A Rapid Electrochemical Technic for Measuring Carbon Dioxide Content of Blood

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An electrochemical technic for measuring carbon dioxide (CO₂) content in whole blood has been devised and evaluated. The method requires a membrane-covered pH electrode for the CO₂ measurements. This electrode system permits rapid determination of CO₂ content in blood samples of less than 1 ml. The measurement is performed by hemolyzing and acidifying a blood sample in such a manner that the released CO₂ goes into physical solution. The increase of tension caused by this physically dissolved CO₂ is measured by exposing the sample to a previously calibrated electrode. While use of the technic requires some compromise with accuracy (standard deviation of replicate samples = 0.76 volume/100 ml. compared with 0.12 volume/100 ml. for the Van Slyke manometric procedure), its convenience may outweigh this consideration in certain routine applications.

Since 1954, when Stow and coworkers (1, 2) first described their membrane-covered electrochemical cell for measuring CO₂ tension in blood, extensive application has been made of the concept of inserting a permeable barrier between a sample being measured and an electrode system. In such a system, the constituent of interest is permitted to diffuse across the barrier to affect the electrical characteristics of an electrochemical detector cell. A number of commercial devices based on this concept have been made available by various manufacturers, and the measurement of CO₂, as well as of oxygen tension, by this means is a fairly common laboratory procedure.

The present efforts were concerned primarily with devising and evaluating a technic for rapidly performing routine blood CO₂ content

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determinations utilizing an electrochemical CO₂ detector. In the course of our preliminary work, it became apparent that the commercial apparatus available to us required considerably more time and attention for its operation than was felt desirable, primarily because of continued drift and the subsequent need for repeated calibration. Consequently, a part of our efforts were devoted to the design of the electrode and cuvet system used in this work and briefly described below. Additional details of the electrode design considerations can be found in a separate communication (3).

The technic for measuring CO₂ content utilizes 0.75 ml. of blood and involves the conversion of bound gas into physically dissolved gas and the measurement of the developed CO₂ tension electrochemically. Severinghaus (4) described a method similar in concept but did not represent validating data. During the preparation of this communication, a report by Linden et al. (5) came to our attention which utilizes the technic described by Severinghaus to measure CO₂ content of dog blood. This technic will be discussed and compared to the one devised here.

Our results were obtained with human blood and compared with the manometric method of Van Slyke and Neill (6).

Method

Electrode System

In evaluating CO₂ electrode systems, primary consideration should be given to the stability of the uncovered glass electrode by testing the electrode with standard pH buffers. Proper evaluation of the characteristics of the pH electrode itself is significantly related to the success obtained with the covered system for CO₂.

Both saturated calomel and Ag-AgCl reference electrodes have been used and they have worked equally well. Although a calomel reference electrode was used in obtaining the data for this report, we now favor the Ag-AgCl reference electrode because of the convenience of its preparation and use.

Figure 1 is a cross-sectional view of the CO₂ electrode assembly designed for these experiments. A glass electrode is enclosed in a Lucite holder, these two units being housed in a stainless steel sheath. The electrode and holders are placed in a water bath in a slanted position approximately 60° with respect to the horizontal. This angle is necessary to prevent gas trapping in the sample cuvet. This sample cuvet, which has a Plexiglas window, threads onto the steel sheath and permits all the active surface of the pH glass electrode to project into the chamber of the cuvet. The glass, active surface is separated from the
Sample by a Silastic membrane which allows CO₂ and other gases to diffuse to the pH electrode.

In Fig. 1, two O-rings (A, B) hold the membrane in place and also help to position the glass electrode within the Lucite holder. A O-ring (C) at the upper edge of the cuvet chamber seals the chamber when the electrode is inserted. A small piece of 0.005-in. thick Silastic membrane is stretched tightly over the tip of the glass electrode. An electrolyte space is provided on the glass electrode by interposing two layers of surgical cotton gauze. Tris (hydroxymethyl) aminomethane buffer, 0.005 M and pH 7.8, was used as the electrolyte fluid in the glass electrode compartment of the CO₂ detector cell. The buffer system has proved very satisfactory from the standpoint of both sensitivity and stability, but no exact comparison with the usual bicarbonate-potassium chloride electrolyte mixture has been attempted.

The two O-rings shown on the Lucite holder in Fig. 1 act to ensure proper alignment of the electrode within the assembly. A bleeder hole for excess electrolyte and an insertion hole for the calomel reference electrode are also shown.
Carbon Dioxide Technic

Carbon dioxide content in blood is measured by hemolyzing and acidifying a blood sample in the following manner: 10 ml. of 1.0 M NaH₂PO₄, pH 4.3 (referred to as Solution B for convenience), is placed in a 10-ml. glass-tipped syringe, with care being taken to avoid bubbles. This solution also contains a hemolytic agent of approximately 0.6 mg./100 ml. digitonin. The pH of the mixture—Solution B and blood—is approximately 4.5. At this pH, the bicarbonate is converted to carbonic acid to the extent of about 98%, which is in equilibrium with dissolved CO₂. The concentration of the phosphate salt is in excess to ensure a constant pH.

Figure 2 shows a sample being introduced into the Solution B-filled syringe containing an unfolded needle-puncture stopper. The outer cavity of the stopper is also filled with Solution B to prevent air bubbles from getting into the syringe when the blood sample is introduced. Two glass beads of known volume are placed in the syringe to aid in the mixing of sample and acid. Mixing was accomplished by rotating slowly by hand for 1 or 2 min. until hemolysis was complete. After Solution B and the sample are mixed, the sample is ready to be introduced into the 1-ml. sample chamber of the CO₂ cell. Care should be exercised throughout the mixing and transfer operations to ensure that no bubbles are formed or introduced.

The results reported here were obtained with freshly withdrawn whole human blood, both heparin and citrate being used as anticoagulants. All the blood samples, except two which were collected anaerobically and measured without further treatment, were equilibrated at room temperature (23°) with gases of known tensions.

Van Slyke Technic

The results obtained with the electrochemical technic were compared with the Van Slyke and Neill (6) method using 1-ml. blood samples. The computed CO₂ contents of the sodium bicarbonate standards used in the determination of solubility and for standardization purposes (see below) were also checked by direct measurements of the Bunsen solubility coefficient of CO₂ in Solution B by means of the Van Slyke method (7).

Solubility Technic and Electrode Calibration

Determination of the Bunsen solubility coefficient for CO₂ in Solution B was made by acidifying standard sodium bicarbonate solutions and measuring the developed CO₂ tension. This is carried out in the same manner as described for blood. The sodium bicarbonate concentrations used were 9.3, 14.0, and 18.6 mM/L.
In the solubility determinations with the manometric technic, Solution B was equilibrated with known CO₂ tensions in a tonometer system. The sample was then transferred to the Van Slyke apparatus by means of a previously cooled Oswald pipet. Cooling the pipet prevented loss of CO₂ from solution during handling of the samples. The water-jacket chamber of the Van Slyke was connected to the water bath of the tonometer to maintain the same temperature in both units.

**Calculations**

From the measured partial pressure of CO₂ in the Solution B-sample mixture, the volume of CO₂ in the original sample can be computed from Henry’s law if the dilution factor and solubility coefficient are known. The average Bunsen solubility coefficient of CO₂ in Solution B was found to be 62.4% of the comparable water value in a temperature range of approximately 21–27°C. The following relation was used for the content determinations:

\[
\text{Volume/100 ml.} = \alpha \times \frac{P_{\text{eq}}}{760} \times \frac{V + v}{v} \times 100
\]  

(1)

where:

- \(\alpha\) = Bunsen solubility coefficient (ml./ml. = 0.624 \(\alpha\) H₂O)
- \(P_{\text{eq}}\) = CO₂ tension of Solution B sample
- \(V\) = volume of Solution B
- \(v\) = volume of sample

The Bunsen solubility coefficient is calculated from the data developed with the standard bicarbonate solutions in similar fashion:

\[
\alpha = M \text{NaHCO}_3 \times 22.26 \times \frac{760}{P_{\text{eq}}} \times \frac{v}{V + v}
\]

(2)


Results

Electrode Calibration

The electrode can be calibrated with either Solution B saturated with known CO₂ concentrations or with water-saturated gases. Figure 3 shows a typical calibration comparing the two methods of standardizing the electrode. While the results have been consistently similar, most of our calibrations carried out in connection with this work were obtained with gas-saturated solutions. For routine purposes, the use of wet gases is probably more convenient. The per cent CO₂ in the gas mixtures used was determined by the Scholander (8) microtechnic.

Solubility

A total of 134 Van Slyke solubility measurements were made on Solution B between temperatures of 22.2° and 27.2° and compared with 70 electrode determinations made at temperatures ranging from 20.6° to 24.0°.

Because the data for the two methods were not comparable on the basis of temperature and also because of the nonlinear relation of the change in solubility with temperature, the results were normalized by converting each measurement to a percentage of the water solubility.
at an equivalent temperature. The electrode determinations yielded a mean value of 63.4% of the water solubility ($S_M = 2.93$) and the Van Slyke 61.6 ($S_M = 2.27$). Since the group mean values determined by the two methods were statistically indistinguishable and since no other rational basis for selection of one or the other presented itself, the final solubility figure utilized (62.4%) was based on the mean value of the pooled data from both methods. Several additional manometric measurements were made to test whether the addition of blood or sodium bicarbonate to Solution B had any significant influence on the solubility of CO$_2$. No differences were observed.

**Comparative Content Data**

Simultaneous determinations of the CO$_2$ content of blood using both the electrode system and the Van Slyke manometric technic are compared in Fig. 4. The heavy center line is the regression line for the data.
which consist of 82 different paired analyses performed over a period of 11 days on 41 separately treated blood samples. The equation for this line is as follows:

\[
\text{Electrode (volumes/100 ml)} = 0.881 + 0.982 \text{ Van Slyke (volumes/100 ml)}
\]

All the data points fell within a range of \(\pm 3\) volumes/100 ml of the regression line. The standard deviation of replicate samples run with the electrode was 0.76 volumes/100 ml, and with the Van Slyke 0.12 volume/100 ml.

The results with five plasma samples measured in the same manner as whole blood gave an average difference of less than 0.76 volume/100 ml.

Discussion and Conclusions

Analysis of the results obtained from the present study indicate that while the electrode technic for determining CO\(_2\) content of blood is certainly convenient, its application should be attempted only where due allowance is made for the error which can be tolerated. The limiting precision of the CO\(_2\) pressure detector (at a 99% confidence level) cannot realistically be set at better than approximately \(\pm 2\) mm. Hg under the conditions applying to the tests reported here. The limiting absolute accuracy is, of course, related to the accuracy of the Scholander microtechnic, which figure of merit is generally quoted as \(\pm 0.2\) mm. Hg. Since a 1 mm. Hg change in final CO\(_2\) tension is equivalent to approximately 1 volume CO\(_2\) content per 100 ml in the original blood sample at the dilution used in these tests, the range of values obtained in the comparisons with the Van Slyke method (Fig. 4) is quite predictable on the basis of the limiting accuracies of the measuring technics used. The deviation of the least squares regression line shown in Fig. 4 from the origin and the lack of a perfect slope of 1.0 is probably accounted for by small, consistent errors in the Scholander microdeterminations of the calibrating gases, as well as the small non-linearity between the log P\(_{co_2}\) and the cell voltage, particularly at the low end of the scale.

Data presented by Linden et al. (5), while appearing to be more uniform than those presented here, actually involve the use of statistically derived "points" in computing the regression line representing the comparison between the electrode and the Van Slyke manometric technic. The precision of the electrode system used by Linden et al. is stated to be "\(\pm 2.5\)% of the P\(_{co_2}\) for a single determination (p = 0.05)," this being the same order of magnitude generally found with the system used in the present study. It should be noted, however, that Linden et al. calibrated their electrode before and after each experimental
run, whereas our present results were obtained using a single daily calibration as a basis for our experimental computations of the CO₂ content of the blood samples. One might conclude from this either that the more elaborate calibration was unnecessary or that the electrode system used in the present study had greater stability than that used by Linden et al. At any rate, it appears reasonably certain that the practical application of the electrode content technic must involve some degree of compromise with accuracy, as compared with the Van Slyke technic. If the nature of the application is such that the wider confidence limits applying to the electrode technic do not endanger conclusions based on the data, the convenience and timesaving obtained with the electrode method may dictate its selection over alternative methods.

References