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A Simplified Method For Sampling Atmospheric Ammonia from Inhalation Chambers During Exposure Studies

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One of the major problems facing investigators in the area of inhalation testing is the ability to quantitatively collect and analyze gaseous samples from test chamber atmospheres during exposures (CAMPBELL 1976). Although many procedures are available for collection of air samples (GAGE 1961), this problem remains especially acute for the collection and determination of atmospheric ammonia (NATIONAL ACADEMY OF SCIENCES 1979).

Most ammonia collection techniques utilize the highly water soluble property of the gas by simply bubbling a sample through some appropriate absorbing media (either water or dilute acid) via aspiration, thereby trapping the ammonia in either the free dissolved form (NH_3) or the ammonium ion (NH_4^+)(GAGE 1961, KATZ 1977) and analyzing via indophenol generation. This procedure has been successfully employed in previous inhalation studies (BARROW & DODD 1979, MALANCHUK et al. 1980), as well as field determinations (PATTY 1963). However, the principle of this collection procedure is confounded by the design of some inhalation systems which do not lend themselves to a pertubation in air flow of sufficient duration to acheive adequate sampling. One alternative is to collect a measured volume of the gas sample using a hand pump or gas-tight syringe. The sample in the syringe is then expelled by hand or infusion pump at a constant, preferably slow rate, through a fritted glass gas dispersion tube, into the absorbing solution. Although theoretically, a high degree of precision should be obtained, our experience indicated that this procedure is in fact quite time consuming and contains a rather high probability of error inherent in the bubbling process. Extreme care must be taken to bubble the sample very slowly and to flush the syringe and dispersion tube for each complete sample collection. In addition, the dispersion tube itself must be washed several times between individual determinations to remove any residual ammonia contamination from the previous sample.

A sampling method is described below which (1) eliminates the use of a fritted glass gas dispersion tube and allows for rapid, efficient and reproducible collection of ammonia in a gaseous sample and (2) utilizes a modified analysis procedure applicable to a wide range of ammonia concentrations. These protocols were developed while examining the toxicity of ammonia under constant flow exposure conditions.

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EXPERIMENTAL

Gas Sample Collection and Ammonia Trapping

As indicated previously, the inhalation chambers employed were of a constant flow design and so constructed as to allow a portion of the flow to be diverted to a sampling port (KAPEGHIAN et al. 1980). Samples were obtained from the port utilizing a 10 mL gas tight syringe (Hamilton 1010-N) fitted with a Luer-Lok(R) stopcock with teflon washers. After several washings of the syringe with the gas to be sampled, a volume of gas approximately 1 mL greater than desired was withdrawn; the stopcock was then closed and removed from the sample port. Two procedures for ammonia trapping were then utilized.

Absorption in Liquid Medium - While still under the hood housing the inhalation chamber, a small volume of the sample was expelled to obtain the desired volume of sample. The syringe assembly was connected to a fritted glass gas dispersion tube (5mm O.D., 135 mm long. Porosity - C) immersed in 3.0 mL of 0.1 N H_2HO_4 solution and the contents of the syringe forced through the system either by hand or with the aid of a constant infusion syringe pump. It was necessary to force an additional 10 mL of room air through the assembly to ensure quantitative removal of the gas from the dispersion tube. The gas dispersion tube was removed, the sample stoppered and held for analysis.

Low Temperature Condensation - The boiling point of ammonia at 1 atm is -33.33°C (BRAKER & MOSSMAN 1971). The gas will condense along the sides of any nonporous vessel at temperatures well below its boiling point. An air sample containing ammonia when slowly expelled along the sides of a test tube placed in dry ice/acetone bath (approx. -70°C) will condense into liquified ammonia (and some water) and can be immediately trapped as NH_4^+ formed by the addition of acid.

This concept was employed for sampling of the gas in the inhalation chambers. While still under the hood housing the inhalation chamber, the gas sampling syringe and stopcock assembly was fitted with a 10 cm stainless steel blunt needle (14 gauge). A small volume of the sample was expelled from the syringe to obtain the desired volume. The contents of the syringe were then expelled through the needle at a moderate rate (0.1 to 0.5 mL/sec) onto the sides of a borosilicate test tube (13 x 100 mm, Kimax(R)) which had been immersed (for 1 min) approximately 3 cm into a dry ice/acetone bath. The test tube was immediately removed and 3.0 mL of 0.1 N H_2SO_4 solution added. At this point, the sample may be stoppered and saved for later analysis.

Analysis of Trapped Ammonia

The analysis of the acid solution for trapped ammonia is a modification of the method of KATZ (1977). The procedure still

employes the classical indophenol generation (Berthelot reaction) but utilizes commercially available reagents and is performed on a much smaller scale than generally reported for atmospheric ammonia.

To the acidic solution containing the trapped ammonia, add 2.0 mL of Na_3PO_4/NaOH solution (KATZ 1977). Vortex well then add 0.5 mL phenol nitroprusside solution (Sigma Chemical Co.) followed by 0.5 mL alkaline hypochlorite solution (Sigma Chemical Co.), vortexing after each addition. Stopper the tube and place in a water bath at 37°C for 30 min (or room temperature for 45 min). Following color development, read the absorbance of the solution at 630 nm against a reagent-room air blank. For concentrated samples where dilutions are required, dilute samples and blanks with distilled water. This protocol was developed using a Spectronic-20 (Bausch and Lomb) however any spectrophotometer may be used.

Calibration Curve

From a stock solution of NH_4Cl (3.18 mg NH_4Cl/ml equivalent to 1.0 mg NH_3/ml), make the following dilutions: Add 1.0, 2.0, 3.0, 4.0 and 5.0 mL of stock solution to 100 mL columetric flasks and dilute the volume to 100 mL with distilled water. This results in a series of 10 - 50 µg/mL NH_3 standards respectively. Add 50 µL aliquots of each standard to a series of test tubes containing 2.95 mL of 0.1 N H_2SO_4 solution. Add 2.0 mL of Na_3PO_4 /NaOH solution to each tube followed by 0.5 mL of phenol nitroprusside solution and 0.5 mL of alkaline hypochlorite solution, vortexing after each addition. To the reagent blank, add 50 µL of distilled water. The final concentration of NH_3 per tube will be 0.5, 1.0, 1.5, 2.0, and 2.5 µg per 6.0 mL. Read the absorbance of each at 630 nm against the reagent blank. Plot the absorbance of each standard vs µg of NH_3 per tube and determine the slope graphically or by linear regression.

Calculation of NH_3 Concentration in Sample

Assuming an investigator chooses to use the reagents in the same proportions listed above, the final volume per tube will be the same in the sample as the standards. There may be situations however, that require modification of the absorbing solution volume, therefore all the steps in the calculations are shown below. Caution must be employed in modification of the assay since the product formation will not take place under acidic conditions.

$$\text{ppm } NH_3 = \left(\frac{ABS}{S}\right)\left(\frac{V}{6.0\text{ mL}}\right)\left(\frac{1}{V_g}\right)\left(\frac{100\text{ ppm}}{70.9 \times 10^{-3}\ \mu g/mL}\right)$$

where ABS = absorbance of test solution,

S = slope of calibration curve (absorbance/µg NH_3),

V = volume of test solution (mL)

and

V_g = volume of original gas sample (mL).

The value of 70.9×10^{-3} µg/mL corresponding to 100 ppm NH_3 is based upon a v/v ratio using the specific volume of NH_3 at 70°F and 1 atm to be 1,411 mL/g (BRAKER & MOSSMAN 1971).

RESULTS AND DISCUSSION

Typical calibration data employing the above procedures are depicted in Figure 1. Linearity is maintained over a wide range of ammonia concentrations. The detection limit of this analysis is estimated to be 0.005 absorbance units or 0.031 µg NH_3 per tube which under conditions specified corresponds to 8.7 ppm in a 5.0 mL gas sample.

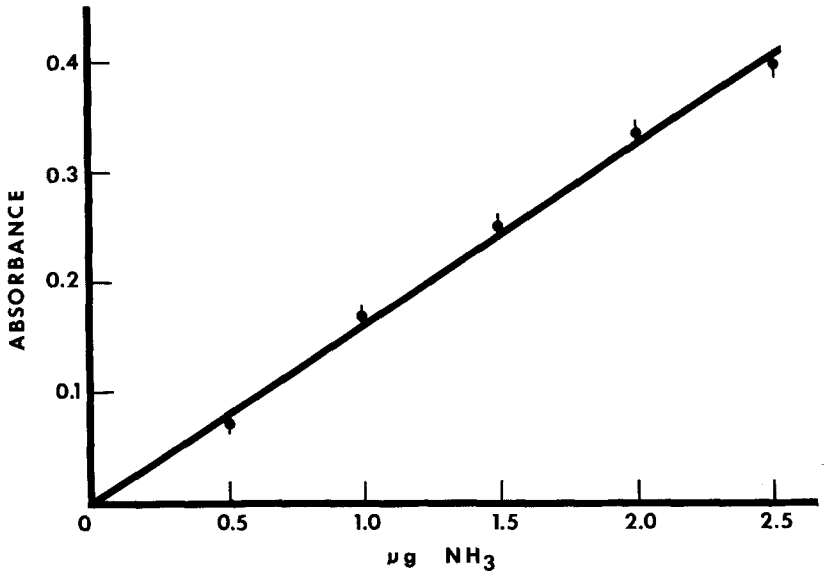

FIGURE 1. Calibration curve for NH_3 using the modified indophenol assay. Each point depicts the mean absorbance from 6 determinations ± standard error. The slope of the regression line is 0.161 absorbance units per µg NH_3, with Y-intercept at zero ($r = 0.997$).

The high degree of correlation ($r = 0.997$) between analytically determined data and the regression line with the y-intercept being essentially zero, allows for the simple conversion from absorbance

units to ppm. Although variations of approximately 3% in repeated calibrations were found, it is suggested that a calibration be performed routinely, especially when fresh reagents are substituted in the assay. In general, all reagents except calibration standards were found to be extrememly stable if well capped and stored in opaque brown bottles. The commercial reagents were quite stable under refrigeration. Color formation remained constant for at least 1 h post incubation with the sample tube tightly capped.

Utilizing calibration data, ammonia concentrations were determined in gas samples collected and trapped by the two procedures. Some typical results of the analyses of samples obtained after equilibrium had been established in the chamber system are presented in Table 1.

TABLE 1 - Comparison of Absorption and Condensation Methods for NH_3 Gas Collection

Method	Nominal Conc[1] (ppm)	Analytical Conc (ppm)	Ratio (Anal/Nom)
Absorption	2950	750, 1400, 1090	0.37
	4550	3670, 3040, 3010	0.71
	5450	1840, 3470, 4510	0.60
Condensation	1350	1280, 1230, 1520	0.99
	2730	2050, 2240, 2330	0.81
	3640	3240, 3450, 3460	0.95
	4650	4860, 4260, 5470	1.04

[1]Nominal concentration refers to a predicted ammonia concentration calculated on a v/v basis from NH_3 and air flow rate data.

Generally, our experience with the absorption procedure has been rather unsuccessful, producing collection efficiencies no greater than 75%, and often much less. Conversely, the condensation approach routinely enabled us to achieve collection efficiencies above 80%. Furthermore, the average interval between individual sample collection and trapping was reduced from 20 to 4 min by switching from the absorption technique to the cryocondensation method.

One of the major advantages of the condensation method resides in the fact that the volume of gas sample does not significantly affect the assay but will increase or decrease the detection limit accordingly. Assuming a 5.0 mL gas sample, the detection limit would be 8.7 ppm, but by increasing the sample volume to 50 mL, the detection limit is reduced to 0.87 ppm. The relative proportions of reagents may also be modified both in the calibration curve and assay, to result in extreme sensitivity over a narrower range of ammonia concentrations, similar to that shown for the more conventional absorption procedure (BARROW & STEINHAGEN 1980). Extremely high NH_3 concentrations may be collected and measured by this procedure by simply diluting the initial sample with water or reducing the gas sample volume.

We feel the condensation method described here will offer a valuable tool to the inhalation research community enabling the investigator to sample and analyze for ammonia levels in constant flow systems, with little or no modification of the chamber air flow, while maintaining a high degree of precision in the analysis. This method also enables an investigator to handle a relatively large number of samples in a short period of time, a factor which is critical to the success of most inhalation studies. It is the hope of the authors that this simple procedure can be utilized in a more general manner with other gases for which these same principles apply.

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