New Sensitive Method for Quantitative Assay of Ammonia in Air

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THERE IS a paucity of sensitive, specific, and simple analytical methods for the assay of ammonia (and aliphatic amines) for use in a variety of applications, such as air and water pollution, clinical chemistry, and oceanography (*1–3*). Nessler's method (*4*) has been employed for the analysis of ammonia, but the method lacks sensitivity and suffers from a variety of interferences and reagent instability (*5, 6*).

Another approach to the assay of ammonia involves the treatment of the sample with hypohalite to form chloramine, which is allowed to condense with an active methylene group conjugated to an auxochrome to produce a colored product. With phenols, an indophenolate dye is formed (*1*); with bispyrazolone, the colored product is rubazoic acid (*2*). These color-forming reactions for ammonia require multistep procedures and careful control of pH and reaction time. In addition, in the case of (*2*), an extraction step is required.

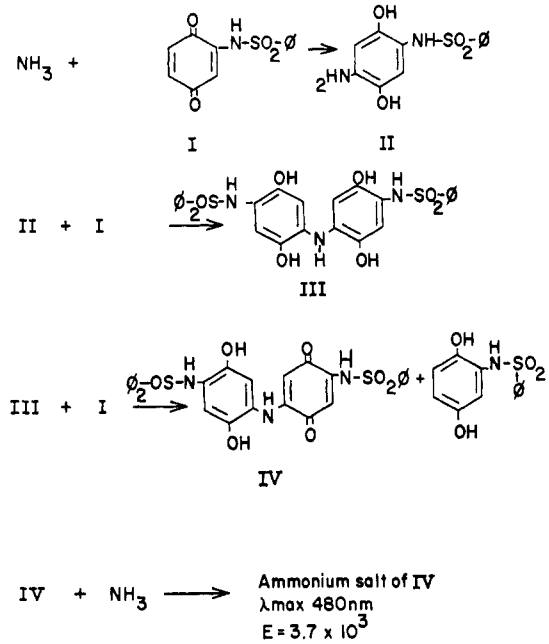
An enzymatic method (*7, 8*) using glutamic acid dehydrogenase is also available for the analysis of ammonia involving its reaction with sodium α-ketoglutarate and the reduced form of nicotinamide adenine dinucleotide (NADH), as shown by the following equation:

$$NH_3 + \alpha\text{-ketoglutarate} + NADH \rightarrow \text{L-glutamate} + NAD^+ \quad (1)$$

The ammonia concentration is determined from the decrease in absorption of light at 340 nm (due to NADH). The method was primarily designed to measure urea in the routine determination of blood and urinary nitrogen. It has the disadvantage that NADH is unstable and expensive.

We wish to report a direct, one-step method for the determination of ammonia (and aliphatic amines). The rate of the reaction is so rapid that instantaneous determinations may be made of the ammonia concentrations being sampled. The method requires collection of ammonia in a suitable neutral solvent (*e.g.*, dioxane) containing a new reagent, *o*-(benzenesulfonamido)-*p*-benzoquinone, I, and measuring the absorption of light at 480 nm. A proposed reaction sequence is given in Scheme I.

Scheme I



EXPERIMENTAL

Apparatus. Spectrophotometric measurements in the ultraviolet and visible regions were made in the Beckman DB Spectrophotometer using 1.0-cm pathlength cells. Infrared spectra were recorded with a Beckman IR-5 Spectrometer. Melting points were obtained using a Fisher Melting Point Apparatus.

Reagents. *o*-(Benzenesulfonamido)-*p*-benzoquinone was synthesized by the chromic acid oxidation of 1-(benzenesulfonamido)-phenol. *o*-(Benzenesulfonamido)-phenol was prepared by reacting equimolar concentrations of benzenesulfonyl chloride (Eastman Kodak Chemical Co., Rochester, N.Y.) and *o*-aminophenol (Pfaltz and Bauer, Inc., Flushing, N.Y.) in the presence of one equivalent of sodium bicarbonate employing the classical Schotten–Bauman procedure. The crude product was recrystallized from water–ethanol (with charcoal) to yield platelets, mp, 131–132 °C in 50% yield (Calcd: C, 57.8; H, 4.45; O, 19.25; N, 5.62; S, 12.86. Found: C, 57.6; H, 4.45; O, 19.25; N, 5.61; S, 12.86). The infrared spectrum (Nujol) revealed OH, 2.89 μ, and $-NHSO_2-$phenyl, 3.16 μ, and $-NSO_2-$, 8.55 μ.

Compound I was obtained by dissolving 5.0 grams of the sulfonamidophenol in 75 ml of glacial acetic acid, and treating this solution with 100 ml of an aqueous solution containing 7.0 grams of $K_2Cr_2O_7$ and 5 ml of concentrated sulfuric acid, added dropwise over 0.5 hour. The resultant dark brown precipitate was collected, washed with water, and air dried. The solid was extracted with 100 ml of benzene, the solvent removed *in vacuo*, yielding I, a bright yellow product which was recrystallized from benzene and petroleum ether (Calcd for $C_{12}H_{10}O_4NS$: C, 54.75; H, 3.42; N, 5.32; O, 24.33; S, 12.16. Found: C, 54.3; H, 3.5; N, 5.30; O, 24.4; S, 12.5). The infrared spectrum (Nujol) revealed $NHSO_2$, 3.16 μ, carbonyl, 5.94, 5.98 μ. The electronic spectrum in dioxane had a λ_{max}, 380 nm, ϵ_{max}, $1.5 \times 10.^3$

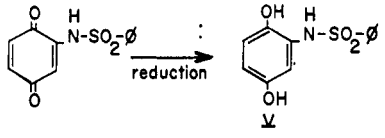
Preparation of the reaction product of ammonia and *o*-(benzenesulfonamido)-*p*-benzoquinone, I: One thousandth mole of I dissolved in 150 ml of benzene was treated with gaseous ammonia at room temperature until the yellow solution was decolorized with the deposition of a brown product. Calcd. for $C_{24}H_{22}O_8N_4S_2$ (ammonium salt of IV): C, 51.8;

(1) J. P. Riley and G. Skinnow, "Chemical Oceanography," V. II, Academic Press, New York, N.Y., 1965, p 368.
(2) L. Prochazkova, ANAL. CHEM., **36**, 865 (1964).
(3) R. T. Emmet, *ibid.*, **41**, 1651 (1969).
(4) J. Nessler, *Chem. Centr.* 27 nene Folge, **1**, 539 (1856).
(5) W. G. James, F. A. Slesinki, and H. B. Pierce, Jr., *Lab. Clin. Med.* **27**, 113 (1941).
(6) L. Rosenthal, *Pharm. Acta. Nelr.*, **29**, 23 (1954).
(7) H. Talka and G. E. Schubert, *Klin. Wochenschr.*, **43**, 174 (1965).
(8) M. Rubin and L. Knott, *Clin. Chem. Acta.*, **18**, 409 (1967).

H, 3.96; O, 23.0; N, 9.71; S, 11.51, Found: C, 51.4; H, 4.4; O, 23.6; N, 9.0; S, 11.6. The infrared spectrum contained a broad absorption in the 2.9–3.05 μ region (–OH); the sulfonamido proton at 3.16 μ; NH^+ stretching (broad) at 3.73 μ; the quinone carbonyls at 5.93 and 5.99 μ. The mass spectral cracking pattern revealed mass peaks at 18 (NH_4^+) and at 265, 264, and 263 which would be expected to occur upon volatilization of the sample. Mass peak 263 corresponds to the parent sulfonamidoquinone. The salt was soluble in water and liberated ammonia in the cold upon addition of alkali. The compound decomposed on melting.

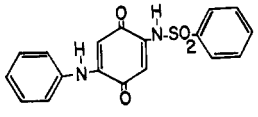
The colorless residue obtained from the benzene filtrate upon recrystallization from water–ethanol was identical with the product obtained upon the reduction of I with HI or bisulfite. Compound V melted at 134 °C and analyzed for $C_{12}H_{11}O_4NS$. (Calcd: C, 54.34; H, 4.15; O, 24.15; N, 5.28; S, 12.1. Found: C, 54.1, H, 4.15; N, 5.12) [Scheme II].

Scheme II



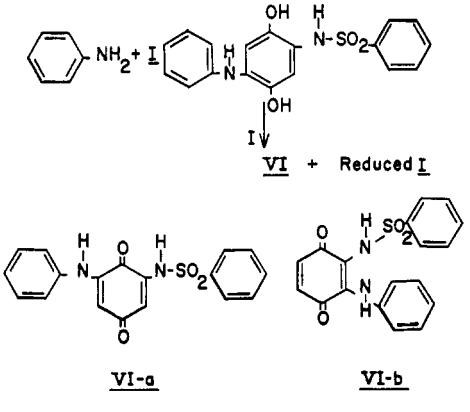
V

Preparation of the aniline adduct of I: One thousandth molar solution of aniline in benzene was reacted at 60 °C with 0.002 molar solution of I in benzene. The solution slowly turned a deep purple and the solvent was removed *in vacuo*. The residue was taken up in 50 ml of aqueous methanol (1:1). A purple solid crystallized on standing. Upon recrystallization from the same solvent, a product was obtained which melted at 165–6 °C. Calcd for $C_{18}H_{14}O_4N_2S$: C, 61.02, H, 3.95; N, 7.91; S, 9.04. Found: C, 61.1; H, 3.9; N, 8.4; S, 9.2. The molecular weight, as determined by mass spectrometry was 354 (parent peak). Also the cracking pattern was in consonance with the following structure:



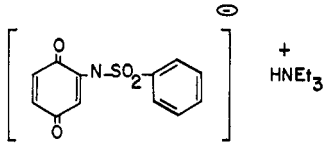
The NMR in $CDCl_3$ revealed two quinoidal hydrogens at 3.98 and 6.49 δ. The IR spectrum showed –NH, 3.36 μ, $-NHSO_2$–phenyl, 3.16 μ, and two carbonyl absorptions at 5.97 and 6.03 μ. The reactions leading to VI are postulated in Scheme III. There is a possibility that VI has the alternative structures VIa or VIb. Structure VIa is ruled out on the basis that the NMR spectrum does not have the expected AB quartet. The choice between VI and VIb is difficult and not entirely unequivocal. However, the difference of 0.51 unit between the two quinoidal hydrogen resonances would lend credence to the postulation that the protons are in the 3- and 6-positions on the quinoidal ring. Whereas, if the hydrogens were 3- and 5-substituents, the chemical shift would not be expected to be of this magnitude since the alpha-hydrogens would be more nearly equivalent in this case as compared to VIa. Moreover, from previous studies on quinoidal compounds (*9*), meta coupling of 2 Hz would be observed if the hydrogens were disposed as in VIa while para-hydrogens display zero coupling. No methyl coupling was observed. Hence, structure IV is preferred.

Scheme III



VI-a VI-b

The reaction product of I and triethylamine: Equimolar solutions of I and triethylamine in benzene solvent were reacted to form a deep red precipitate. After filtration and thorough washing with benzene, the water soluble red product analyzed for the salt VII as shown below:



VII

The water solubility, IR, NMR, and elemental analysis support this structure (Calcd for $C_{18}H_{24}O_4N_2S$: C, 59.34; H, 6.59; O, 17.58; N, 7.69; S, 8.79. Found: C, 59.3; H, 6.8; N, 7.4; O, 17.4; S, 9.1). Salt formation would be expected since the pK_a of the sulfonamide proton was 4.5. The eletronic spectra: λ_{max} 480 nm and $\epsilon_{max} = 1.98 \times 10^3$.

Procedure. Standard source of a low ammonia concentration: An ammonia dilution apparatus was employed as described by (*10*) and the source was standardized by means of Nessler's reagent according to standard procedures (*11*).

Determination of Ammonia. Ten milliliters of a solution of $10^{-3}M$ of I in reagent grade dioxane is introduced in a bubbler (glass) of a 50-ml capacity. Two of these bubblers in tandem are attached to the ammonia source and samples are taken at a sampling rate of 1 liter per minute. At the completion of the sampling time, the contents of each bubbler are transferred to a 10-ml volumetric flask and diluted to the mark to compensate for evaporation. The solution is then read spectrophotometrically at 480 nm against a reagent blank. The solution may be allowed to stand for an hour without significant change. The readings in both bubblers are added to give the final absorbance reading. By using different sampling times, a calibration curve may be set up relating absorbance values and ammonia concentrations.

RESULTS AND DISCUSSION

As expected from Scheme I, two moles of ammonia react with I to yield one mole of dye. This was inferred by isolation and characterization of the dye, as the ammonium salt of IV. The extinction coefficient of the dye formed in the ammonia determination was 3.5×10^3 as compared to the found extinction of IV, ammonium salt; 3.7×10^3 (see Figure 1). This is equivalent to a 95% conversion.

A linear relationship was found for ammonia concentration and molar absorbance in the range of 1.7×10^{-5} to 6.0×10^{-5} m/l. in solution, where the air sample contained ammonia

(9) D. N. Kramer and G. M. Gamson, *J. Biol. Chem.*, **235**$_8$ 1785 (1960).

(10) W. L. Robb, "Thin Silicone Membranes—Their Permeation Properties and Some Applications," General Electric Research Laboratory Report No. 65-C-031, October 1966.

(11) ASTM Designation D-1426-58, "Ammonia in Industrial Water and Industrial Waster Water," "Book of ASTM Standards," Pt. 23, Philadelphia, Pa., 1968, pp 363–373.

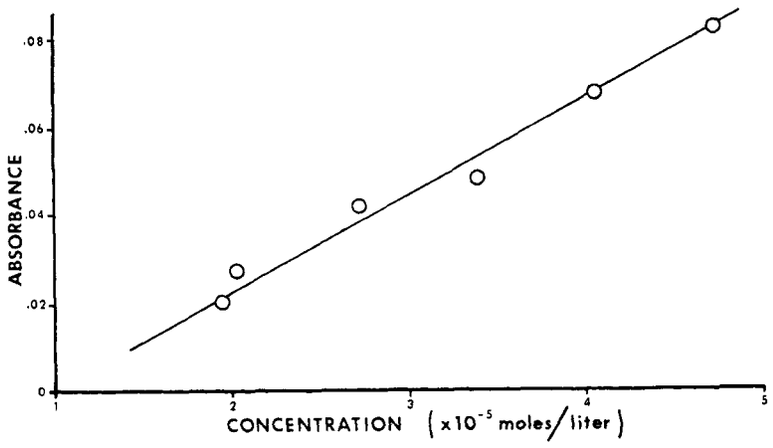


Figure 1. Calibration curve of ammonia

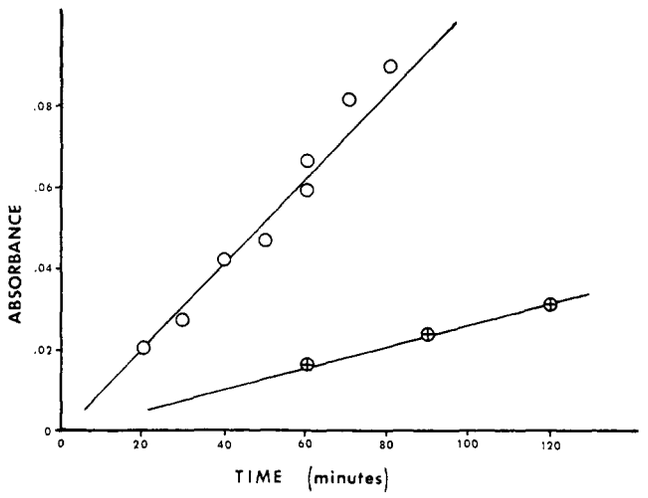


Figure 2. Comparison of new method to Nessler's method (concentration of NH_3 in air stream 10×10^{-8} g/l.; sampling rate: one l./min)

○ New reagent
⊕ Nessler's reagent

Sampling time, min	New method	Nessler's
30	0.031	0.008
40	0.042	0.011
50	0.053	0.013
60	0.063	0.016
70	0.074	0.019
80	0.085	0.021

in a concentration of $0.59 \times 10^{-8}M$ NH_3/liter air. Also, higher concentrations of ammonia in dioxane can be analyzed, *e.g.*, 10^{-4} *M*/l. For more dilute ammonia concentrations in the atmosphere, longer sampling times may be employed.

The present method was found to be 3 to 4 times more sensitive than the Nessler's reagent, as shown in Figure 2.

The method is readily adaptable to the analysis of other primary, secondary, and tertiary amines. Aromatic primary and secondary amines react more slowly with I and require heating to obtain stoichiometric dye production.

The stability of the dye formed in the ammonia reaction is shown in Figure 3, with very small changes occuring over a 2-hour period.

The reaction of ammonia with the *o*-(benzenesulfonamido)-*p*-benzoquinone to form the product IV, has not been previously observed in the reaction of ammonia with quinones.

In general, the addition of ammonia to a substituted quinone (*e.g.*, X = Cl) is as follows, Scheme IV.

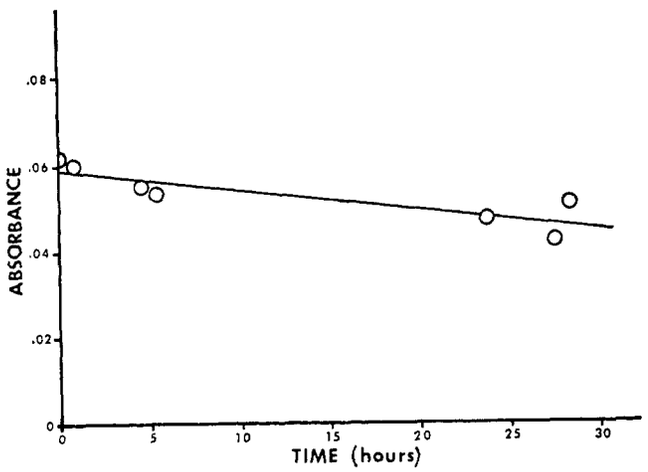
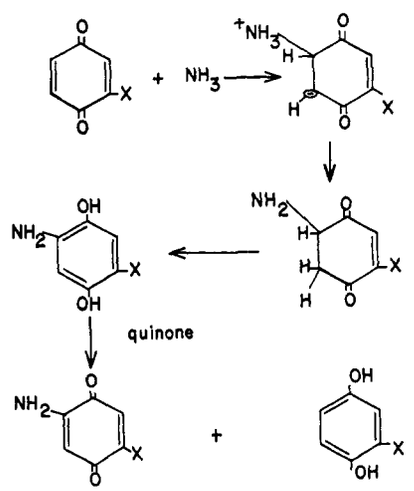


Figure 3. Stability of dye formed in the ammonia reaction (6.9×10^{-8} gram NH_3/10 ml of $10^{-3}M$ solution of I)

Scheme IV



This is general except for the case of cyanide addition to quinone where the 2,3-di-adduct is formed (*12*).

In our case the para position to the amine substituent is occupied, preventing further addition. In addition, the enhanced electrophilic character of the reagent facilitates the reaction of a second molecule of the quinone with the amine adduct to form the product.

(12) J. Thiele and J. Meisenheimer, *Ber.*, **33**, 675 (1900).

This addition was also found for the aniline and methylamine adducts. The formation of the dimer, IV, was therefore unexpected. The ammonium salt of IV was obtained synthetically in the presence of excess ammonia in benzene solvent, the base reacting to form the salt or ion-pair. Similarly, carrying out the reaction under analytical conditions, using dioxane as solvent and the ammonia at low concentrations, a similar salt or ion-pair is presumed to be formed. The acidic proton of IV (pK_a 4.5) would be expected to react with a molecule of ammonia. Thus, two moles of ammonia react with two moles of I to yield one mole of dye in the analysis of ammonia in air.

It would be expected that acidic vapors would have a negative interference effect on the analysis and detection of ammonia and amine vapors. This could possibly be overcome by employing an alkaline prefilter which would selectively remove these vapors. The acidic vapors, HCl, oxides of nitrogen, sulfur dioxide, and sulfuric acid do not react with the reagent to yield the colored dye.

The reagent has been used as a spray on surfaces for detection of amines, *e.g.*, in the thin layer chromatography of proteins and amino acids.

The reagent may be employed in the analysis of aromatic primary amines by suitably modifying the procedures, *e.g.*, employment of a higher boiling solvent and running the analysis at 60 °C.

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New Reaction Mixture for Spectrophotometric Determination of *N*-Acetylhexosamines

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N-ACETYLHEXOSAMINES UPON HEATING in alkaline solutions are converted to heterocyclic compounds (*1*, *2*) which, in turn, can react with *p*-dimethylaminobenzaldehyde (*p*-DMAB) to produce a pink color. The intensity of the color is proportional to the concentration of *N*-acetylhexosamines. Based upon this reaction, Zuckerkandl and Messiner-Klebermass (*1*) developed a colorimetric method for quantitative estimation of *N*-acetylglucosamine. Modifications of the method were made by Morgan and Elson (*2*) and Aminoff *et al.* (*3*) in order to increase accuracy and precision, and by Reissig *et al.* (*4*) to increase the sensitivity of the test. All these methods, however, yield inaccurate results for samples containing lipids and proteins which may cause turbidity in the final solution, and are susceptible to interference by reducing sugar–amino acid mixtures. This paper is a report on the utilization of a reaction mixture based upon formic acid, permitting the determination of *N*-acetylhexosamines in the presence of proteins and lipids.

EXPERIMENTAL

Apparatus. Hitachi Perkin-Elmer UV-Vis Model 139 and Recording Model 202 Spectrophotometers were used for the spectrophotometric measurements.

Reagents. Borate buffer was prepared by dissolving 4.95 grams of boric acid in about 80 ml of water and with dropwise addition of 30% KOH to a final pH of 9.1. The volume was adjusted to 100 ml with distilled water.

The *p*-DMAB reagent was prepared by dissolving 4 grams of *p*-dimethylaminobenzaldehyde (Matheson, Coleman and Bell, Norwood, Ohio) in 100 ml of chloroform. This reagent was stable for at least one month when stored in amber bottles. Formic acid (J. T. Baker Chemical Company, Phillipsburg, N.J.) was reagent grade (90–92%). *N*-Acetylglucosamine, *N*-acetylmannosamine, and *N*-acetylgalactosamine (Calbiochem., Los Angeles, Calif.) were used without further purification.

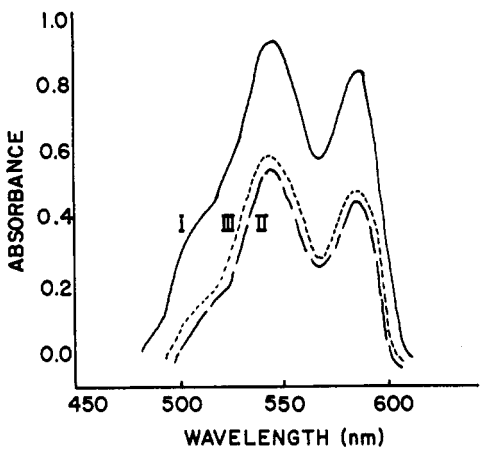

Figure 1. Absorption spectra for the *N*-acetylhexosamines

I. *N*-acetylglucosamine
II. *N*-acetylgalactosamine
III. *N*-acetylmannosamine

Procedure. A 0.50-ml portion of the sample was placed in a screw-cap test tube and 0.10 ml of the borate buffer was added. The mixture was heated in boiling water for 10 minutes. After cooling the mixture in ice-water, 2.0 ml of the *p*-DMAB reagent was added followed by slow addition of 4.0 ml of formic acid. The tubes were shaken for 1 minute and incubated at 37 °C. After 20 minutes, the tubes were cooled in ice-water to 2–4 °C for 10 minutes, and then centrifuged immediately at 1000 × G for 5 minutes. The absorbance of the clear supernatant was measured at 545 nm. The blank consisted of distilled water in place of the sample.

Standard curves were prepared using aqueous solutions containing 20–200 μg of the individual *N*-acetylhexosamine

(1) F. Zuckerkandl and L. Messiner-Klebermass, *Biochem. Z.*, **236**, 19 (1931).
(2) W. T. J. Morgan and L. A. Elson, *Biochem. J.*, **28**, 988 (1934).
(3) D. Aminoff, W. T. J. Morgan, and W. M. Watkins, *Biochem. J.*, **51**, 379 (1952).
(4) J. L. Reissig, J. L. Strominger, and L. F. Leloir, *J. Biol. Chem.*, **217**, 959 (1955).