Rapid, Automated, Simultaneous Determination of Serum CO₂ and Chloride with the "AutoAnalyzer I"

Margaret A. Kenny and Mary H. Cheng

A method for simultaneous, automated determination of serum CO₂ and chloride is presented, in which the dialyzers of the more expensive AutoAnalyzer II system are combined with the usual components of the AutoAnalyzer I system. Thus, the innovative features of the AutoAnalyzer II system are used at low cost.

The "AutoAnalyzer 12/60" system (Technicon Corp., Tarrytown, N. Y. 10591) (1) incorporated new design features to improve performance of some continuous-flow assay techniques. Marketed later, the "AutoAnalyzer II" system (Technicon) used components and methodologies of the 12/60 system that permitted the automation of fewer than 12 different analyses, either singly or simultaneously. Innovative features that particularly appealed to us were: (a) silicone-rubber membranes are used for the CO₂ determination; (b) the miniature dialyzers are conveniently located, easy to service, and bubble patterns through them are completely visible at all times; and (c) the "air bar" device introduces air bubbles in a precise amount and sequence. As a result of these changes, gaseous CO₂, liberated by acid from serum, may be dialyzed directly into a buffered cresol red solution through the silicone-rubber membrane.

We present here a similar method for measuring serum CO₂ and chloride with use of the older AutoAnalyzer I components, to which the dialyzers are added. To set up this system, one need only purchase one 15.2-cm (6-inch) and one 30.4-cm (12-inch) dialyzer—a total additional cost of only $268 (Evergreen Scientific Co., Los Angeles, Calif. 90011). Although we are using a Pump III with the "air bar" device, it is not essential and one can use Pumps I or II instead. Only 0.2 ml of serum is necessary for simultaneous determination of CO₂ and chloride. An analysis is completed in only 6 min after sample pick-up, 60% less time than required by the conventional AutoAnalyzer I system.

In addition to easier maintenance, simpler reagents, and faster measurement than with the conventional AutoAnalyzer I system, this method permits the rapid, accurate, and precise microscale determination to be made. It is well suited for pediatric, routine, or emergency laboratory use.

Materials and Methods

Equipment

The AutoAnalyzer modules used are the Sampler II; Pump I (or II); Miniature Dialyzer,² 15.2-cm (6-inch) path, with silicone-rubber dialysis membrane² for CO₂; Miniature Dialyzer,² 30.4-cm (12-inch) path, with type C membrane for chloride; Colorimeters, with 430-nm and 480-nm filters for CO₂ and chloride, respectively; and the Double-Pen Recorder.

Reagents

Only de-ionized water was used to prepare reagent and standard solutions.

\[ \text{CO}_2 \text{ determination.} \]

\[ \text{Cresol red stock solution, 0.025 mol/liter:} \]
\[ \text{Dissolve 1.0 g of the sodium salt of cresol red in 100 ml of water.} \]

\[ \text{Tris buffer, 0.9 mol/liter, pH 10.0: Mix 900 ml of tris(hydroxymethyl)aminomethane (Tris,} \]
\[ \text{1 mol/liter) with 100 ml of ammonium hydroxide} \]
\[ \text{(1 mol/liter). Store at 4°C.} \]

\[ \text{CO}_2 \text{ color reagent: Dilute 1.8 ml of stock} \]
\[ \text{cresol red solution, 0.8 ml of stock Tris buffer, 10} \]
\[ \text{ml of ammonium hydroxide (30 mmol/liter), and} \]
\[ \text{0.2 ml of "Brij-35" (30 ml diluted to 100 ml} \]
\[ \text{Technicon) to 1 liter with water. Adjust the pH to} \]
\[ \text{9.2 with HCl, 1 mol/liter.} \]

\[ \text{Chloride determination.} \]

\[ \text{Satulated mercuric thiocyanate solution:} \]
\[ \text{Dissolve 2 to 3 g of mercuric thiocyanate in 1 liter} \]
\[ \text{of water, and heat to boiling while stirring.} \]

\[ \text{Ferric nitrate solution, 0.5 mol/liter:} \]

² Component of SMA 12/60 or AutoAnalyzer II.
Dissolve 202 g of ferric nitrate \([\text{Fe(NO}_3\text{)}_3 \cdot 9\text{H}_2\text{O}]\) in 22.2 ml of concentrated nitric acid and dilute to 1 liter with water.

**Mercuric nitrate solution**: Dissolve 6.85 g of mercuric nitrate in 0.9 ml of concentrated nitric acid in a 100-ml volumetric flask and dilute to volume with water.

**Color reagent**: Mix 500 ml of mercuric thiocyanate solution with 100 ml of ferric nitrate solution and filter. Before use, add 2.5 ml of the mercuric nitrate solution and 0.2 ml of 30% Brij-35 to the filtrate.

**Reference standards**.

**Stock standard solutions**: Anhydrous sodium carbonate, 1.0 mol/liter; sodium chloride, 1.0 mol/liter.

**Working standard solutions**: The stock standard solutions are diluted to give five working standards containing the following respective millimolar concentrations of carbonate and chloride: (a) 40 and 130 (b), 30 and 120, (c) 20 and 110, (d) 10 and 100, and (e) 5 and 90.

**Procedure**

The flow diagram for the automated method is shown in Figure 1. Serum is aspirated at a sampling rate of 50 ml/h, with a 2:1 water-wash ratio, and split into two streams.

**CO₂ determination**. The CO₂ sample stream is mixed with an acid diluent (H₂SO₄, 0.25 mol/liter), which liberates all CO₂ from the sample into the CO₂-free air segment. The stream then enters a 6-inch dialyzer containing a silicone-rubber gas-dialysis membrane, where the CO₂ diffuses through the membrane and is absorbed by the alkaline solution. The resulting decrease in pH causes a corresponding decrease in color intensity of the indicator solution. Absorbance of the indicator is measured at 430 nm in a 15-mm flowcell.

The CO₂ color reagent can be protected from prolonged exposure to the CO₂ in air by filling the container to the top to exclude any air during storage. The bottle used to hold the reagent during analysis is closed with a two-hole rubber stopper bearing a long arm, which delivers the reagent to the manifold. A short arm, extending through the cap and above the reagent level, is connected to a tube containing "Mallcosorb" (Mallinckrodt Chemical Works, St. Louis, Mo. 63160), a CO₂ absorbent with indicator, bringing CO₂-free air into the bottle as liquid is displaced. The air stream used in the CO₂ method is also freed of CO₂ by passing it through a tube containing CO₂ absorbent.

Thus protected, the working CO₂ color solution is most stable. However, significant color intensity and hue variation was observed in red sodium salt purchased from different manufacturers, and also between different lots of the chemical from a single source. Salt that is too impure or several months old requires a different Tris buffer concentration and should not be used. Other minor variations in color intensity between stock cresol red preparations are correctable by use of different colorimeter apertures; more severe ones require an increased volume of buffer.

**Chloride determination**. The chloride sample stream is mixed with an acid diluent (H₂SO₄, 0.125 mol/liter) and dialyzed in a 12-inch dialyzer directly into the color reagent. Note in Figure 1 that the chloride color reagent enters the dialyzer in the lower or bottom stream. The analysis is less sensitive if the sample is introduced in the lower chamber. The change in transmittance between 90 mmol/liter and 130 mmol/liter standards was not the expected 40% increment, but only 32%, although the curve was still linear.

Mercuric nitrate solution is added to chloride color reagent to suppress the first 60 mmol/liter of chloride in the sample, to bring the range of most clinical samples (90–130 mmol/liter) into the most sensitive part of the recorder scale. Absorbance of the color complex in the indicator stream is measured at 480 nm in a 15-mm flowcell. A "0.5" aperture is used in the colorimeter.

**Results and Discussion**

Values obtained for CO₂ and chloride by the described method were simultaneously compared to those obtained by use of the SMA 12/30, for fresh serum.¹ The data (Table 1) for 80 sera illustrate the good agreement in results so obtained. Statistical analysis of the data by use of the paired t test indicate no significant difference between results obtained by the two procedures. Precision of the micro-scale method was ex-

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¹ The CO₂ method on the SMA 12/30 also used cresol red as an indicator.
Table 1. Comparison of Chloride and CO₂ Values Obtained by SMA 12/30 and the Present Method on 80 Sera

<table>
<thead>
<tr>
<th>Differences</th>
<th>Mean (mmol/liter)</th>
<th>SD</th>
<th>t-value</th>
<th>Limits</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride</td>
<td>0.025</td>
<td>1.59</td>
<td>0.14</td>
<td>0.8 &lt; P &lt; 0.9</td>
<td></td>
</tr>
<tr>
<td>CO₂</td>
<td>1.037</td>
<td>1.11</td>
<td>0.30</td>
<td>0.7 &lt; P &lt; 0.8</td>
<td></td>
</tr>
</tbody>
</table>

* Range: chloride, 85-111 mmol/liter; CO₂, 19-34 mmol/liter.

Table 2. Precision of Micro-Scale vs. SMA 12/30 Chloride and CO₂ Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>( \bar{x} )</th>
<th>SD</th>
<th>CV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride, SMA 12/30</td>
<td>107</td>
<td>1.0</td>
<td>0.9</td>
<td>50</td>
</tr>
<tr>
<td>Chloride, this method</td>
<td>106</td>
<td>0.8</td>
<td>0.9</td>
<td>120</td>
</tr>
<tr>
<td>CO₂, SMA 12/30</td>
<td>11.6</td>
<td>1.0</td>
<td>8.2</td>
<td>50</td>
</tr>
<tr>
<td>CO₂, this method</td>
<td>28</td>
<td>0.8</td>
<td>2.9</td>
<td>120</td>
</tr>
</tbody>
</table>


Examinined by assaying control sera daily for one month in the emergency laboratory. Table 2 compares the results with daily assays of a control serum evaluated for two weeks on a Technicon 12/30. Standard deviations for results of the micro-scale CO₂ and chloride determinations were 0.8 and 0.8 mmol/liter respectively. Relative standard deviations (coefficients of variation) for CO₂ and chloride were 0.9% and 2.9%, respectively. In general, the respective data from the two instruments correlated excellently for both determinations.

The sensitivity of the chloride assay has been discussed previously. The CO₂ assay is as sensitive as the the 12/30 methodology from which it is derived. The 60% difference in transmittance observed between 5 and 40 mmol/liter is significantly more sensitive than the corresponding 7% change observed when the 12/60 chemistry was applied on the AutoAnalyzer I without modification. Results for both analyses obey Beer's law over the range of standards used, except that occasionally the highest chloride standard deviates from linearity with different color-reagent preparations.

This method for the simultaneous determination of CO₂ and chloride has been in satisfactory operation in our emergency laboratory for five months. We were thus able to refine existing methods at an additional equipment cost of less than $300.

Reference