOUNTS OF DIMETHYLAMINE IN BIOLOGICAL FLUIDS

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LÖFFLER [1935] has stated that, of the volatile bases in human urine, ammonia and dimethylamine are present in greatest amount and that other methylamines are present only in traces. Attention has now been paid to dimethylamine in cow's urine; a suitable method for its determination in the presence of other nitrogenous bases is described in this paper.

Up to the present, the method of Weber & Wilson [1918] has been most successfully used for determining the distribution of volatile bases. In this method ammonia is absorbed with mercuric oxide, and methylamine is then determined by Van Slyke's amino-nitrogen method. Another portion of the solution is treated with nitrous acid which destroys ammonia and methylamine; dimethylamine is converted into the nitrosamine and trimethylamine is left unchanged. Steam distillation of the alkaline solution into standard acid enables the trimethylamine to be determined, while the nitrosamine which also distils over is reduced by Zn-HCl to a volatile base (stated by Weber & Wilson to be dimethylamine) which can be determined by difference after another distillation. The method is laborious and likely to yield misleading results if the many precautions given by the authors are not followed. The method is unsuitable for the quantities of amines usually found in biological material and is inaccurate in the presence of trimethylamine.

Katcher & Voroshilova [1934] have determined $\text{NH}(\text{CH}_3)_2$ in the presence of NH_3 by conversion into the NH_4 salt of dimethyldithiocarbamic acid (shaking the alkaline solution for 15 min. with carbon disulphide). The dithiocarbamate is then precipitated quantitatively as its insoluble copper salt by titration with 0.5 N copper sulphate. This method is claimed to be accurate for quantities of about 0.25 g. of amine. Although $\text{N}(\text{CH}_3)_3$ forms an addition compound with CS_2 , the colourless compound formed does not react with copper salts; $\text{NH}_2(\text{CH}_3)$ behaves in a similar manner to $\text{NH}(\text{CH}_3)_2$.

By modifying the dithiocarbamate technique, a simple sensitive method unaffected by the presence of $\text{NH}_2(\text{CH}_3)$ has been developed. The method does not distinguish between $\text{NH}(\text{CH}_3)_2$ and other secondary alkylamines; the latter, however, do not usually occur in biological material [Sidgwick, 1937]. The Cu salt of dimethyldithiocarbamic acid is insoluble in water but slightly soluble in organic solvents such as amyl alcohol, benzene and carbon tetrachloride. If a 5% solution of CS_2 in amyl alcohol is shaken with an alkaline aqueous solution containing $\text{NH}(\text{CH}_3)_2$ and a Cu salt, the upper layer rapidly assumes an amber tint. $\text{NH}_2(\text{CH}_3)$ forms a similar colour but of decreasing intensity as the alkalinity of the aqueous phase increases; this effect is more marked with C_6H_6 from

which the Cu salt of methyldithiocarbamic acid can be removed entirely by shaking with 0.1 *N* NaOH solution; the effect on the Cu dimethyldithiocarbamate is negligible under these conditions. There does not appear to be any decomposition of the monomethyl compound, which collects as a scum at the interface of the two liquids.

Before a solution of CS₂ in C₆H₆ will react with an aqueous solution of NH(CH₃)₂, a solvent mutually soluble in both phases must be added; amyl alcohol and ammonia are two such reagents. Since amyl alcohol reduces the effectiveness of separation of the copper salts, ammonia has been chosen for the method about to be described, with ammonium acetate as a source of buffering ammonium ions. A final shaking after the addition of acetic acid removes ammonia from the benzene layer, assists the clarification and increases the stability of the colour.

Description and examination of the method

Solutions required:

- (A) A 5% solution of carbon disulphide in benzene.
- (B) A solution of 20 g. ammonium acetate and 0.2 g. CuSO₄ · 5H₂O in 30 ml. H₂O is mixed in the cold with 10 g. NaOH in 25 ml. H₂O; to this are added 20 ml. NH₄OH (sp. gr. 0.880) solution and the volume is made up to 100 ml.
- (C) A 30% solution of acetic acid in water.
- (D) A standard solution of NH(CH₃)₂ containing 10 mg. dimethylamine N per l.

To 10 ml. of a neutral aqueous solution containing 2–50 γ dimethylamine nitrogen are added 1 ml. solution B and 10 ml. solution A. This mixture (in a large test tube) is warmed for 1 min. at 40°, stoppered and shaken vigorously for 30 sec. 1 ml. solution C is added and the mixture is shaken again for 30 sec., cooled, and centrifuged or allowed to stand until the upper layer is clear. The upper layer is decanted into a colorimeter cup and the depth of colour compared with that prepared in a similar way from a known amount of dimethylamine.

Some results given by this method when applied to solutions of dimethylamine hydrochloride are given in Table I.

Table I. *Determination of dimethylamine alone in solution*

Standard contained 3.0 γ N		Standard contained 12.0 γ N		Standard contained 28.0 γ N	
γ N added	γ N found	γ N added	γ N found	γ N added	γ N found
2.0	1.94	8.0	7.84	20.0	18.5
3.0	3.12	10.0	9.92	24.0	23.9
4.0	4.02	13.0	12.72	29.0	29.1
6.0	6.16	17.0	16.80	34.0	33.2
—	—	—	—	40.0	39.8

It will be seen that the interaction of amine with carbon disulphide and copper has proceeded to completion, and that the depth of colour obeys Beer's law. If the dimethylamine nitrogen exceeds 50 γ this agreement ceases owing to the supersaturation of the benzene with copper salt and its subsequent precipitation.

Before it could be decided whether this method was affected by the presence of mono- and tri-methylamines, it was necessary to free solutions of these bases

from dimethylamine. The 33% aqueous solutions which were used for these experiments were found to contain only a trace (0.05%) of dimethylamine in the case of the primary amine, but over 1.5% in the case of the tertiary amine. Purification of the former was readily accomplished by recrystallization of its hydrochloride from alcohol, but this method could not be applied to trimethylamine. This base was purified by treatment with nitrous acid, alkaline distillation into hydrochloric acid, evaporation of the distillate to dryness and recrystallization from alcohol-ether. All traces of dimethylnitrosamine had to be removed since its aqueous solution appeared to be decomposed by sunlight to dimethylamine. The purified amine solutions were found to be free from formaldehyde and to give no reaction with Schryver's reagent. This is in agreement with the finding of Reay [1936, 1] that the positive reaction of these amines with this reagent previously observed by him [1936, 2] and by Tankard & Bagnall [1926], was due to the use of impure solutions and not to any fault in the Schryver technique.

Table II gives the results for the above method with dimethylamine in the presence of larger concentrations of the other methylamines and of ammonia. Each result is calculated from the mean of 4 colorimeter readings. The NH_3 , $\text{NH}_2(\text{CH}_3)$ and $\text{N}(\text{CH}_3)_3$ were added as solutions containing 100 γ N per ml., and the mixed solutions were adjusted by dilution or concentration to 10 ml. in all cases.

Table II. *Determination of dimethylamine in a mixture of amines*

	Bases added (γ N)				Dimethyl- amine found γ N
	NHMe_2 γ N	NH_3	NH_2Me	NMe_3	
2.0	1000	—	—	—	1.98
	—	1000	—	—	2.08
	—	—	1000	—	2.01
	10	10	10	10	2.01
	100	100	100	100	2.14
10.0	500	500	500	500	2.47
	500	—	—	—	9.95
	—	—	—	500	9.76
	10	10	10	10	9.95
	100	100	100	100	9.95
50.0	500	500	500	500	10.10
	10	10	10	10	50.05
	100	100	100	100	50.10
	500	500	500	500	50.25
	500	500	500	500	50.25

Table II shows that in mixtures containing large amounts of amines other than $\text{NH}(\text{CH}_3)_2$ slightly higher values than theoretical for that base are given. A mixture containing in 10 ml. 2.5 mg. N as $\text{N}(\text{CH}_3)_3$ and 0.1 mg. N as $\text{NH}_2(\text{CH}_3)$ only, gave a coloration equivalent to 0.83 γ N as dimethylamine, although the separate constituents gave no coloration. It seems that the molecular rearrangement $\text{NH}_2\text{Me} + \text{NMe}_3 = 2\text{NHMe}_2$ occurs probably only during the shaking with carbon disulphide and copper solution when the dimethylamine is removed as soon as formed.

Application of the method to urine

To establish as far as possible that no normal urinary constituents interfere with this determination some samples of urine were examined before and after the addition of known amounts of dimethylamine. The method consisted of

distilling 5 ml. with 50 ml. H_2O and 10 ml. N NaOH until 40 ml. of distillate had been collected. This was made up to 50 ml. and 5 ml. were taken for the determination of dimethylamine. At the same time a direct determination, omitting the distillation, was made on 5 ml. of urine diluted 10 times. The results (Table III) showed in the case of cow's urine that little advantage was gained by distillation.

Table III. *Dimethylamine in urine (mg. N/litre)*

Source of sample	Method	Original $NHMe_2$ content	$NHMe_2$ added	Total $NHMe_2$ found	Added $NHMe_2$ found
Cow A	Direct	27.6	5.0	32.2	4.6
	Distilled	28.1	5.0	33.3	5.2
Cow B	Direct	40.4	10.0	50.3	9.9
	Distilled	40.4	10.0	50.0	9.6
Cow C	Direct	18.8	6.0	25.3	6.5
	Distilled	19.4	6.0	25.8	6.4
Human	Direct	5.8	10.0	14.5	8.7
	Distilled	7.6	10.0	17.2	9.6

Reduction of the nitrosamine in the Weber & Wilson method

It was found that the nitrosamine obtained by the action of nitrous acid on dimethylamine was not reduced to the parent amine by $Zn-HCl$, as stated by Weber & Wilson, but mainly to unsymmetrical dimethylhydrazine ($N(CH_3)_2 \cdot NH_2$) which is strongly dissociated, and can be determined by steam distillation from an alkaline solution. Of many methods examined that of Jones & Kenner [1932] was alone found applicable to the reduction of the nitrosamine to the original amine. If 2 ml. of a 20 % solution of Cu_2Cl_2 in HCl and 2 ml. conc. HCl are added to a solution of the nitrosamine in 5 ml. water, the nitrosamine is converted quantitatively into $NH(CH_3)_2$ when the mixture is slowly raised to the boiling point, and the base can then be isolated by distillation from alkaline solution. (The distillation should be done in an all-glass apparatus as dithiocarbamates and derivatives of $NH(CH_3)_2$ are used as rubber "accelerators".) In the presence of $N(CH_3)_3$ more than the theoretical amount of $NH(CH_3)_2$ could be recovered owing to the oxidation of the tertiary amine to the nitrosamine. On standing at room temperature about 1 %, and at 70° 13 %, of the tertiary amine was thus changed. Wegler & Frank [1936] have observed the same effects on bornyl dimethylamine and some aromatic amines and they state that the reaction is slowest at low temperatures and in the presence of strong mineral acids. Taylor [1928; 1929] has shown that the activity of nitrous acid towards primary and secondary alkylamines is decreased in the presence of mineral acids and it is probable that the conditions stipulated in the Weber & Wilson method are the most suitable.

SUMMARY

A colorimetric method for the determination of small amounts of dimethylamine, in the presence of other volatile bases, as copper dimethyldithiocarbamate in solution in organic solvents is described. The interference of methylamine is avoided by making the aqueous phase alkaline.

The oxidation of small amounts of trimethylamine to dimethylnitrosamine by nitrous acid is reported.

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