Improved gasometric methods for estimating oxygen content and percentage saturation in haemoglobin dissociation curve determinations

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By using carbon monoxide as a reagent for releasing oxygen from blood solutions (instead of the customary potassium ferricyanide) and by combining the techniques of the Van Slyke constant-volume gasometric apparatus and the Scholander gas analysis apparatus, a precise method has been devised of estimating oxygen content and carbon monoxide capacity simultaneously on one and the same sample of blood solution. The procedure eliminates various errors inherent in the determination of oxygen percentage saturation, when oxygen content and capacity are successively measured on separate samples.

The method has been extensively tested on sheep blood solutions containing 3 to 4 g haemoglobin/100 ml. and is considered to give an accuracy of ±0.5 in the oxygen percentage saturation of such solutions. In its present form the method is only applicable to solutions containing a negligible amount of dissolved nitrogen, but it is probable that this restriction could be removed by the simple addition to the technique suggested in the text.

Modifications are also described in the usual tonometer technique for equilibrating blood solutions with various pressures of oxygen, and for transferring the equilibrated blood solution to the Van Slyke apparatus for analysis.

The techniques of the present paper have been specially used for obtaining oxyhaemoglobin dissociation curve data of higher accuracy than hitherto available. From these improved data it has for the first time been possible to calculate directly, and in many cases with fair precision, the values of the equilibrium constants of the four successive reactions of haemoglobin with oxygen.

INTRODUCTION

In most work on the oxyhaemoglobin dissociation curve it has been customary to obtain points within the main range (i.e. 5 to 95% saturation) to an accuracy of 1 to 2% saturation. In a series of recent papers Roughton and his colleagues (Paul & Roughton 1951; Roughton 1954; Roughton, Otis & Lyster 1955) stress the need to improve this accuracy several-fold and also to obtain specially precise determinations at the two extreme ranges of the curve, if the intermediate compound hypothesis is to be incisively tested. An appropriate technique for the upper range (98 to 100% saturation) has recently been worked out by Roughton (1954); for the lower range (0 to 2% saturation) the method of Paul & Roughton (1951), as amended recently by Roughton et al. (1955), is suitable. In the present paper we describe a gasometric method for the simultaneous determination, in one and the same sample of sheep haemoglobin solution, of the oxygen content and the gas-combining capacity. The method, which was especially designed for our dissociation curve work, but which has also other possible applications, is based on the fact that when vertebrate haemoglobin is shaken with carbon monoxide gas at sufficient partial pressure, the latter combines almost completely

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with the former, replacing all but a minute, but accurately calculable, proportion of the oxygen initially combined with the haemoglobin. From a knowledge of the amount of carbon monoxide initially present in the gas phase and from the measurement of the volume and composition of the final gas phase, the volumes of carbon monoxide taken up and of oxygen given off by the blood solution can then be obtained. From these figures, the oxygen percentage saturation of the sample is readily calculated, provided that allowances are made for the volumes of the gases in physical solution. No correction is included for the incomplete extraction of the oxygen, since this is compensated by incomplete absorption of carbon monoxide, with the result that the calculated percentage saturation with oxygen is at most only 0·04 % too low (see worked example later). Allowances should, however, be made if exact figures are needed either for oxygen content or for carbon monoxide capacity separately. It should be emphasized that the technique, in its present form, is only applicable to blood solutions in which the content of dissolved nitrogen is negligible. This condition has been met in the dissociation curve work to which the method has so far been applied, for the gas phases in the tonometers in which the blood solutions were equilibrated contained no significant amount of dissolved nitrogen. Modifications would be required if the nitrogen content of the blood solution is appreciable.

Although carbon monoxide has been and is frequently employed in standard methods for determining the gas-combining capacity of blood, its use as a reagent for releasing oxygen has not, to our knowledge, been practised in recent years. It is of historical interest, however, to note that some of its advantages for this purpose were pointed out by Claude Bernard (1859) nearly a century ago.

Experimental details

The simultaneous gasometric determination of O₂ content and CO capacity of sheep haemoglobin solutions (3 to 4 g Hb/100 ml.) will first be described; details will then be given of the equilibration of such haemoglobin solutions for dissociation curve determination, prior to the transfer of samples of the equilibrated haemoglobin solutions to the Van Slyke apparatus for the subsequent gasometric determination.

Apparatus and reagents required

1. Van Slyke ‘constant volume’ manometric apparatus with chamber having marks at 0·5, 2, 10 and 50 ml.
3. A modified Hempel pipette (Peters & Van Slyke 1932, p. 109; see also Van Slyke & Hiller 1928) containing CO over Krogh’s solution, an oxygen absorbing reagent, having the following composition:

\[
\begin{align*}
\text{Na}_2\text{S}_2\text{O}_4 & \quad 8 \text{ g} \\
\text{KOH} & \quad 7 \text{ g} \\
\text{Na-anthraquinone-2-sulphonate} & \quad 1.5 \text{ g} \\
\text{H}_2\text{O} & \quad 50 \text{ g}
\end{align*}
\]
Glass U-tube (1.5 mm internal diameter) with rubber tips for transfer of gas from the Van Slyke apparatus to the Scholander apparatus.

Gas-free $\text{NaOH}$. 

Acid rinse for Scholander apparatus (Scholander 1947).

Oxygen absorber for Scholander apparatus. This reagent is prepared in the manner described by Scholander (1947) and is then saturated with CO and stored in a glass syringe in contact with a bubble of CO.

**Procedure with Van Slyke and Scholander apparatus**

1. The Van Slyke chamber is first freed completely of $O_2$ by repeated evacuation and shaking within it of 10 ml. of water plus a few drops of octyl alcohol.

2. The water is ejected and exactly 10 ml. of the blood solution, whose $O_2$ content is required, are then drawn from the tonometer into the Van Slyke chamber, use being made of the 10 ml. mark on the latter.

3. Approximately 1.5 ml. of CO are then slowly and smoothly transferred from the modified Hempel pipette to the Van Slyke chamber, in the manner described by Van Slyke & Hiller (1928). During this process the gas/blood interface in the chamber is disturbed as little as possible, so as to avoid absorption of CO by the solution (see later). Both stopcocks of the Van Slyke apparatus are now closed and the Hempel pipette is removed and sealed with mercury. The Van Slyke levelling bulb is lowered and by operation of the lower stopcock the blood meniscus is brought gently down to the narrow segment of the chamber below the 2 ml. mark. By careful manipulation of the upper stopcock the CO contained in its bore is displaced by mercury from the cup. The blood meniscus is then brought exactly to the 2 ml. mark and the pressure, $P_1$, is read on the manometer.

4. The mercury is brought to the 50 ml. mark, the chamber is covered with opaque paper and the apparatus is shaken for 15 min at a speed of about 200 per min.

5. During the shaking period the Scholander apparatus is prepared for use and left with the mercury adjusted to the top of the capillary and the compensating chamber half full of acid rinsing fluid.

6. At the end of the shaking period the opaque paper is removed, the gas pressure in the chamber is raised to about 150 mm Hg.

7. The $CO_2$ in the chamber is then absorbed in the usual way with 1 ml. of deaerated $\text{NaOH}$. The pressure at the 2 ml. mark, $P_2$, is read.

8. With about 5 ml. of mercury covered with a little distilled water in the Van Slyke cup, the U-tube is seated in the cup and secured in place by a rubber band, as indicated in figure 1. At this stage the other end of the tube is not seated in the Scholander apparatus, but is merely immersed in the acid rinsing solution contained in the upper bulb of the Scholander apparatus.

9. The Van Slyke levelling bulb is hung at the upper level and the gas in the chamber is compressed by temporarily opening the main stopcock. The upper stopcock is then cautiously opened and the compressed gas is allowed to expand into the U-tube pushing the mercury and a small amount of water along ahead of it.

10. The Van Slyke levelling bulb is placed at such a level as has been previously
determined to produce a pressure in the chamber a few mm of mercury above atmospheric. The U-tube is held by the right hand in readiness to seat it in the Scholander cup, and the main cock of the Van Slyke apparatus is slowly opened with the left hand. When all liquid has been cleared from the U-tube and gas begins to bubble from its tip the latter is seated and held in place with the right hand.

(11) The duty of holding the U-tube in place is now transferred to the left hand and gas is introduced into the Scholander apparatus by manipulation of the micrometer screw with the right hand, a zero setting being made in the manner described by Scholander (1947). During the transfer the pressure must be kept within a few mm of mercury of atmospheric. This is facilitated by resting the Van Slyke levelling bulb in a ring, the level of which is easily adjustable by a worm gear, and crank or similar arrangement.

(12) When the desired amount of gas has been removed to the Scholander apparatus (20 units on the micrometer is customarily introduced), the U-tube is removed and any residual gas is expelled from the Van Slyke chamber without loss of fluid. The blood meniscus is again brought exactly to the 2 ml. mark and the pressure reading, \( P_3 \), is then taken.

(13) Excess fluid is removed from the compensating chamber of the Scholander apparatus, the stopcock closing the compensating chamber put in place and the reading of the initial volume \( V_1 \) is taken. \( \text{O}_2 \) absorber is introduced from the side arm and the apparatus is shaken until absorption is complete. The final volume reading \( V_2 \) is taken.

**Calculations**

I. \( \text{O}_2 \) content:

\[
\frac{V_1 - V_2}{V_1} \times (P_2 - P_3) \times F = \text{O}_2 \text{ content of sample in volumes } \% \text{ s.t.p.,}
\]

where \( F \) is the Van Slyke factor for the particular conditions.
II. Dissolved $O_2$:

\[
\frac{P_{O_2}}{760} \times a_{O_2} \times 100 = \text{dissolved } O_2 \text{ in sample in volumes } \% \text{ s.t.p.}
\]

where $P_{O_2}$ = partial pressure of $O_2$ with which the sample was equilibrated before removal from tonometer to Van Slyke apparatus.

$a_{O_2} = \text{solubility coefficient for } O_2 \text{ in the blood solution at the temperature of equilibration.}$

III. Combined $O_2$:

I-II = $O_2$ in combination with haemoglobin in the sample in volumes $\%$.

IV. Total CO taken up by sample:

\[
\left[(P_1 - P_3 - c) - \frac{V_2}{V_1} \times (P_2 - P_3)\right] \times \frac{2}{760} \times \frac{273}{T} \times \frac{100}{V_s} \times (1 - 0.000173t)
\]

= total CO taken up by sample in volumes $\%$ s.t.p.

where $c$ is the correction factor for the addition of 1 ml. of n-NaOH, as determined by a blank analysis.

$T = \text{temperature of the Van Slyke chamber in degrees absolute,}$

$V_s = \text{volume of blood sample,}$

$t^\circ C = T - 273,$

$1 - 0.000173t = \text{correction factor for unequal expansion of glass and mercury (Peters & Van Slyke, p. 282).}$

V. CO taken up by physical solution:

\[
100a_{CO} \times \frac{P_2 - P_3}{760} \times \frac{V_2}{V_1} \times \frac{2}{50 - V_s} = \text{CO dissolved in sample in volumes } \%
\]

where $a_{CO} = \text{solubility coefficient of the blood solution for carbon monoxide at the temperature of the analysis.}$

VI. CO-combining capacity:

IV-V = combining capacity of the sample for CO in volumes s.t.p.

This calculation assumes that the CO content of the sample before introduction into the Van Slyke apparatus is negligible. If such is not the case the CO content must be independently measured by the method of Roughton & Root (1945) and added to the expression IV-V.

VII. Percentage saturation:

\[
\frac{\text{III}}{\text{VI}} \times 100 = \text{percentage saturation of the sample.}
\]
Note on the magnitude of errors due to incomplete extraction of oxygen and incomplete absorption of carbon monoxide

(a) For a blood solution 99% saturated with oxygen.

Suppose that:

(i) The true gas combining capacity of a 3·3% haemoglobin solution is 0·044 ml. of O₂ (or CO) per ml. solution.
(ii) The haemoglobin solution also contains initially 0·006 ml. dissolved O₂ per ml. solution.
(iii) The volume of carbon monoxide introduced into the Van Slyke chamber initially is 1·5 ml.
(iv) The volume of haemoglobin solution in the chamber is 10 ml.

Then, at the end of the shaking period

\[ \frac{\text{p}_\text{CO}}{\text{p}_\text{O}_2} \] 

is approximately

\[ \frac{1·5 - (10 \times 0·044)}{10 \times (0·044 + 0·006)} = 2·12. \]

In a haemoglobin solution, in equilibrium with mixtures of O₂ and CO at such pressures that the amount of reduced haemoglobin is negligible (i.e. below 0·1%) the proportion of CO\(\text{Hb}\) to O\(\text{2Hb}\) is given by the equation

\[ \frac{[\text{COHb}]}{[\text{O}_2\text{Hb}]} = M \frac{\text{p}_\text{CO}}{\text{p}_\text{O}_2}. \] 

In the sheep haemoglobin solutions currently used in the present dissociation curve work \(M\) averages about 240. The ratio of \([\text{COHb}]\) to \([\text{O}_2\text{Hb}]\) at the end of the shaking period in the present case is thus approximately 240 \(\times\) 2·12, i.e. 509. The haemoglobin solution at the end of the shaking period is thus 99·8% saturated with CO, and 0·2% saturated with O₂, and the CO capacity and O₂ content are thus each about 1 part in 500 too low.

The calculated value of percentage saturation, however, is far less in error, being, in fact, equal to

\[ 100 \times \frac{99 - 0·2}{99·8}, \text{ i.e. 98·997%}, \]

as compared with the true value, assumed in this case, of 99%, i.e. a quite negligible discrepancy.

(b) For a solution with a true O₂ percentage saturation of 50, similar calculation show that the end of the shaking period the haemoglobin solution is 99·92 saturated with CO and 0·08% saturated with O₂. The absolute errors both in the CO capacity and O₂ content are thus only about one-half those in the previous instance, but the error in the calculated value of this percentage saturation, i.e.

\[ 50 - 100 \times \frac{50 - 0·08}{99·92} \text{ or } 0·04\%, \]

is larger, but still is insignificant in comparison with the experimental error due to other causes (+ 0·5% saturation).
For the general case of 10 ml. of a $y'$ percentage saturated solution, of gas-combining capacity equal to $\phi$ ml. $O_2$ per ml. of solution, the error in $y'$ due to neglect of the unextracted $O_2$, can be shown to be

$$\frac{y'\phi(100-y')}{10M(\theta-10\phi)},$$

where $\theta$ is the volume of CO gas in ml. introduced into the Van Slyke chamber in stage 3 of the procedure. It is easy to show that with given values of $M$, $\phi$ and $\theta$ the error in $y'$ is at a maximum when $y' = 50\%$.

**Equilibration procedure for middle range of oxyhaemoglobin dissociation curve**

The general procedure for obtaining points in the middle region of the dissociation curve was as follows: A portion of the stock blood solution (usually sheep blood solution containing 3 to 4 g Hb per 100 ml., pH 7.1 to 9.1) was given a preliminary equilibration with air, and accurately measured volumes of this aerated solution were introduced from a weighing tube into tonometers containing known amounts of $O_2$ so calculated as to give the desired partial pressure of $O_2$ on final equilibration. The equilibrated samples were finally analyzed for $O_2$ content and combining capacity in the manner described above. The aerated blood solution was similarly analyzed.

**Preparation of tonometers for final equilibration**

The tonometers used for the final equilibration of the blood solution had accurately known capacities of approximately 200, 600 or 800 ml. They were similar in design to those described by Hecht & Morgan (see Barcroft 1928), except that the main body of those of the largest size was cylindrical instead of spherical. Each tonometer to be used was first prepared by thorough evacuation on a Cenco Hyvace pump, according generally to the procedure of Paul & Roughton (1951).

The charging of an evacuated tonometer with $O_2$ was accomplished as follows. The approximate amount of $O_2$ (or equivalent amount of air) desired was introduced into the chamber of the Van Slyke monometric apparatus, the upper stopcock sealed with mercury, and the manometer reading ($P_1, v$) taken with the gas at one of the calibrated volumes ($v$) of the chamber. The temperature was also recorded. The stem of the tonometer to be charged with gas was fitted with a rubber tip, by means of which it could be snugly seated in the bottom of the Van Slyke cup (see Roughton 1954, figure 1B). After all the air in the stem had been displaced with mercury, the stem was firmly seated in the cup and the whole of the gas in the Van Slyke chamber transferred to the tonometer, without any admixture of outside air. A final reading ($P_2, v$) was taken with the mercury in the Van Slyke chamber at the same volume as for the initial reading. From the two Van Slyke readings the exact volume of gas introduced into the tonometer was calculated at standard temperature and pressure.

**Equilibration of blood solution in tonometer**

Usually about 20 ml. of the aerated blood solution were introduced from the weighing tube into each tonometer, the actual amounts being weighed to 0.01 g.
The tonometer was then rotated for 1 h in a water-bath regulated at the desired temperature. At the end of this equilibration the blood solution was transferred from the tonometer to the Van Slyke apparatus as described above (see procedure with Van Slyke and Scholander apparatuses) and its O₂ content and CO capacity measured.

*Calculation of the partial pressure of oxygen with which blood sample was finally equilibrated*

This is given by the formula

$$p_0 = \frac{V_0 + V'O_2}{V_f - V_b} \times \frac{760}{273} \times T = \text{partial pressure of oxygen in tonometer at equilibrium,}$$

where $T = \text{temperature of equilibration (in degrees absolute),}$

$V_f = \text{total volume of tonometer,}$

$V_b = \text{volume of blood solution in tonometer}$

$= \text{weight of blood solution} \times \text{specific volume,}$

$V_0 = \text{volume of O}_2 \text{ s.t.p. introduced initially into the tonometer,}$

$V'O_2 = \text{volume of O}_2 \text{ s.t.p. released from blood during equilibration}$

$= \text{total O}_2 \text{ content of aerated blood initially introduced into tonometer minus total O}_2 \text{ content of final equilibrated solution.}$

Supplementary details of the experimental procedure are given in an Appendix to this paper, which has been deposited in the Archives of the Royal Society. These details may be of help to workers who are not well-versed in gasometric procedure.

**Tests and accuracy of the method**

*Comparison with standard methods*

A. Oxygen content of blood solutions

The method has been checked against the standard Van Slyke procedure on samples of blood solution containing 3 to 4 g Hb/100 ml. and of varying percentage saturation, i.e. 10 to 90%. The samples contained no dissolved N₂. The results are

<table>
<thead>
<tr>
<th>O₂ content (volumes %)</th>
<th>discrepancy in volumes %</th>
<th>in % saturation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>standard method</td>
<td>present method</td>
<td>in % saturation*</td>
</tr>
<tr>
<td>------------------------</td>
<td>--------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>0-307</td>
<td>0-317</td>
<td>+0-010</td>
</tr>
<tr>
<td>0-398</td>
<td>0-409</td>
<td>+0-011</td>
</tr>
<tr>
<td>1-457</td>
<td>1-450</td>
<td>-0-007</td>
</tr>
<tr>
<td>2-215</td>
<td>2-229</td>
<td>+0-014</td>
</tr>
<tr>
<td>3-415</td>
<td>3-370</td>
<td>-0-045</td>
</tr>
<tr>
<td>4-034</td>
<td>4-025</td>
<td>-0-009</td>
</tr>
<tr>
<td>4-230</td>
<td>4-230</td>
<td>±0-000</td>
</tr>
</tbody>
</table>

average discrepancy (regarding signs) | -0-004 | -0-10 |
average discrepancy (disregarding signs) | ±0-014 | 0-35 |

* Assuming an average figure for the gas combining capacity of 4 volumes %.
shown in Table 1. No systematic difference between the two methods is indicated, the agreement being generally excellent. The greatest deviation between any pair of analyses is only 0.045 volume %, i.e. ca. 1.1% saturation, and even this discrepancy is rather an isolated one, being 3 or more times greater than that seen in any of the other pairs of analyses in Table 1.

Table 2. Comparison of results of analyses of blood solutions for CO capacity by standard method and new method

<table>
<thead>
<tr>
<th>Carbon monoxide capacities (volumes %)</th>
<th>Standard method</th>
<th>Present method</th>
<th>Discrepancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.97</td>
<td>3.92</td>
<td>-0.05</td>
<td></td>
</tr>
<tr>
<td>4.00</td>
<td>4.02</td>
<td>+0.02</td>
<td></td>
</tr>
<tr>
<td>3.97</td>
<td>3.95</td>
<td>-0.02</td>
<td></td>
</tr>
<tr>
<td>4.00</td>
<td>3.97</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td>4.40</td>
<td>4.37</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td>4.36</td>
<td>4.39</td>
<td>+0.03</td>
<td></td>
</tr>
<tr>
<td>Average discrepancy (regarding signs)</td>
<td>-0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(disregarding signs)</td>
<td>0.030</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. Carbon monoxide capacity

At the end of several analyses (of O₂ content and CO capacity) by the present method, the blood solution left in the Van Slyke chamber was analyzed for CO by the standard method of Houghton & Root (1945). Table 2 shows that the discrepancies are only very small (i.e. less than 1% of the CO capacity) though they are on the average 2 to 3 times greater than those of Table 1. There is a slight indication, though the results are perhaps not numerous enough to be significant, that the results by the present method may be perhaps about 1 in 300 lower than by the standard method. Three possible causes suggest themselves for such an effect, if it were definitely established:

(i) In the figures for CO capacity by the present method in Table 2 it was assumed that the CO content of the sample before introduction into the Van Slyke apparatus was negligible (see above). Unfortunately, the initial CO contents were not measured independently, but in sheep blood solution similarly treated the CO content usually averages about 0.004 volume %. One-third or so of the average discrepancy (regarding signs) of 0.013 volume % might thus be due to this factor.

(ii) When the method was first conceived, the possibility of estimating CO combining capacity as well as O₂ content was considered doubtful. It was feared that appreciable amounts of CO might be taken up by the blood solution before the initial pressure reading, P₁, could be obtained. If this were the case, the calculated value of the CO capacity by the present method would also be too low. Though a systematic error might thus be due to this factor, the results given in Table 2 indicate that, as long as reasonable care is taken to avoid agitation of the blood/gas interface during the introduction of the CO and during the adjustment of the meniscus previous to the P₁ reading, the uptake of CO during this period is barely, if at all, significant.

(iii) The blood solutions were all assumed to be perfectly free from dissolved N₂. Any accidental contamination of the blood solutions with N₂ previous to, or
during their transfer into the Van Slyke chamber, would lead to evolution of this N₂ during the subsequent shaking and hence to an equivalent underestimate of the volume of CO taken up during the shaking.

Reproducibility of the method

In the course of applying the method to experimental work we have had occasion to make several replicate determinations of the CO capacity of each of numerous samples of blood. Table 3 shows results of such analyses selected at random from our data. The difference between extreme values in a given series was usually no greater than 0.05 volume %. Spans greater than 0.1 volume % were rare, and

<table>
<thead>
<tr>
<th>sample no.</th>
<th>CO capacity (volumes %)</th>
<th>sample no.</th>
<th>CO capacity (volumes %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
<td>3.94</td>
<td>619</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td>3.93</td>
<td></td>
<td>4.47</td>
</tr>
<tr>
<td></td>
<td>3.96</td>
<td></td>
<td>4.49</td>
</tr>
<tr>
<td></td>
<td>3.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.93</td>
<td>629</td>
<td>4.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.07</td>
</tr>
<tr>
<td>51</td>
<td>4.39</td>
<td></td>
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<tr>
<td></td>
<td>4.41</td>
<td></td>
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<tr>
<td></td>
<td>4.42</td>
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</tr>
<tr>
<td></td>
<td>4.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.40%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>4.94</td>
<td>713</td>
<td>4.34</td>
</tr>
<tr>
<td></td>
<td>4.99</td>
<td></td>
<td>4.36</td>
</tr>
<tr>
<td></td>
<td>4.78</td>
<td></td>
<td>4.35</td>
</tr>
<tr>
<td></td>
<td>4.33%</td>
<td></td>
<td>4.33</td>
</tr>
<tr>
<td>613</td>
<td>4.10</td>
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<td>4.37</td>
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<td></td>
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<td></td>
<td>4.36</td>
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<tr>
<td></td>
<td>4.10</td>
<td></td>
<td>4.33</td>
</tr>
<tr>
<td></td>
<td>4.11</td>
<td></td>
<td>4.34</td>
</tr>
<tr>
<td></td>
<td>4.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

when they did occur could usually be attributed to known faults in technique. From one sample of blood three successive determinations of O₂ content and percentage saturation were made. The results, as summarized in table 4, indicate a reproducibility for O₂ content within a range of 0.025 volume %. The difference between the extreme values for percentage saturation is 0.57 unit. Extensive experience of the method in dissociation curve work, as described in the previous paper by Roughton et al. (1955), leads us to believe that the method, with solutions
containing 3 to 4 g Hb/100 ml., is usually accurate to about ±0.5% O₂ saturation as in table 4. Occasionally, as in all gasometric procedure requiring meticulous accuracy, errors two or three times as great may be made, due presumably to human fallibility.

Table 4. Triplicate determinations on same sample of blood solution

<table>
<thead>
<tr>
<th>trial</th>
<th>combined O₂ (volumes %)</th>
<th>CO capacity (volumes %)</th>
<th>based on own capacity</th>
<th>based on average capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>2.724</td>
<td>5.02</td>
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</tr>
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<td>(2)</td>
<td>2.711</td>
<td>4.95</td>
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<tr>
<td>(3)</td>
<td>2.699</td>
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<tr>
<td>mean</td>
<td>2.711</td>
<td>4.97</td>
<td>54.53</td>
<td>54.53</td>
</tr>
</tbody>
</table>

Control experiments and comments

Necessity for saturation of oxygen absorber with carbon monoxide

During the early development of the method, samples of pure CO over Krogh's solution in the Van Slyke chamber were transferred to the Scholander apparatus in order to check on possible contamination during the transfer process. When O₂ absorber was introduced from the side arm of the Scholander apparatus and the latter shaken, the samples consistently showed a small diminution of volume (of the order of one small division on the micrometer). At first this was thought possibly to be due to reaction of CO with the O₂ absorber to produce formate. At this stage of the work, however, the O₂ absorber was saturated with N₂ instead of CO during its preparation and calculations indicated that the volume change in question could be accounted for by the difference in solubility between CO and N₂ and the consequent unequal exchange between these gases during the shaking. This explanation was confirmed by saturating the O₂ absorber with CO before loading it into the side arm of the Scholander apparatus. Under these conditions no volume change was observed when blank analyses of pure CO were performed.

Time required for shaking of the Van Slyke chamber

Tests showed that under the conditions employed (shaking rate about 200 per min) 15 min. were required to ensure complete equilibrium between O₂, CO₂ and haemoglobin solution. Other shaking rates were not tested, but a faster speed would doubtless diminish the time required. At any rate this factor should be tested for the particular conditions to be employed.

Reason for introduction of sodium hydroxide in Van Slyke chamber

In the procedure as originally tried the step of introducing NaOH into the Van Slyke chamber at the end of the shaking period was not included. Instead CO₂ absorber was introduced from the side arm of the Scholander previous to O₂ absorption. No difficulty was experienced in obtaining reproducible results by this procedure when the blood solution analyzed was at a pH of 9.1. With solutions at
pH 7, from which more CO₂ would be extracted, however, results were very erratic, probably because of variable reabsorption of CO₂ during the transfer process from Van Slyke to Scholander. This difficulty was eliminated when the addition of NaOH at the end of the shaking was included in the procedure.

Comments

The combined estimation of O₂ content and CO capacity on the same sample by the present method required meticulous care, and takes nearly twice as long as is required in the separate estimation of either of these quantities by the usual Van Slyke methods. The estimation of both quantities on the same sample, however, leads to some economy of blood, but—more important—it eliminates errors arising from changes in combining capacity, which occur under certain conditions with time (e.g. during the rotation of blood solutions in tonometers, a specially important point in dissociation curve work) as well as errors arising from uncertainties in volume measurement of separate samples. Another advantage, from the technical point of view, is that gummy precipitates of denatured protein are avoided. Consequently the blood meniscus is always sharp and the Van Slyke chamber is easily cleaned after an analysis merely by a few flushings with distilled water.

In the estimation of O₂ content, the gain of precision obtained by use of the Scholander apparatus as an adjunct to the Van Slyke technique may be thought of as equivalent to that which would be secured by reading the manometer of the latter instrument accurately to ± 0.1 mm Hg. This figure is based on the fact that the Scholander apparatus is capable of analyzing for oxygen to ± 0.02% and if, as in the present experiments, the total pressure of CO and O₂ in the Van Slyke chamber was about 500 mm, then ± 0.0002 x 500 = ± 0.1 mm. Another feature of the method is that, although a ‘c’ connexion is necessary for the added NaOH, it affects only the CO estimation and not that for O₂ which, in the present application, was the more critical.

Although the method has so far been applied to dilute solutions of sheep blood, it should be possible by minor modifications of the procedure to use it for analysis of whole blood or of more concentrated haemoglobin solutions. In the introduction it was, however, stressed that the method, in its present form is limited to (and has only been applied to) blood solutions, in which the content of dissolved N₂ is negligible. This limitation could, however, be removed if, in addition, the N₂ content of the gas sample at the end of the shaking process in the Van Slyke chamber could be accurately determined. The following example shows how this might be done. Suppose that the 10 ml sample before transference to the Van Slyke chamber for analysis had been in equilibrium with N₂ at 0.8 atmosphere pressure (as in ordinary air) instead of at zero pressure. The total dissolved N₂ content of the sample would then be about 0.12 ml., and of this about 99.5% would be liberated into the gas phase during the 15 min shaking of the Van Slyke chamber. After transfer of an aliquot of the gas to the Scholander apparatus and the absorption of its O₂ content, the residual gas should then consist of 90 to 95% CO and 10 to 5% N₂. All that would then remain would be to determine the percentage of
$N_2 (= x)$ in the residual gas to within $\pm 0.1\%$. This could be done by ejecting a portion of the residual gas from the Scholander apparatus into a Scholander–Roughton (1943) syringe-capillary apparatus, modified by addition of a bulb of accurately known volume (0.05 or 0.1 ml.). After absorption with Winkler’s solution, the residual $V_2$ should all be measurable in the capillary to an accuracy of $\pm 0.1\%$ of the whole sample, in exactly the same manner as that described by Hall (1944) for determining the $\% N_2$ in $O_2$ to within $\pm 0.1\%$.

For calculation of the CO capacity it would then only be necessary to replace $V_2$ by $V_2(1-x/100)$ in equations IV and V of the section ‘calculations’. No modification would be necessary in the calculation of $O_2$ content by equation I of the section ‘calculations’.

References


Otis, A. B. & Roughton, F. J. W. 1951 *J. Physiol.*, 115, 3P.


