A Direct CO₂ Gasometric Apparatus

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ABSTRACT

An apparatus which retains the accuracy and precision of the classical method, yet obviates the disadvantages (time, space, mercury) of the Van Slyke method,¹ is described. The apparatus for the gasometric determination of CO₂ content consists of a sample chamber for a one ml serum sample, a syringe arrangement for injecting lactic acid and collecting CO₂ evolved, and a clamp for joining the two. The chemical procedure used is based on the classical Van Slyke CO₂ content determination. The syringe technique is similar to that of Scholander and Roughton.² A sodium bicarbonate standard is used to calibrate the apparatus to atmospheric conditions. The measurement requires approximately one minute. The reagents appear to be stable indefinitely at room temperature.

The method is linear up to a concentration of 40 mM CO₂ per liter. Day-to-day reproducibility studies (duplicates on each of 20 days) showed a CV of 2.99 percent for elevated standard values (50 mM CO₂ per liter) and 2.9 percent for normal standard values (30 mM CO₂ per liter). Recovery of CO₂ added to serum averaged 99 percent.

Comparison with the classical Van Slyke method gave a slope of 0.92, a Y-intercept of 2.35 mM CO₂ per liter, and a CV between the methods of 8.4 percent. Good agreement was obtained in comparisons with several other methods. It is concluded that this system is acceptable for routine laboratory use.

Introduction

There are many pathological conditions which are accompanied or caused by a disturbance of the electrolyte composition of the body. These changes are generally reflected in the anion-cation pattern on the extracellular fluid. The acid-base shift is of particular interest since it can be a metabolic (a deviation in fixed, non-volatile acid or base content) or respiratory (change in pCO₂) or a combination of both. These disturbances are generally compensated to some extent by renal or respiratory mechanisms. The final pH of the blood is the net effect of these two shifts.

The chemical equilibrium in question is as follows:

\[
\text{H}_2\text{CO}_3 (\text{CO}_2) \rightleftharpoons \text{H}^+ + \text{HCO}_3^- 
\]
In order to analyze the above equilibrium, it is necessary to make two measurements. One method is to measure CO₂ content \( (H_2CO_3(CO_2) + HCO_3^-) \) and pH. A second approach is to measure pH and pCO₂. If two of the three measurements are known, the third value can be calculated using the Henderson-Hasselbalch equation. However, there are several constants in the equation which are doubtful at times and, therefore, all three parameters pH, pCO₂ and HCO₃⁻ should be measured.

It is relatively easy to measure pH and, with the advent of new instrumentation, it has become relatively easy to measure pCO₂. However, the methods for measuring total CO₂ still require equipment that is relatively complex in structure and operation and expensive. In addition, the measurements demand a large amount of the technician's time.

In this paper a newly developed apparatus is described which permits quick, reliable, total CO₂ measurements. The method used is a modification of the classical Van Slyke method for CO₂ content. The apparatus used is similar to that devised and used by Scholander and Roughton.²

**Principle**

The CO₂ apparatus (figure 1) permits the standard and the unknown to be measured under the same conditions of temperature and pressure. The apparatus has a gas-tight syringe in which is enclosed a plunger. The chamber of the syringe is in direct contact with a vial in which the sample and a measured quantity of lactic acid have been mixed. As carbon dioxide is given off from the sample, the plunger of the syringe is displaced and the extent of the displacement is indicated by markings on the graduate syringe. The amount of the displacement is read and from this reading is calculated the CO₂ content.

**Reagents**

The CO₂ reagent set consists of the carbon dioxide liberating solution; lactic acid, 22 percent solution; and sodium bicarbonate standard, 30 mM CO₂ per liter.

The lactic acid acidifies bicarbonate to carbonic acid which breaks down to release CO₂. The concentration was chosen to give a pH low enough to completely liberate all the CO₂ in a one ml serum sample.

The test sample may consist of either whole blood, serum or plasma. Either serum or plasma is recommended because of problems in cleaning the apparatus when whole blood is used. However, if cleaning the equipment is not considered a problem, then whole blood may be used.

**Standard Solution**

The standard consists of a bicarbonate solution of known carbon dioxide content. The standard is used to compensate for fluctuations in atmospheric conditions and temperature.

**Special Apparatus**

The CO₂ apparatus consists of a sample chamber for the one ml serum sample, a
syringe for injecting lactic acid and collecting CO₂ evolved and a clamp for joining the two. Reaction vessels, stoppers, and reagents are needed with the apparatus. In figure 1 is shown the complete apparatus.

Procedure

The procedure for measuring CO₂ content is described. Both the sample and standard are treated in the same manner.

One ml of serum or plasma is pipetted into one of the reaction vessels and the vessel is stoppered. Carbon dioxide liberating solution is drawn into the CO₂ apparatus a few lines above the zero mark. The syringe is inverted and the plunger brought to the zero mark to permit escape of air bubbles. The CO₂ apparatus is assembled and the entire system clamped together. The plunger of the syringe must be braced so that it cannot change position. The plunger is depressed to completely expel the carbon dioxide liberating solution into the reaction vessel.

The sample is mixed by shaking the entire apparatus using a Vortex-Genie* mixer at full speed. The apparatus must be held upright in the mixer until the plunger stops rising (about 15 seconds). From the scale on the syringe, the reading at the bottom of the plunger is recorded and the CO₂ content of the sample calculated.

Calculations

The formula used to calculate the CO₂ content of a serum sample using the CO₂ apparatus is:

\[
\text{CO}_2 \text{ content (mM CO}_2/\text{liter)} = \frac{\text{mEq CO}_2/\text{liter}}{\text{Reading of the Unknown}} \times \frac{\text{Reading of 30 mM Standard}}{30 \text{ mM CO}_2/\text{liter}}
\]

An accurate measurement of carbon dioxide content is obtained by using this formula. The sodium bicarbonate standard provided with the set is used to calibrate the instrument to the varying atmospheric pressure and temperature under which the test is performed.

Discussion

The CO₂ apparatus was evaluated for reproducibility, recovery and linearity. A within day reproducibility study, using a normal serum pool tested 10 times, showed a CV of 1.6 percent. The day-to-day reproducibility used as a test sample a normal standard (30 mM CO₂ per liter) and an elevated standard (50 mM CO₂ per liter). The tests were run in duplicate for twenty days. A CV of 2.9 percent was determined for the normal standard; a CV of 2.99 percent, for the elevated standard. The recovery study used sodium bicarbonate standard solutions added to both an aqueous medium and a serum medium. Recovery of CO₂ was calculated by taking the ratio of the reading for serum medium to the reading for the aqueous medium and multiplying by 100. Recovery was 99 percent. Linearity was observed to 40 mM CO₂ per liter and was 5 percent low at 50 mM CO₂ per liter.

In figures 2 and 3 are data of two comparison studies using the Van Slyke method.

and the AutoAnalyzer SMA® 6/60.* The information in each figure represents test results collected by independent investigators. Additional comparison studies utilizing other instruments were also completed. Data are not shown since good comparison values were obtained between the CO₂ apparatus and the other methods.

Using 93 syringes for a syringe to syringe evaluation, the mean was determined to be 25.1; the standard deviation, 1.0; and the coefficient of variation, 3.8 percent.

Sources of Error

Erroneous readings will be avoided with proper handling of the equipment and reagents. Therefore, by observing some precautions, accurate results should be easily obtained.

Reagents should be stored at room temperature. The syringe should be rinsed and drained with distilled water after each series of tests. The 30 mM standard must be run with each series of CO₂ determinations to compensate for varying atmospheric pressure. However, when running a series of samples over a period of a few hours or less, the content of only one standard need be determined. The plunger must be lubricated by drawing carbon dioxide liberating solution into the syringe several times prior to use. If the plunger does not move freely, results will be erroneous.

The sodium bicarbonate standard must be tightly recapped immediately after use. Care must be taken when assembling the CO₂ apparatus that the clamp used to fit all units together is on all the way to insure proper attachment with maximum clamping pressure. Excessive pressure on the clamp handles during mixing must be avoided, otherwise, the apparatus will come apart. If under normal conditions of temperature and atmospheric pressure, the syringe displacement number for the 30 mM CO₂ per liter standard falls outside the range of 28 to 32 units, the reagents should be replaced with fresh ones.

Normal Range

The normal range for CO₂ content, according to Tietz,² of venous plasma or serum is 23 to 30 mM CO₂ per liter. Since the principle behind releasing the CO₂ from serum or plasma is not being challenged with this apparatus, the normal range of CO₂ level will remain as previously established by Tietz.

Résumé of Clinical Interpretations

The clinical significance of total CO₂ measurement is quite apparent when major vital systems are affected by a deviation in acid-base balance. Therefore, an instrument that is relatively simple to operate, inexpensive in structure and operation and yields a quick, accurate total CO₂ result is preferred. It has been shown that the CO₂ apparatus meets these criteria by combining the chemistry procedure of Van Slyke with a syringe technique similar to that of Scholander and Roughton. In addition, other benefits gained are more laboratory space since the apparatus is compact and more technician time for other clinical work.
since the procedure is fast and requires only one pipetting.

The reagents are stable for at least 16 months at 25°, 37° and 56°. The method is linear up to a concentration of 40 mM CO₂ per liter. Comparison with the classical Van Slyke method gave a slope of 0.92, a Y-intercept of 2.35 mM CO₂ per liter and a CV between methods of 8.4 percent. Recovery of CO₂ added to serum averaged 99 percent. Therefore, it is concluded that the CO₂ apparatus is acceptable for routine laboratory use.

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References