Analysis of Oxygen, Carbon Dioxide and Nitrous Oxide Mixtures with the Scholander Apparatus

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Mixtures of nitrous oxide, oxygen, and carbon dioxide can be analyzed by the Scholander apparatus with a 95 per cent confidence of 1 per cent of the gas concentration. The technique follows the description of Scholander and differs principally in the exposure of two samples of the gas to the two absorbing reagents, as well as a determination of the solubility coefficient of nitrous oxide in the reagents. The precision of this technique is not as good as the Scholander analysis of mixtures of oxygen, nitrogen, and carbon dioxide, but compares favorably with other methods appropriate to anesthetic mixtures. The apparatus is economical, durable, and available.

DETERMINATION of oxygen and carbon dioxide content in mixture of gases by chemical analysis ordinarily depends on the insolubility of the balance of the gases (usually nitrogen). If the background gas is only nitrous oxide, accurate analysis of the three components can be made by a modification of the Scholander microanalytic method. Reproducibility is 0.06 per cent or better (95 per cent confidence limits) and accuracy is better than 1 per cent of the concentration of each gas. The method differs from the usual analysis of gases in air, requiring measurement of the solubility coefficient of nitrous oxide at the temperature of the water bath, and by exposure of separate samples to each absorbing solution.

Principle

When a mixture of nitrous oxide, oxygen, and carbon dioxide is exposed to the oxygen

absorber (alkaline sodium hydrosulfite), all carbon dioxide and oxygen is chemically absorbed. Nitrous oxide partitions between the gas phase and the absorber. The volume of nitrous oxide in simple solution is determined by its solubility coefficient in the absorber at the temperature of the water bath, the volume of the absorber, and by atmospheric pressure.

When a second sample of the same gas mixture is exposed to the carbon dioxide absorber (sodium hydroxide solution), carbon dioxide is chemically absorbed and some nitrous oxide passes into simple solution, again depending on its solubility and concentration, the temperature, the volume of the absorber, and pressure. If pure nitrous oxide is exposed to the two solutions, the Ostwald solubility coefficient for nitrous oxide in the reagents may be determined. From the four exposures of gas to reagents, two pairs of simultaneous linear equations are derived; the first permits the calculation of nitrous oxide concentration in the sample, and the second permits calculation of oxygen and carbon dioxide content.

Method

Modifications of the Scholander method. The technique generally follows that described by Scholander.[1] Attention to a number of details is important in achieving reproducibility. A list of these details, along with a derivation of the equations for calculating final concentrations appear in the Appendix.

Initially the micrometer scale is set at approximately 5 units. The gas sample-mercury interface is brought to the mark on the capillary tube and micrometer reading M_1 made. Approximately 0.5 ml. of gas (about 15 micrometer units) is admitted to the reaction chamber, followed by an indicator drop of acid rinsing solution (ARS). The compensating chamber is emptied, the gas-ARS interface brought to the mark, the open stopcock put in

place, and micrometer reading M_2 recorded. An estimated 4–5 micrometer units of oxygen absorber are introduced into the reaction chamber. After absorption the ARS drop is brought to the mark and the third micrometer reading M_3 made. As the gas sample is next expelled, the gas absorber interface is stopped at the mark and M_4 read on the micrometer. Finally, the absorber is expelled, the absorber mercury interface brought to the mark, and micrometer read, M_5. The volumes of the gas sample, of gas absorbed, of gas remaining, and of absorber are calculated by difference.

The procedure is repeated on a second sample of gas using the carbon dioxide absorber. This enables the calculation of the fractional concentrations of carbon dioxide, oxygen, and nitrous oxide in the gas mixture, provided the solubility of nitrous oxide in the absorbing solutions is known.

Ostwald Solubility Coefficients of Nitrous Oxide in Absorbing Solutions. A modification of the method of Saidman *et al.*[2] is used to measure the solubilities of nitrous oxide in both absorbing solutions at the water bath temperature used. The measurement must be made for each new batch of reagents. The mean of three measurements of Ostwald solubility coefficient with a range of not more than 0.02 is substituted in the equations. The relation between solubility coefficient and temperature are presented in figures 1 and 2. Means of six to ten solubility measurements are plotted against temperature from 19° C. to 30° C.,
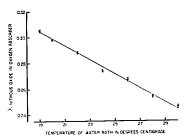

FIG. 1. Relation between Ostwald solubility coefficient (λ) and temperature of the oxygen absorber. The points are means of triplicate determinations. The vertical bars represent the standard errors.
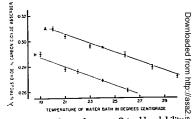

FIG. 2. Relation between Ostwald solubility coefficient and temperature of the carbon dioxide absorber. The points of line A are means of triplicate measurements. Line B represents triplicate estimates of λ for the same container of carbon dioxide absorber one month later. The difference is thought to represent small change in solute concentration, and demonstrate the importance of individual determination of λ. The vertical bars represent the standard errors.

within the range an analysis is likely to be performed. Below 19° C., the ARS crystallized, making the analysis impractical with the usual solutions.

Minor changes in the solute content of the absorbers produce a major change in the solubility coefficient as illustrated by lines A and B in figure 2. The same absorber from the same container was used in all analyses, the difference being that the measurements on line B were determined one month following the measurements on line A. Evaporation of a small amount of water from the reagent had increased the concentration of solutes creating a salting-out effect. Deliberate dilution of the reagents and measurement of the change in solubility coefficient has confirmed this change. Because of the variation we recommend direct determination of the solubility coefficient in the reagents used, rather than reliance on graphs of figures 1 and 2.

Calculations. In the equations, V refers to a volume of gas measured in micrometer units. The first subscript identifies the volume of sample gas injected, GI; the volume of gas absorbed, GA; or the final gas volume, GF. V_A is the volume of absorber admitted to the reaction chamber. A second subscript, CO_2, O_2, N_2O describes that portion of a volume expressed as pure carbon dioxide, oxygen, or nitrous oxide. Lambda (λ), with subscripts

OA and CA refers to the Ostwald solubility coefficient of nitrous oxide in the oxygen and the carbon dioxide absorber respectively, at the temperature of the water bath. F refers to the fractional concentrations in the dried sample gas modified by subscripts as is V. Thus, F_{IO_2} refers to the fractional concentration of oxygen in the initial sample, while FF_{N_2O} refers to the final fractional concentration of nitrous oxide in the gas at the end of analysis. The equations are:

$$F_{IN_2O} = \frac{V_{IN_2O}}{V_{GI}} = \frac{(\lambda OA \times V_A) + V_{GF}}{V_{GI}} \quad (1)$$

$$F_{ICO_2} + F_{IO_2} = \frac{V_{GA} - (\lambda OA \times V_A)}{V_{GI}} \quad (2)$$

$$V_{GF} \times F_{ICO_2} - \lambda CA \times V_A \times F_{IO_2}$$
$$= \frac{V_{GF}(V_{GA} - \lambda CA \times V_A)}{V_{GI}} \quad (3)$$

Equation (1) gives the fractional concentration of nitrous oxide directly. Equations (2) and (3) enable calculation of fractional concentrations of oxygen and carbon dioxide by the solution of simultaneous equations. Equation (3) assumes no solution of oxygen in the reagent. As long as the sample contains less than 50 per cent oxygen this assumption results in no appreciable error. If more oxygen is present, the carbon dioxide absorber should be equilibrated with oxygen before use, as suggested by Scholander in the original method.

TABLE 1. Repeated Analysis of Poynting Gas Sample

Sample	N_2O	$O_2 + CO_2$	CO_2
1	79.05	20.88	−0.15
2	79.16	20.78	−0.12
3	79.04	20.86	−0.07
4	79.10	20.77	−0.09
5	79.08	20.81	−0.11
6	79.08	20.83	−0.07
Mean	79.08	20.82	−0.10
n	6		
S.E.	0.16	0.08	0.01

Water bath temperature was 21.5° C. λOA 29.1 ± 0.2, λCA 26.4 ± 0.2. The negative values for CO_2 are discussed in the text.

TABLE 2. Comparison of Four Samples by Three Analytic Methods

Sample	Gas	Rotameter Analysis	Physical Analysis	Scholander Analysis
1	CO_2	4.64	4.76	4.91
	O_2	20.9	20.3	19.5
	N_2O	74.4	74.9	75.5
2	CO_2	3.56	3.54	3.72
	O_2	26.7	26.8	26.1
	N_2O	65.3	65.1	65.6
3	CO_2	3.73	3.76	3.80
	O_2	26.7	26.8	26.1
	N_2O	69.5	69.4	70.1
4	CO_2	0.0	0.0	0.09
	O_2	27.5	27.1	26.7
	N_2O	72.5	72.9	73.1

Rotameters, calibrated with bubble flowmeter and oxygen analyzer were read before and mid-sample point and after sample collection. Carbon dioxide analysis was continuous during sampling. Scholander analysis is the mean of two measurements with 0.04 agreement or better in all cases.

Reproducibility and Accuracy

A cylinder of Poynting gas (a mixture of nitrous oxide and oxygen) analyzed by a paramagnetic analyzer and by an oxygen electrode contained 21.0 ± 0.3 per cent oxygen and 79.0 per cent nitrous oxide (the limit of accuracy for these techniques). Table 1 shows the results of repeated analysis by the Scholander technique; the mean was 79.08 per cent nitrous oxide. A similar set of analyses of the same cylinder one month later gave a mean of 79.16 per cent nitrous oxide, a difference well within the standard error of the measurements reported below. The full analytic procedure was carried out on these samples and regularly yielded a negative value for carbon dioxide of the order of one-tenth of one per cent. The bulk of this could be explained by evolution of nitrogen dissolved in the carbon dioxide absorber during analysis. This can be avoided by equilibrating the carbon dioxide reagent with helium or another insoluble gas. We believe, however, that this would complicate the analysis without a significant improvement in the results.

Mixtures of nitrous oxide, oxygen, and carbon dioxide were prepared by rotameters cali-

brated with a bubble flow meter. The carbon dioxide concentration varied from 0 to 5 per cent oxygen from 20 to 30 per cent, and nitrous oxide from 65 to 75 per cent. A mixed sample from the rotameters was collected over mercury during a one minute period. Simultaneously, an aliquot of the mixed gases was passed through a Beckman C2 paramagnetic oxygen analyzer (0 to 50 per cent O_2 scale) and a Godart Kapnograph. The oxygen analyzer was calibrated with air and nitrogen, and a correction made for the slightly diamagnetic property of nitrous oxide. The Kapnograph was calibrated with carbon dioxide in oxygen mixtures and correction made for both the spectral overlap of nitrous oxide and the collision-broadening effect. Thus, there were three independent estimates of the gas composition: chemical analysis with micro-Scholander technique; physical analysis of infrared and paramagnetic properties; and volumetric analysis from the rotameter readings. Table 2 contains the results of these analyses. The volumetric analysis by rotameter probably has an accuracy of ± 1 per cent of the gas composition. The accuracy of the infrared analysis for carbon dioxide was of the order of one-half of one per cent of the gas concentration, when the appropriate corrections for nitrous oxide were made.[3] The Beckman C2 oxygen analyzer had an accuracy of ± 2 per cent of the oxygen concentration used in this study. Table 2 shows that the Scholander analysis of the four gas samples agreed with the rotameter and physical analyses within the order of accuracy of the latter two techniques. In the absence of a primary standard with which to compare the chemical analysis, we can only say that the accuracy of the Scholander technique when nitrous oxide, oxygen, and carbon dioxide mixtures are being analyzed, is at least of the order of 1 per cent of the concentration of the gases being analyzed.

Discussion

The method gives reproducible analysis of dry gas concentrations of mixtures of nitrous oxide, oxygen, and carbon dioxide. All three gas concentrations are measured during the same procedure. An analyst already familiar with the Scholander apparatus will require a slightly longer time to perform the analyses. Minor errors due to nitrogen and oxygen content of the reagents are acknowledged. During measurement of solubility coefficients, small volumes of oxygen and nitrogen pass into the gas phase. With samples of nitrous oxide, oxygen, and carbon dioxide, similar diffusion takes place. When the sample oxygen concentration is 21 per cent there is no exchange of oxygen; when F_{IO_2} is greater than 0.21, a small volume of oxygen passes from the gas into the liquid phase of the carbon dioxide absorber. The exchange of oxygen and nitrogen gives rise to small errors, which can be disregarded if F_{IO_2} is less than 0.5. This is part of the explanation for the negative value for carbon dioxide in Table 1.

The negative value for carbon dioxide is also explained in part by the slight deviation from a direct relationship between the concentration of nitrous oxide in a mixture and its solubility in liquids.[4] The higher the concentration, the greater the solubility. F_{N_2O} in the range of 0.6 to 0.9 causes an underestimation of F_{CO_2} by 0.02 to 0.01.

References

1. Scholander, P. F.: Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples, J. Biol. Chem. 167: 1, 1947.
2. Saidman, L. J., Eger, E. I., II, Munson, E. S., and Severinghaus, J. W.: A method for determining solubility of anesthetics utilizing the Scholander apparatus, ANESTHESIOLOGY 27: 180, 1966.
3. Smith, T. C., Colton, E. T., and Dripps, R. D.: Mixed venous-arterial carbon dioxide tension difference in anesthetized man (Abstract), Fed. Proc. 26: 2, 1967.
4. Findlay, A., and Craighton, N. J. M.: Some experiments on the solubility of gases in ox blood and ox serum, Biochem. J. 5: 294, 1911.

Appendix

Supplementary Details of the Method

1. Temperature equilibrium must be established within the water bath. Water bath temperature must be measured at the start of the analysis, and thereafter kept constant (±0.1° C.). The water bath can be mixed with a slow stream of air bubbles.

2. Before each gas sample is introduced, the mercury is brought into the capillary tube. The analyst must then wait for 60–120 seconds to

allow ARS to escape from the reaction chamber, where a small volume tends to remain temporarily between the mercury and the side walls.

3. The gas sample must be kept under slight positive pressure during transfer from the tonometer by allowing the mercury column in the pipette to fall slowly. Use of a syringe is less easy and not recommended.

4. An effort is made to exclude all ARS from the capillary tube during introduction of the gas sample. If present, ARS dissolves some gas before the initial gas volume can be measured, and the absorber subsequently admitted is diluted with ARS.

5. Micrometer reading M_1 should be within the range 4.8 to 5.2.

6. After bringing the gas sample into the reaction chamber and drawing an indicator drop into place, the ARS is removed from the compensating chamber. Air must not be sucked through the chamber as evaporative cooling becomes significant. The stopcock is then replaced. It is left open for 20–30 seconds until the slight movement of the indicator drop ceases. During this time temperature equilibration and humidification of air in the compensating chamber take place. A minute volume of gas dissolves in the ARS of the indicator droplet. With a small indicator drop, this change in volume should never be greater than 0.020 micrometer units, and often is near zero. As a check, M_2 should not change when the stopcock is closed.

7. Four to five micrometer units of absorber is the optimum volume; 3.5 to 5.5 units is acceptable. Outside this range, analysis is unsatisfactory. With volumes greater than 5.5 units, the initial movement of the indicator drop tends to be very rapid. Also, a much longer time than 90–120 seconds is required for complete solution and equilibration to take place. With volumes less than 3.5 units, errors in micrometer reading and dilution with the minimal drop of ARS become proportionately greater.

8. The shaking of the apparatus should be controlled so that the absorber covers the side walls of the reaction chamber up to a level just below the capillary tube. Control of the agitation can be achieved manually with the analyst's right hand on the dowel attached to the micrometer handel and his left hand on the horse-shoe part of the micrometer burette units, or by adjusting the tension in the spring connected to the eccentric pin on the counterpulley. This provides the maximum gas-absorber interface, and allows for the most rapid and complete absorption. Complete absorption is confirmed by the meniscus remaining stationary in the capillary tube during the last 10 seconds of agitation. Ninety seconds for carbon dioxide absorption and 120 seconds for oxygen is usually sufficient. If during shaking the absorber passes into the capillary tube, the analysis should be discontinued.

9. The indicator drop must be kept as stationary as possible during exposure to the absorber. This minimizes diffusion of nitrous oxide across the drop. In the absence of movement of the indicator drop, experiments with 100 per cent nitrous oxide showed less than 0.1 per cent of initial gas volume lost in 10 minutes. If the drop was deliberately moved continuously, the loss was never more than 2.4 per cent in 10 minutes. Shaking of the apparatus brought about the loss of less than 0.1 per cent of the initial gas volume during three minutes. An alternative approach to minimize diffusion recommends a continuous flow of nitrous oxide through the compensating chamber during analysis.[2] The disadvantages are that it is impossible to judge an appropriate flow rate which will replace the air present, but will not produce appreciable evaporative cooling. It is also less convenient than the usual method. As a direct comparison, the solubility of nitrous oxide in carbon dioxide absorber was measured at constant temperature, first with air in the compensating chamber, and then with nitrous oxide flowing through slowly. The means of 6 analyses by each method differed by only 0.16 per cent, the variability of each group being of the same order. An attempt was made to use mercury in the indicator drop, but it proved unsatisfactory owing to its high specific gravity and surface tension.

10. Throughout the analysis it is unnecessary to bring the indicator drop down to the piccolyte coating of the reaction chamber before reading the micrometer. Mixing of the gas in the reaction chamber is rapid. There is no evidence that a significant volume of ARS adheres to the side walls of the capillary tube, or that such ARS as there may be is removed by bringing the indicator drop down to the piccolyte.

11. When M_1 is read, the gas absorber interface is brought to the meniscus. This reading should be made without delay, as 0.010–0.020 micrometer units of ARS may slide down the capillary tube on the top of the absorber. The stopcock should, of course, be open for this reading.

Derivation of Equations

A. Exposure to Oxygen Absorber

$$V_{GA} = V_{ACO_2} + V_{AO_2} + V_{AN_2O}$$
$$= V_{ICO_2} + V_{IO_2} + V_{AN_2O} \tag{1}$$

Because of complete removal of CO_2 and O_2,

$$V_{IN_2O} = V_{GF} + V_{AN_2O} \tag{2}$$

Also,

$$V_{AN_2O} = \lambda_{OA} \times V_A \tag{3}$$

From (2) and (3)

$$V_{IN_2O} = V_{GF} + (\lambda_{OA} \times V_A)$$

$$\therefore \quad F_{IN_2O} = \frac{V_{IN_2O}}{V_{GI}} = \frac{(\lambda_{OA} \times V_A) + V_{GF}}{V_{GI}} \tag{4}$$

From (1) and (3)

$$V_{GA} - (\lambda_{OA} \times V_A) = V_{ICO_2} + V_{IO_2}$$

Dividing both sides by V_{GI} to convert to fractional concentration:

$$\therefore \quad F_{ICO_2} + F_{IO_2} = \frac{V_{GA} - (\lambda_{OA} \times V_A)}{V_{GI}} \quad (5)$$

B. *Exposure to Carbon Dioxide Absorber*

$$V_{GA} = V_{ACO_2} + V_{AN_2O}$$
$$= V_{ICO_2} + \lambda_{CA} \cdot V_A \cdot F_{FN_2O}$$
$$= V_{ICO_2} + \lambda_{CA} \cdot V_A \frac{(V_{GF} - V_{IO_2})}{V_{GF}}$$

Multiplying both sides by V_{GF}, rearranging, and converting to fractional concentration gives:

$$V_{GF} \cdot F_{ICO_2} - \lambda_{CA} \cdot V_A \, F_{IO_2}$$
$$= \frac{V_{GF} (V_{GA} - \lambda_{CA} \, V_A)}{V_{GI}} \quad (6)$$

Equation (4) gives the fractional concentration of N_2O directly. Equations (5) and (6) enable calculation of F_{O_2} and F_{CO_2}. Equation (6) assumes no solution of oxygen in the reagent. As long as the sample contains less than 50 per cent oxygen this assumption results in no appreciable error. If more than 50 per cent oxygen is present, the CO_2 absorber should be equilibrated with oxygen before use, as suggested by Scholander in the original method.

Surgery

POSTOPERATIVE HEPATITIS Fifteen patients are reported who developed postoperative hepatitis after receiving halothane anesthesia. Nine of these had multiple administrations of the agent; eight of these cases became jaundiced within 8 days of the last surgical procedure. In the reported cases transfusion was essentially ruled out as a causal agent as were other possible hepatotoxic medications. Two of the patients had pre-existing liver disease; none gave historic evidence of recent exposure to infectious hepatitis. The evidence of these cases is suggestive but not conclusive that hepatotoxicity is more frequent after multiple exposures to halothane and in those patients with a history of previous liver disease. (*Mendel, E., and Trostel, R.: Hepatitis following Halothane Anesthesia, Pacific Med. 75: 28 (Jan.) 1967.*)

STERILIZATION OF APPARATUS The use of anesthesia equipment results in contamination of that equipment in as high as 80 per cent of the items employed. (No protection is offered by the soda-lime cannister on anesthesia machines, although this has been reported.) Adverse reactions to potent germicidal agents applied to apparatus have long since been reported and have eliminated otherwise satisfactory methods of sterilization. No difficulty of this sort was experienced with buffered glutaraldehyde which was found to be superior to hexachlorophene and a 70 per cent alcohol soak. The sterilizing action of ethylene-oxide gas has been reported and its application to decontamination of anesthesia apparatus has been suggested. Because of the various requirements of processing, especially the time element and the bulkiness of quite large amounts of rubber articles, it often is not feasible to adopt this method. A carefully documented relationship between contaminated anesthesia apparatus and the incidence of postoperative infections should be studied further. In the interim, decontamination by use of buffered glutaraldehyde is superior to many presently used techniques. (*Meeks, C. H., and others: Sterilization of Anesthesia Apparatus, J.A.M.A. 199: 276 (Jan.) 1967.*)