Determination of Total CO₂ in Plasma by Automated Flow-Injection Analysis

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We describe a procedure for measuring total CO₂ in plasma, based on the principles of the flow-injection analysis technique, which makes use of unsegmented fast-flowing reagent streams, as developed by Růžička and co-workers. The further methodological design resembles the silicone-rubber membrane technique of Kenny and Cheng. CO₂ in the sample is released by reaction with H₂SO₄. Appropriate amounts of CO₂ permeate through the membrane that separates the acid reagent stream and a buffered cresol-red indicator stream. The experimental set-up and functioning of this system are described.

Additional Keyphrases: silicone-rubber membrane technique • blood gases • intermethod comparison

We were faced with the need to replace the instrument used in our determination of plasma CO₂. Basically, this procedure consisted of a mechanized back-titration to pH 7.4 of acidified, degassed plasma samples. This procedure was slow (20 duplicate estimations per hour) and required continuous attendance of analytical personnel.

Attracted by such qualities as simplicity, speed, and high sampling rates, we decided to apply the innovative features of the flow-injection methodology developed by the group of Růžička (1–4) to the well-established Technicon AutoAnalyzer CO₂-procedure as modified by Kenny and Cheng (5). In the original continuous-flow procedure, the sample is aspirated and mixed with an acid diluent, which is segmented with CO₂-free air. Released CO₂ then enters the air segments, from which aliquots are aspirated and used in turn to segment an alkaline-buffered pH indicator stream, the absorbance of which is continuously measured. In the newer methodology (5), a gas-permeable membrane is fitted into a short dialyzer module, which separates the acidified sample stream from the color-indicator stream. Released CO₂ in the air segments permeates the membrane and produces the anticipated color changes in the indicator stream.

The most important difference of the flow-injection technique is the use of unsegmented continuously flowing reagent streams, transported through tubings with relatively narrow internal diameters (0.5–1.0 mm). After certain time intervals the sample solution is rapidly injected into the carrier stream, either manually through a septum device (1) or by some automatic injection system. The injected samples form discrete zones, which are then transported towards the flow-through detection system. During this transport, the sample zones are mixed with the carrier reagent stream and thus can react with its components. One of the striking advantages of applying this technique to bicarbonate chemistry is that there is no need to maintain a CO₂-free air-segmentation procedure, with its inherent complications. Moreover, the system has a higher capacity than the conventional continuous-flow procedures. Its design shows much in common with the silicone-rubber membrane methodology, as described before (5).

Materials and Methods

Reagents

Only de-ionized water was used. Standard solutions are made up with boiled, CO₂-free water.

Acid diluent, 0.18 mol/L H₂SO₄. Mix 10 mL of concentrated H₂SO₄ with about 900 mL of water and further dilute to 1 L. Add 0.5 mL of Brij-35 surfactant (Merck 10522).

Stock cresol-red solution. Dissolve 1 g of cresol-red (Merck 5225) in 100 mL of 0.1 mol/L NaOH.

Color reagent. Dilute 10 mL of NaHCO₃ (0.2 mol/L), 5 mL of Na₂CO₃ (0.2 mol/L), 4 mL of stock indicator solution, and 0.5 mL of Brij-35 to 1 L. Adjust the pH of this solution with HCl (1 mol/L) to pH 9.2. Fill the flask for this reagent to the top, and during use exclude CO₂-containing air by venting the flask to the atmosphere via a tube containing anhydrous CaCl₂ connected to a short arm extending through the top cap.

Standard solutions. Prepare 10, 20, 30, and 40 mmol/L solutions of anhydrous NaHCO₃ in CO₂-free water.

Procedure

Figure 1 shows the flow diagram for the flow-injection system. Fit a peristaltic pump (Cenco B.V., Breda, The Netherlands) with two Tygon pump tubings with a capacity of 2.9 mL/min for both the acid-reagent and color-indicator streams and one tubing with a capacity of 0.5 mL/min for transport of the sample to the sample injection system. Make all further tubings and reaction coils from coiled polyethylene tubes of 0.5 mm i.d. The dialyzer consists of a 15-cm module, equal to that used for standard continuous-flow purposes, except for the radial depth of the grooves, which are 0.5 mm deep instead of the ordinary 1.0 mm. The dialysis unit is equipped with a non-wettable, gas-permeable membrane of dimethyl silicone-rubber (Corning Scientific Instruments, Medfield, MA). The colorimeter is a Skalar module (Skalar B.V., Delft, The Netherlands) fitted with a Hellma flow-through cuvet (Hellma Benelux, The Hague, The Netherlands) with an internal diameter of 1 mm and a pathlength of 10 mm. S in Figure 1 denotes the point of sample injection, which is done automatically by means of a sampling-loop device, as shown in Figure 2. The hardware of this system is formed by Flarefit modular elements (Flarefit System, Durham Instrument Corp., Sunnyvale, CA). This system consists

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of a Sample Injection Slide Valve 24139, two pneumatic Slide Valve Actuators 24146, and two Solenoid Valves 24099, to trigger the actuators. To minimize dead volume, we use bores of constant 0.8 mm i.d. for all inserts and passages in this system. The system has been electronically incorporated into the time-control unit, which drives the movements of the sample turntable.

As shown in Figure 2a, the sample S is aspirated from the turntable T through the sample loop L. During this sample cycle, the acid-reagent stream bypasses through line B into the analytical system shown in Figure 1. At the moment this cycle has ended, the turntable moves to the flush position and the slide valves switch to the flow pattern position of Figure 2b. The sample zone, which has then completely filled the sample loop L, will be carried along with the reagent stream as a well-defined sample plug into the analytical system. The variability of the hold-up volume of the sample loop enables accommodation of the other variables such as reagent-line length (and associated dilution of the sample), reagent pump-rates, residence time in the dialysis unit, and photometric linearity. With the system described here, the sample loop had a volume of 50 μL.

The specimens were assayed with the following machine settings: sample cycle 15 s, wash cycle 25 s. This gave a capacity of 90 samples per h. When four standards are analyzed after every 16 samples, the average throughput becomes about 70 samples from patients per h.

Results

Analytical Variables

Figure 3 shows a representative recorder output. With the data given in the experimental section, the time required for the ascending part of the recorder peaks amounted to 5 s. The total residence time—from the moment of sample injection (S, Figure 3) till the moment of reaching the detector (and giving maximum recorder output)—of the sample plug was 18 s. W₀.₆, the peak-width duration at 50% of peak height, which according to Ržička and Hansen (4) is a measure of the total dispersion of the sample plug in the reactor system, amounted to 6.0 s. After reaching maximum deflection, the recorder took another 33 s to return to the baseline. This means that with the settings used there was no carry-over from one sample to another.

Within-run precision was assessed by making 15 replicate determinations on two lots of pooled serum with different CO₂ values. The low-value pool (mean CO₂ content, 16.4 mmol/L) showed a coefficient of variation (CV) of 2.5%. For the normal-value pool, the corresponding figures were 23.2 mmol/L and 1.8%.

Day-to-day precision was evaluated by assaying on 15 different days a particular lot of lyophilized control serum (Seronorm, Batch 138; Nyegaard, Oslo, Norway). A mean value of 11.5 mmol/L was found, with a CV of 3.4%.

Linearity. As mentioned in the introduction, the flow-injection technique comprises a whole set of variables, all of which are interdependent. These include the volume of the injected sample, line length, internal diameter of the reagent tubings and reaction coils, and reagent pump rates. The final dilution of the sample with the reagents is a function of each of these variables. In the case of this particular application, the linear working range of the indicator system puts a further restraint on the practical variations of the parameters mentioned. So, in practice one has to choose a combination that suits one's particular analytical needs. With the data given in the experimental section, the standard curve for the system was linear to 50 mmol/L.

Correlation

Because with this method all forms of carbon dioxide are converted into CO₂ gas and thus are all included in the determination, we decided to compare this proposed method with the Harleco CO₂ apparatus (Harleco, Philadelphia, PA). For 75 determinations on serum specimens with CO₂ concentrations ranging from 7 to 32 mmol/L, statistical evaluation showed a correlation coefficient of r = 0.981, with y (flow-injection) = 0.957x (Harleco) + 1.05. In a similar comparison study by Hicks et al. (6), the Beckman CI/CO₂ analyzer was compared with the Natelson microgasometer. They obtained
a very similar relation of $y$ (Beckman microgasometer) = 0.967$x$ (Natelson microgasometer) + 0.8. In the Beckman technique liberated CO$_2$ passes through a silicone-rubber membrane and affects the pH of a bicarbonate solution, which is monitored with a pH electrode. This resembles the flow-injection approach described here. In contrast, both the Harleco apparatus and the Natelson microgasometer essentially are based on a volumetric CO$_2$-displacement procedure. Thus the agreement between these studies, in our view, demonstrates the usefulness of our procedure.

Interferences

Increasing amounts of bilirubin were added to a pooled serum specimen to give values ranging from 50 to 400 μmol/L. No measurable effect was noticed. Likewise, serum was supplemented with increasing amounts of a washed, hemolyzed erythrocyte concentrate to give hemoglobin values of 0.06 to 0.48 mmol/L (0.96−7.68 g/L). Again, no interfering effect was noticed.

We studied the influence of protein content by mixing a particular serum pool with increasing amounts of a primary bicarbonate standard that was concentration-matched with the serum CO$_2$ concentration. Thus the protein content was altered, while the CO$_2$ value was kept constant. The results showed no systematic change, and the bias remained within the range of the within-day precision established earlier.

Discussion

Although the most striking difference with conventional continuous-flow analysis seems to be the absence of air segmentation, Růžička and Hansen (4) point out that the flow-injection technique is based on a combination of three fundamental principles: (a) sample injection rather than continuous aspiration of sample into a carrier stream, (b) reproducible residence time of the sample on its way from injection point to detection system, in order to provide (c) a controlled dispersion with associated mixing of the sample along the analytical line, rather than a dispersion limited as far as possible as in classical continuous-flow analysis.

A further advantage over the conventional procedure is that the flow-injection technique generally allows a much higher sampling frequency; with a suitable design of the manifold, virtually no carry-over or sample interaction can be obtained because the signal still reaches the baseline after every sample. This is another difference with the classical methodology, the abandonment of the “steady-state signal” concept.

The results confirm the usefulness of the method presented. The methodological design excels in simplicity, which makes the method easily accessible. Apart from the modules used to make the automated injection device, the method requires only extremely simple components. All features guarantee the stability of the analytical response. Finally, the correlation studies reveal that one can confidently change from the conventional continuous-flow technique to the new concept of unsegmented flowing reagent streams, combined with flow-injection.

References