

Determination of Carbon Dioxide in Gas Mixtures

A Potentiometric Method

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THE need for a rapid, accurate method of estimating carbon dioxide is common to many branches of science. A large number of studies in both theoretical and applied chemistry demand simple methods for determining small percentages of carbon dioxide in air and other gaseous mixtures. It is not surprising that many ingenious devices have been developed in an effort to satisfy this need. The majority of the proposed methods can be classified into the following types: (1) those in which the $PV = K$ property of gases is utilized; (2) those in which the carbon dioxide in a known volume of gas is absorbed in alkali and subsequently measured by chemical or physical means. The first class includes those methods in which special apparatus is required—e. g., the Haldane, Van Slyke, or Warburg gas analysis apparatus. Methods of the second class are more frequently used because the apparatus is less expensive and the determination is simple. However, an accurate gas meter is often required, and this is not standard equipment in most laboratories.

A method that belongs to neither of these classes is available but because of certain technical difficulties, little work has been reported on it. This method depends upon the measurement of the equilibrium between the carbon dioxide in an atmosphere and a sodium bicarbonate solution of known concentration. The equilibrium is usually determined by estimating the hydrogen-ion activity or pH. If this is done by means of the hydrogen electrode, elaborate precautions must be maintained to avoid displacement of the equilibrium, hence the method becomes too tedious for routine analyses. Recourse to colorimetric methods, such as proposed by Haas (4), Higgins and Marriot (7), and McClendon (11), has proved of value, but this expedient has sacrificed accuracy to ease of operation. Since colorimetric standards can be read to less than 0.1 pH only with difficulty, the error involved in a determination may be considerable. The accuracy of this method has been increased by determining the pH potentiometrically with the glass electrode, and thus the qualities necessary for routine analyses—i. e., rapidity, simplicity, and ease of operation—have been preserved. The method as finally developed is accurate to 0.02 pH (one millivolt) which is the limit of accuracy of the potentiometer set-up in this laboratory. The only apparatus required is a vacuum-tube potentiometer, or any other devices suitable for measuring pH with the glass electrode. Since the glass electrode method for the determination of pH is gaining favor in biological and chemical laboratories, this method should be available to many without the expense of additional equipment.

A rapid, accurate method is described for measuring low percentages (0.03 to 7 per cent) of carbon dioxide in gas mixtures. It consists of bubbling the gas mixture through about 10 cc. of dilute sodium bicarbonate solution, then determining the pH of the solution with the glass electrode. From a calibration curve the $p\text{CO}_2$ can be readily ascertained. The theory upon which the method is based is discussed. For routine analyses a set-up is described which combines speed and ease of operation with a maximum error of 4 per cent. For a greater accuracy, certain refinements are indicated. The method can be modified for use with a pH colorimeter, if a suitable potentiometric set-up is not available.

THEORETICAL DATA

The principle of the method is based on the buffering effect of a salt of an acid when added to a solution of the acid; for example, in an aqueous solution of carbonic acid and sodium bicarbonate the pH will be a function of the activities of the bicarbonate ion and carbonic acid present. The latter depends on the partial pressure of the carbon dioxide in the atmosphere which is in equilibrium with the solution. If the activity of the bicarbonate ion is fixed, then the pH is a function solely of the partial pressure of the carbon dioxide that is in equilibrium with the sodium bicarbonate

solution. Since carbonic acid is only very slightly ionized in solution, the effective concentration (or activity) of the bicarbonate ion is sensibly that of the sodium bicarbonate present in the solution. It follows that if a dilute solution of sodium bicarbonate of known concentration is brought to equilibrium with an atmosphere containing carbon dioxide, the final pH of the solution measures the $p\text{CO}_2$ in the atmosphere and is independent of the volume of gas passed through or the volume of sodium bicarbonate used.

Because of the importance of this sodium bicarbonate-carbonic acid equilibrium in physiological chemistry, intensive studies of the reaction have been made by a large number of investigators. Hasselbach (5) studied this equilibrium and derived the equation

$$\text{pH} = \text{p}K + \log \delta + \log \frac{(\text{HCO}_3^-)}{(\text{CO}_2)} \quad (1)$$

where $\text{p}K$ represents the negative log of the first dissociation constant of carbonic acid, and δ the degree of dissociation of sodium bicarbonate in the solution used.¹ Warburg (13) discussed the equation and the data of Hasselbach in light of the new theory of complete dissociation of strong electrolytes, and pointed out that Hasselbach's treatment was merely a first approximation, since concentrations rather than activities were employed. Warburg then developed the corrected mathematical relations for use under various assumptions and conditions. He showed that the bicarbonate ion due to the dissociation of the carbonic acid can be neglected if a concentration of sodium bicarbonate is used such that

$$\frac{cH}{cM} < 0.01$$

where cH is the concentration of hydrogen ions and cM is the concentration of the sodium bicarbonate. Also, he

¹ If δ is the activity coefficient of sodium bicarbonate, this equation is correct and is independent of any theory (see Equation 2).

demonstrated that if the measured pH was less than 8.0, the error arising through neglect of the second dissociation constant of carbonic acid is negligible. Hastings and Sendroy (6) studied the application of the Debye-Hückel theory of electrolytic dissociation to the sodium bicarbonate-

extended this work and verified the value of pK_{∞} . These workers also discuss the variation of the value of this constant with temperature. The value of $\Delta pK_{\infty}/\Delta T$ varied from 0.005, based on e. m. f. measurement, to 0.0069 based on Thomsen's colorimetric determinations; the best agreement was among the e. m. f. measurements.

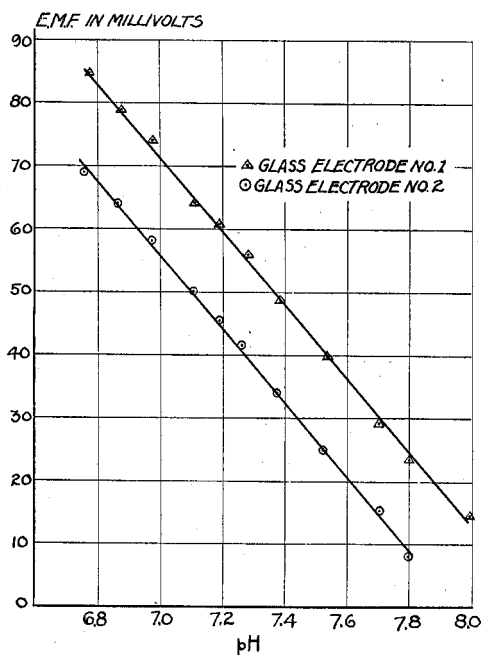


FIGURE 1. CHANGE OF E. M. F. WITH pH USING GLASS ELECTRODES

carbonic acid system in the presence and absence of other ions—e. g., sodium chloride. The theoretical equation applicable to the system that was derived by these investigators is:

$$pK_1 + \log \gamma_1 = \text{pH} - \log (\text{NaHCO}_3) + \log p\text{CO}_2 + \log \frac{\alpha_{\text{CO}_2}}{760 \times 22.4} \quad (2)$$

where $pK_1 = -\log K_1$
 K_1 = apparent first dissociation constant of carbonic acid
 γ_1 = activity coefficient of sodium bicarbonate in concentration used
 (NaHCO_3) = molal concentration of NaHCO_3
 $p\text{CO}_2$ = partial pressure of CO_2
 α_{CO_2} = solubility coefficient of carbon dioxide in water at temperature used

If $pK_1 + \log \gamma_1$ is designated by pK_1' , this latter will be a constant for a given concentration of sodium bicarbonate, and according to the Debye-Hückel theory should vary with the concentration in dilute solutions according to the following equation:

$$pK_1' = pK_{\infty}' - 0.5 \sqrt{\mu} \quad (3)$$

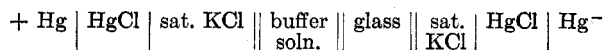
where pK_{∞}' = value of pK_1' at infinite dilution
 μ = ionic strength = $\frac{1}{2} \sum c_i v_i^2$
 v_i = valence of ions
 c_i = molal concentration of ions

Using very exact experimental technic, Hastings and Sendroy determined the value of pK_1' over a range of concentrations of sodium bicarbonate extending from 0.01 to 0.03 normal with and without added sodium chloride, so that the ionic strength varied from 0.01 to 0.18. Their results closely followed the equation predicted by the theory and gave a value of pK_{∞} of 6.33 at 38° C. Stadie and Hawes (12)

EXPERIMENTAL PROCEDURE

DETERMINATION OF pH. The method was developed to measure the carbon dioxide in air supplied to plants grown in atmospheres containing 0.1 to 1 per cent of this gas. It was necessary to use 0.001 N solutions of sodium bicarbonate for the lower concentrations of carbon dioxide and 0.01 N for the higher. An attempt was made to use the quinhydrone electrode to measure the pH, but this proved unsatisfactory because of the drifting of the e. m. f. Glass electrodes were next tried and proved very satisfactory. The vacuum-tube potentiometer used to measure the e. m. f. was a modification of the type described by Allyn and Baldwin (1). This set-up employs a UX-199 vacuum tube, the grid bias of which is adjusted as closely as possible to the value at which there is zero grid current (floating grid potential). The actual grid current obtained, although varying considerably, was never greater than 10^{-12} amperes. This potentiometer is simple in construction, inexpensive, and gives reproducible results with a minimum of care. It is sensitive to one millivolt which was sufficiently accurate for these purposes. Glass electrodes as described by MacInnes and Dole (8, 9) were first tried, but because of the necessity for frequent handling, transportation, etc., these were found to be too fragile. The electrode finally adopted was made by sealing about 3 cm. of 6-mm. Corning 015 glass (9) to 20 cm. of ordinary soft glass, then blowing a fairly thin bulb about 10 mm. in diameter on the special glass end. The thickness of this bulb is limited to the sensitivity of the potentiometer employed and can best be determined by trial. The desiderata of these electrodes are that they should be sturdy enough to withstand considerable shock in handling and yet give a constant, reproducible potential when used to measure the pH of a standard buffer solution. As the bulbs increase in thickness, the potentials become less reproducible, because of changes in body capacity, leakage currents in the circuit, variations in grid current, etc. With a little practice the desirable thickness is readily ascertained; an actual test is the final criterion as to whether or not a suitable bulb has been blown. Measurements on a typical bulb indicated that the glass membrane was about 50 microns in thickness; the resistance of these bulbs is of the order of 200 to 250 megohms.

After a suitable bulb has been blown, the electrode is filled with saturated potassium chloride solution and aged in this solution for 3 weeks. It is then tested daily for about a week against a standard buffer solution. In these tests the e. m. f. measurement is made on the cell:



Since the two calomel half-cells cancel each other, the observed e. m. f. is due to difference in pH of the solutions on either side of the glass membrane plus the "asymmetrical potential"—i. e., the potential in the glass (3, 9). An ordinary type of calomel half-cell is used in the above cell, and contact is made with the glass electrode by filling the latter completely with saturated potassium chloride solution. When the electrodes were first prepared, the asymmetric potential was found to vary, but after aging, it remained very constant in the majority of the electrodes.

If, after aging, the electrode is found to have a constant potential against a standard buffer solution (constant asymmetric potential), it is sealed to a calomel half-cell as shown in Figure 2. This half-cell is readily made by sealing a side arm of 6-mm. glass tubing into about 10 cm. of 10-mm. glass tubing, the lower end of which has had a platinum wire sealed in as shown in the figure. Near the upper end of this 10-mm. tube, a constriction is made. The glass electrode is now sealed to the side arm, after which the mercury calomel and saturated potassium chloride are added. In order to fill completely the side arm and electrode, it is necessary to apply suction occasionally to remove trapped air. After filling, the half-cell is sealed off at the constriction. Electrodes made at the same time and aged in the same way give fairly constant values with the same buffer solutions, as shown in Table I. These measurements were made on Sorensen's phosphate buffer mixtures in the range covered by this method. The results indicate that, although the apparent asymmetric potential may differ to some extent among various electrodes, the difference is independent of the pH that is measured.

TABLE I. COMPARISON OF DIFFERENT GLASS ELECTRODES FILLED WITH SATURATED POTASSIUM CHLORIDE

BUFFER pH	ELECTRODE			
	1 E. m. f.	2 E. m. f.	3 E. m. f.	4 E. m. f.
7.78	0.022	0.022	0.030	0.026
7.53	0.033	0.034	0.044	0.039
7.38	0.043	0.044	0.053	0.049
7.18	0.053	0.054	0.063	0.059
6.87	0.072	0.073	0.082	0.078

MacInnes and associates (9, 10) have shown that from pH 1 to 9 the glass electrode described by them gives potentials which vary with the hydrogen-ion activity exactly as the hydrogen electrode. To determine if the electrode described behaved as a hydrogen electrode in the range employed in this method, potentials were taken on Sorensen's phosphate buffer solutions ranging in pH from 6.7 to 8.0. The data for two electrodes which differ in the asymmetric potential by 15 mv. are given in Figure 1. The points fall on a straight line which has the correct slope, 0.059. These data indicate that the behavior of the electrodes is quite consistent in this range of pH.

A calibration curve was prepared for the method by determining the pH of sodium bicarbonate solutions of known concentration which had been brought to equilibrium with an air mixture containing a known percentage of carbon dioxide. The air mixtures were prepared by the addition of pure carbon dioxide from a cylinder to air in a 20-liter Pyrex bottle. Acidulated water was used as a confining liquid, and each mixture was allowed to stand 2 hours with occasional shaking to allow the gas to come to equilibrium with the water. The mixture was displaced with acidulated water, passed through two Truog towers in series containing 0.1 N sodium hydroxide, then through a tell-tale containing bromothymol blue as an indicator, and finally measured by a gas meter. In order to reduce the error arising from the solubility of the carbon dioxide in the displacing liquid, the latter was run in quite rapidly so that the time required for a determination was about 10 minutes. The gas mixture was divided as it came from the mixing bottle and part passed through a sodium bicarbonate solution of known concentration. The sodium bicarbonate solution was prepared by diluting a standard solution of sodium hydroxide and adding carbon dioxide until a pH of about 6.0 was reached. Phenol red indicator (75 cc. of a 0.01 per cent solution per liter) was added to the sodium hydroxide solution during dilution to aid in the estimation of the end point. The apparatus used for equilibrating the bicarbonate solution is shown in Figure

2. The gas passes through the inlet *a* which is drawn to a fine point, bubbles through the sodium bicarbonate solution, and out at *b*. The presence of phenol red in the bicarbonate helps to decide when equilibrium is reached. Five minutes' bubbling at a rapid rate suffices to bring about 10 cc. of solution to equilibrium. During the aëration, the Pyrex tube containing the bicarbonate was kept in a water

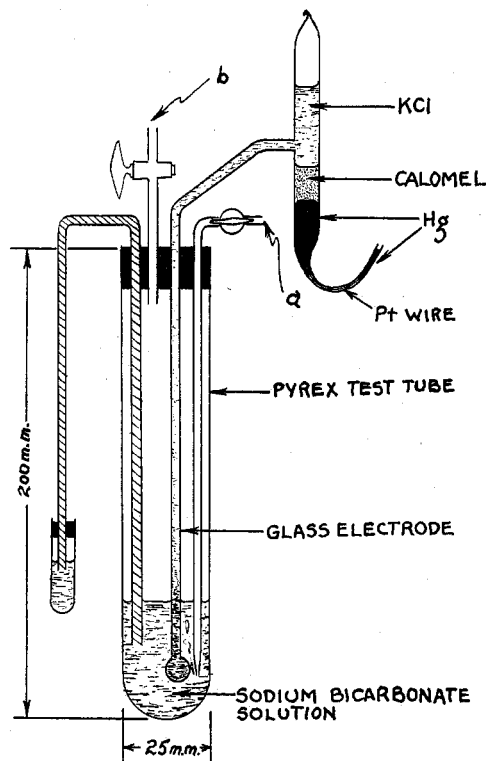


FIGURE 2. DIAGRAM OF APPARATUS

bath maintained at $26^\circ \pm 0.1^\circ \text{C}.$ After equilibrium was reached, the stopcocks were closed and the pH of the solution determined. It was found that the pH does not change even though the bicarbonate solution is allowed to stand 1 to 2 hours after equilibrium is reached.

After obtaining the reading on the bicarbonate solution, the electrode was checked by determining the e. m. f. of a known buffer solution. The pH of the bicarbonate solution was calculated from the formula:

$$\text{pH}_1 = \text{pH}_2 + \frac{\text{e. m. f.}_2 - \text{e. m. f.}_1}{0.0593} \text{ at } 26^\circ \text{C.} \quad (4)$$

where subscript 1 refers to the bicarbonate solution and subscript 2 to the standard buffer solution. All pH determinations were made in duplicate, and checks with 0.02 pH units were constantly obtained. The carbon dioxide in the gas was determined by a double titration modification of the official volumetric method (2). With the volume of gas passed through the towers and the temperature and pressure known, the partial pressure of carbon dioxide in the gas mixture can readily be calculated. Assuming that the concentration of the bicarbonate ion is equal to that of the sodium bicarbonate solution used and taking α_{CO_2} equal to 0.738, Equation 2 reduces to:

$$\text{p}K_1' = \text{pH} + \log \text{pCO}_2 - 1.362$$

Fourteen determinations gave a value for $\text{p}K_1'$ of 6.31 ± 0.007 , indicating that the results were quite consistent.

* This temperature was used because the incubator room in which the potentiometer was set up was kept at this temperature.

From the results obtained by Hastings and Sendroy (6) at 38° C. and using $\Delta pK_1'/\Delta T = 0.005$, the value of pK_1' at 26° C. and for 0.001 *N* sodium bicarbonate concentration should be 6.375. The agreement is certainly all that could be expected in view of the differences in technic. Hastings and Sendroy determined the carbon dioxide in the gas mixtures by the Haldane apparatus, estimated the free and bound carbon dioxide in the bicarbonate solution by means of the Van Slyke apparatus, and in general made use of refinements not available to the authors. It is believed that

dioxide. If 0.01 *N* sodium bicarbonate is used, a pH of 7.67 represents a pCO_2 of only 9.33 mm.³ Use of Equation 3 shows that if 0.0107 *N* sodium bicarbonate is used, a pH of 7.67 will represent 10.0 mm. of pCO_2 , and hence one line will serve for a ten-fold increase in concentration. The points designated by triangles were obtained by use of 0.0107 *N* sodium bicarbonate and were placed on the graph after dividing the pCO_2 by 10—i. e., subtracting 1.00 from the log pCO_2 .

METHOD

APPARATUS. The apparatus and solutions required for the determination of carbon dioxide in a gas mixture are: a glass electrode fitted in the aëration device shown in Figure 2, a potentiometer capable of measuring the pH of a solution with the glass electrode, and a standard solution of 0.001 or 0.0107 *N* sodium bicarbonate. The construction of the glass electrode has been given in detail in the text. The sodium bicarbonate solution is made by dilution of standard solution of sodium hydroxide and passing in carbon dioxide until a pH of about 6.0 is reached; phenol red indicator can be added to the solution during dilution to aid in the estimation of this end point.

PROCEDURE. For the analysis of a gas mixture containing 0.03 to 0.7 per cent carbon dioxide, about 10 cc. of 0.001 *N* sodium bicarbonate are placed in the aëration device, (Figure 2), and the mixture to be analyzed passed through at the rate of 4 to 5 bubbles per second. After approximately 5 minutes of aëration, the stopcocks are closed and the e. m. f. determined by a suitable potentiometric set-up. The pH of the bicarbonate solution is calculated from Equation 4, and the partial pressure of carbon dioxide determined from the calibration curve, Figure 3. For concentrations of carbon dioxide ranging between 0.7 and 7 per cent, 0.0107 *N* sodium bicarbonate solution is used. If the "asymmetric potential" of the glass electrode remains constant as shown by e. m. f. readings on a standard buffer solution, the calibration curve can be constructed to read pressures of carbon dioxide directly from the e. m. f. determination.

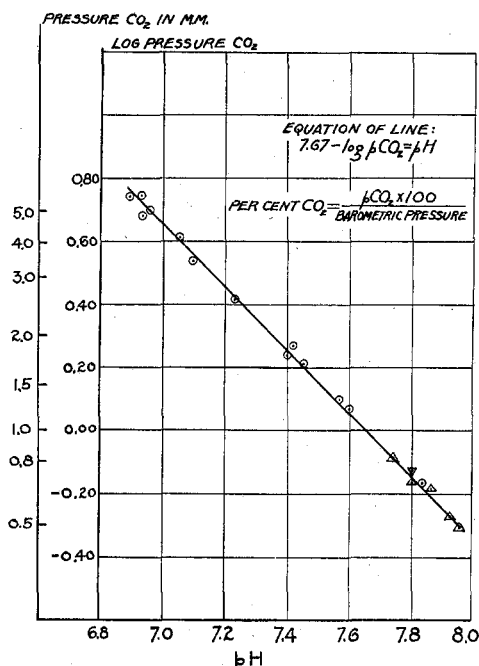


FIGURE 3. CALIBRATION CURVE FOR 0.001 *N* AND 0.0107 *N* SODIUM BICARBONATE
(For 0.0107 *N* sodium bicarbonate solution, multiply ordinate reading by 10)

use of this empirical calibration curve constructed from the results obtained under the conditions in use in the laboratory (in regard to saturation, e. m. f. measurement, etc.), is more satisfactory than would be the use of a theoretical curve based on data obtained under more ideal conditions. It is desirable that anyone adopting the method construct his own calibration curve, but if this is not possible and if the analyses are made exactly as described, the graph in Figure 3 can probably be used without introduction of appreciable error. The points located by use of the 0.001 *N* sodium bicarbonate are marked in Figure 3 by circles.

If the pCO_2 in the gas mixture is plotted against the equilibrium pH for a given concentration of sodium bicarbonate, a logarithmic curve results. For the most accurate results (speed and stability of equilibrium), it is desirable to keep the pH measurements on the part of the curve at which the curvature is greatest. This can be done by increasing the concentration of the sodium bicarbonate solution, whenever the pCO_2 is such that the pH measured lies on the flat part of the curve. Thus for mixtures containing more than 0.7 per cent carbon dioxide, it is advantageous to increase the concentration of the sodium bicarbonate solution to about 0.01 *N*. Since the theoretical pK_1' for this strength of bicarbonate is 6.34, it is necessary that a slightly stronger solution of sodium bicarbonate be used in order that the same line will represent partial pressures of carbon dioxide that are ten times as strong as those indicated by the ordinates. For example, if 0.001 *N* sodium bicarbonate is used, a pH of 7.67 represents a partial pressure of 1 mm. of carbon

APPLICATION AND MODIFICATIONS

The method was tested by determining the carbon dioxide in gas mixtures that were used in the greenhouse; these mixtures were prepared by adding pure carbon dioxide to air, controlling the proportions of each by flowmeters. The tubes of the aëration apparatus were kept in a cheap and easily built water bath made from a large battery jar placed in a box and insulated with shavings. The temperature was maintained at 26° C. by adding cold water or by heating with a blackened 200-watt electric lamp. Temperature control of 0.1° to 0.2° C. for 10 to 15 minutes is readily obtained, and this suffices for routine analyses. At the same time that the equilibrium measurements were taken, check analyses were made by absorbing from a known volume of gas the carbon dioxide present by the method already described. The results were quite satisfactory, as the average recovery on mixtures whose carbon dioxide content ranged from 0.15 to 0.9 per cent was 103 ± 1.29 per cent. The accuracy of this method is limited by the accuracy of the pH determination, which is about 0.02 of a pH unit, corresponding to a maximum error of about 4 per cent.

Whenever a determination is made of the carbon dioxide content of an air mixture, the electrode is checked after the

³ Assuming that the pK_1' corresponding to the authors' technic would be 0.035 less than that found for 0.001 *N* sodium bicarbonate—i. e., 6.275. The mean of seven determinations of 0.0107 *N* sodium bicarbonate was 6.28 ± 0.005 , which is consistent with the values for 0.001 *N* sodium bicarbonate.

determination by means of a standard buffer solution. This added reading requires only a few minutes and serves to determine if the asymmetric potential is unchanged. Over a period of weeks, no change in two electrodes used in analysis has been observed, and hence this added determination may appear to be redundant. However, the extra labor is repaid by increased confidence in the results.

The method as described here was developed for the specific purposes of routine analyses of air samples in greenhouse work. Great accuracy was not essential for this purpose, since 5 per cent error could be tolerated. It is apparent that the method as described can be readily applied to other problems similar to this. If higher accuracy is desired, certain modifications can be made—e. g., increased accuracy of the potentiometer readings, analysis of the sodium bicarbonate solution for bound carbon dioxide content, etc. It might also be desirable to use a silver-silver chloride electrode in 0.1 *N* hydrochloric acid instead of the calomel half-cell. If the electrodes are not required to stand much shock in handling, the glass electrode described by MacInnes and Dole (9) and MacInnes and Belcher (10) might be advantageous. The calibration curve should also be determined by more refined technic, if high accuracy is desired.

Laboratories which do not have the facilities for use of the glass electrode can readily adapt the method for their needs by use of an accurate colorimetric method. With one of the newer types of pH colorimeter, an accuracy of 0.05 pH can be obtained, and this would suffice for most routine work. For example, the $p\text{CO}_2$ in an atmosphere could readily be determined by the insertion of the apparatus (Figure 2) without the glass electrode in the line supplying the gas mixture. Whenever desirable, colorimetric readings could be taken and the $p\text{CO}_2$ read from Figure 3, and in this way it would be possible to maintain a close check on the carbon dioxide content of the atmosphere. The accuracy of the results would not be as high as with the glass electrode, but a high degree of accuracy is usually achieved by the expenditure of considerable time and care. Often for routine work, ease and speed of manipulation are more desirable than the next decimal place.

DISCUSSION

It appeared to be of interest to check the results against those of other investigators who have made use of this principle for the determination of carbon dioxide. Higgins and Marriot (7), who determined the pH colorimetrically, state that they arrived at the carbon dioxide values of the pH standards used by empirical methods. An examination of the values obtained indicate that these are in great error. For example, a plot of their data (assuming a constant barometric pressure of 760 mm.) gives points that lie on a fairly straight line except at the higher concentrations of carbon dioxide. The equation of the line for 0.001 *N* sodium bicarbonate is:

$$\text{pH} = 7.42 - 0.89 \log p\text{CO}_2 \quad (5)$$

Calculations of the value of pK_1' from this line show that this "constant" varies from 5.99 to 6.12. If a barometric pressure of 740 mm. is assumed, these values are lowered slightly. The variation in the value of this constant is due to the slope of the line differing from unity. As already noted, the correct value for the constant at 26° C., according to the careful work of Hastings and Sendroy, is 6.375. Some difference would be expected if less careful technic were used, but the difference noted is larger than one might expect from mere difference in procedure and suggests that the results were subjected to a rather large systematic error. Higgins and Marriot advise use of 0.0085 *N* sodium bicarbonate for concentrations of carbon dioxide ten times as

great as those determined with the 0.001 *N* sodium bicarbonate. It has already been shown that this concentration should be 0.0107 *N*. Calculations of pK_1' for 0.0085 *N* sodium bicarbonate from Higgins and Marriot's data give values ranging from 6.05 to 6.19—i. e., values higher than with 0.001 *N* sodium bicarbonate. Reference to Equation 3 shows that this value should decrease with increasing concentration of the bicarbonate.

McClendon (11) also gives graphs showing plots of the pH of sodium bicarbonate solution of varying concentration in equilibrium with atmospheres containing carbon dioxide. He suggests a method for determining carbon dioxide in air, similar to that of Higgins and Marriot. The slopes of the lines are around 0.8, hence the values of pK_1' calculated from them vary greatly and in all cases are low. McClendon, however, states that this report is preliminary and great accuracy is not claimed for the data.

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A Composite Reagent for Calcium

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IN AN extensive study involving the determination of calcium in bone ash dissolved in dilute hydrochloric acid, a combination of some of the separate solutions used in the precipitation of calcium oxalate was found convenient.

The reagent employed is prepared by dissolving 200 grams of oxalic acid ($\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$) and 500 grams of ammonium chloride in 3500 cc. of water. Then 1000 cc. of glacial acetic acid and 10 cc. of 0.04 per cent methyl red are added, and if any precipitate is formed, the solution is filtered. For the amounts of calcium concerned, 50 cc. of the reagent were measured in a cylinder and added to the properly diluted calcium solution. The mixture was heated to boiling and ammonium hydroxide added to make the reaction slightly alkaline to methyl red.

This composite reagent is satisfactory for the precipitation of calcium oxalate from bone ash solutions. For use in the analysis of other materials, different proportions may be necessary. The combination of the four reagents into one solution is obviously always advantageous because of the consequent saving in time and the uniformity of procedure without loss of precision.

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