Phase Equilibrium of Oxygen in “Nitrogen-Filled Vacutainers”

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Relationships describing the exchange of oxygen between the liquid and gas phases in a “Vacutainer” are derived and substantiated. The use of Vacutainers for determining P(O₂) and percent oxyhemoglobin saturation is condemned.

Nitrogen-filled “Vacutainers” (Becton-Dickinson, Rutherford, N. J. 07070) have been proposed for the anaerobic collection of specimens for blood gas determinations (1). We have indicated (2) that these devices, as currently supplied by the manufacturer, contain oxygen and may produce spuriously high P(O₂) and percentage oxyhemoglobin (HbO) values.

We had previously indicated preference for the use of common (air-filled) heparinized Vacutainers for percentage HbO determinations (3). Our preference was based on the conclusion of several authors (4–7) that data on blood gas for samples drawn in syringes and Vacutainers were comparable. The primary difference between these two blood drawing methods is contact of the blood sample with a gas phase in the Vacutainer.

The fluids present in a Vacutainer will be subject to the fundamental gas laws; the behavior of the liquid and gas phases will be influenced by concentration gradients and solubilities.

A liquid and a gas in a closed container tend to come to equilibrium. The P(O₂) of the gas and liquid phases in a nitrogen-filled (oxygen-free) Vacutainer tends to equalize, some oxygen going from the liquid to the gas phase. If the liquid phase is blood, this oxygen is derived from two sources, which are also in equilibrium: the physically dissolved and the hemoglobin-bound oxygen. The consequent transfer of oxygen can be estimated.

To simplify this estimation we will consider two examples: (a) oxygen in physical solution in water, and (b) oxygen in both physical solution and hemoglobin-bound, as found in blood. In the former, changes in oxygen concentration will be reflected only in the P(O₂), while in the latter, both P(O₂) and percentage HbO will be affected.

Example a:

Consider the behavior of 4.5 ml of water drawn into a Vacutainer and equilibrated with a 1.1-ml bubble of nitrogen at 37 °C. These volumes approximate those of commercially available nitrogen-filled Vacutainers.

The amount of gas dissolved in a given volume of solvent is proportional to the pressure of that gas with which it is in equilibrium, at constant temperature (9). The absorption coefficient of oxygen in water at 37 °C is 0.024 (10). Then 1 ml of water will dissolve v ml of oxygen at a P(O₂) of P Torr (1 Torr = 133 Pa), such that:

\[ v = \frac{0.024 \times P \times ml}{760} \]  

(1)

The volume V dissolved in 4.5 ml of water is given by:

\[ V = \frac{0.108 \times P}{760} \]  

(2)

If the gas phase and liquid drawn into the Vacutainer come to equilibrium, the total volume of oxygen originally present in the liquid will be distributed between that volume that remains in the liquid phase and a volume that enters the gaseous phase.

The volume of oxygen in the liquid phase at the original P(O₂) (Peq) and equilibrium P(O₂) (P(eq)) can be calculated by Equation 2.

The volume of oxygen present in the 1.1-ml gas phase at equilibrium is given by:

\[ V_{gas} = \frac{1.1 \times P_{eq}}{760} \]  

Then, where

\[ V_{total} = V_{liquid} + V_{gas} \]  

(4)

\[ \frac{0.108 \times P}{760} = \frac{0.108 \times P_{eq}}{760} + \frac{1.1 \times P_{eq}}{760} \]  

(5)

which reduces to:

\[ P_{eq} = 0.089 \times P \]  

(6)

Equilibration of 4.5 ml of water containing oxygen with a 1.1-ml bubble of nitrogen should reduce the P(O₂) of the aqueous phase to 8.9% of its original value. Example b:

Oxygen dissolved in blood is either hemoglobin-bound or present in physical solution. The relationship between oxygen content (volume/dl), oxyhemoglobin saturation (% Sat), and hemoglobin concentration (Hb, g/dl) is (11)

\[ O₂ \text{ content} = (134) \left( \frac{\% \text{ Sat}}{100} \right) \text{Hb} \]  

(7)

The volume of hemoglobin-bound oxygen in 4.5 ml of blood (V_Hb) is

\[ V_{Hb} = (6.03 \times 10^{-8}) \left( \% \text{ Sat} \right) \text{Hb} \]  

(8)

The volume of physically dissolved oxygen in 4.5 ml of blood is given by Equation 2.

Consider the behavior of 4.5 ml blood drawn into a Vacutainer and equilibrated with a 1.1-ml bubble of nitrogen at 37 °C. Let the blood have some hemoglobin concentration Hb, original P(O₂) = P_o, and original % Hb_s = % Sat_o. The sum of the original hemoglobin-bound volume V(Hb_o) and original physically dissolved oxygen volume V(P_o) is equal to the sum of the hemoglobin-bound volume V(Hb_eq) physically dissolved volume V(P_eq) and volume in the gas phase, V_{gas} at equilibrium.

\[ V_{(Hb_o)} + V_{(P_o)} = V_{(Hb_eq)} + V_{(p_eq)} + V_{gas} \]  

(9)

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Equation 9 can be simplified by expressing the volumes as functions of \( P(O_2) \) or \% Sat.

\[ V(Hb_0) \] and \[ V(Hb_{eq}) \] can be expressed by Equation 8, \( V(p_0) \) and \( V(p_{eq}) \) by Equation 2, and \( V_{gas} \) by Equation 3. Equation 9 becomes

\[
(6.03)(10^{-4})(Hb)(\% Sat_0) + \frac{0.108 \ P_o}{760} = \\
(6.03)(10^{-4})(Hb)(\% Sat_{eq}) + \frac{0.108 \ P_o}{760} + \frac{11 \ P_{eq}}{760}
\]

which reduces to:

\[
6.03 \ Hb(\% Sat_0) + 1.42 \ P_o = \\
6.03 \ Hb(\% Sat_{eq}) + 15.92 \ P_{eq}
\]

where \( \% Sat_0 \) is the original percentage HbO and \( \% Sat_{eq} \) is the equilibrium percentage HbO. If Hb, \% Sat_0, and \( P_o \) are known, the expression becomes a linear relationship between \% Sat_{eq} and \( P_{eq} \). Substitution of \( \% Sat_{eq} \) as a function of \( P_{eq} \) will permit the calculation of \( P_{eq} \).

The normal oxyhemoglobin dissociation curve is a straight line in the region 30-60\% HbO (12). The least-square line of Severinghaus’ data [reference (12) page 1111, Table 2] in this region is described by

\[
\% Sat = 2.63 \ P - 19.7
\]

For a sample calculation and solution of Equation 11, let the sample aspirated into the nitrogen-filled Vacutainer have \% Sat_0 of 50\%, \( P_o \) of 26.5 Torr, and Hb of 14 g/dl. Substituting, Equation 11 becomes

\[
(6.03)(14)(50) + (14.2)(26.5) = \\
(6.03)(14)(26.83 \ P_{eq} - 19.7) + 15.92 \ P_{eq}
\]

and

\[
P_{eq} = 24.9
\]

Although this shift in \( P(O_2) \) from 26.5 to 24.9 torr is small, the corresponding shift in percentage HbO should be from 50 to 45.8\% (Equation 12), which is significant.

**Methods and Materials**

**Apparatus**

All measurements were performed on a Model 313 Blood Gas Analyzer, and a Model 182 Co-Oximeter (Instrumentation Laboratory, Lexington, Mass. 02173).

"Nitrogen-filled" Vacutainers (No. 3206 XF 516) were obtained from Becton, Dickinson & Co., Rutherford, N. J. 07070).

**Procedures**

Nitrogen-filled, oxygen-free tubes were prepared in our laboratory by purging the commercially available "nitrogen-filled" Vacutainers with pure nitrogen (National Cylinder Gas Division, Chemetron Corp., Chicago, Ill.) at 50 ml/min for 5 min. The flushing gas entered and left the Vacutainer via two hypodermic needles inserted through the stopper. Samples of blood or demineralized water were introduced into these purged tubes with a syringe, nitrogen flowing during the introduction of the sample into the tube. Care was taken to avoid bubbling nitrogen through the aqueous phase.

Demineralized water was equilibrated at 37 °C with room air (Water A) and 95% oxygen-5% carbon dioxide (Instrumentation Laboratory, Lexington, Mass. 02173) (Water B) by using the apparatus previously described (2). Water samples were aspirated directly from the equilibration flask into a glass syringe, sealed, and subjected to the measurement and equilibration described below.

Four 10-ml blood samples were obtained by venipuncture, and transferred to four 30-ml glass syringes, each containing three Teflon boiling stones to facilitate mixing. Blood samples A and B were analyzed without adjustment of \( P(O_2) \).

The \( P(O_2) \) of blood samples C and D were increased by gently rolling the sealed syringe containing the sample and 20 ml of either room air (Sample C) or 95% oxygen (sample D). The room air and 95% oxygen were vented and exchanged several times. Finally, all gas bubbles were removed, the syringes sealed, and the samples subjected to the measurement and equilibration described below.

The water and blood samples in the sealed syringes were subjected to an initial determination of blood gas values (Reading 1). An 18-gauge 38-mm hypodermic needle was then placed on the syringe hub, and a drop of sample expressed to rinse and fill the needle. The needle was then inserted through the stopper of the Vacutainer, which was being purged with nitrogen as described above, and 4.5 ml of sample was carefully introduced. The syringe-needle combination was removed from the stopper, and the syringe again sealed. The nitrogen inlet and outlet needles were withdrawn, and the Vacutainer tube and sealed glass syringe were placed on a 60 rpm rotator in a 37 °C incubator for 5 min. The Vacutainer and syringe were removed from the incubator, and blood gas values of the sample in the syringe (Reading 2) and the tube (Reading 3) were determined (Table 1).

**Results and Discussion**

The data in Table 1 show that equilibration of samples of high initial \( P(O_2) \) with a nitrogen bubble will significantly decrease the \( P(O_2) \). Equilibration will, however, have little effect on percentage HbO in blood with a high initial \( P(O_2) \). This can be seen from the data on water samples A and B, and blood sample D.

**Table 1. Blood Gas Values for Syringe and Tube**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reading a</th>
<th>( P(O_2), ) Torr b</th>
<th>HbO saturation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water A</td>
<td>1</td>
<td>132.0</td>
<td>—</td>
</tr>
<tr>
<td>Water A</td>
<td>2</td>
<td>138.6</td>
<td>—</td>
</tr>
<tr>
<td>Water A</td>
<td>3</td>
<td>29.1</td>
<td>—</td>
</tr>
<tr>
<td>Water B</td>
<td>1</td>
<td>578</td>
<td>—</td>
</tr>
<tr>
<td>Water B</td>
<td>2</td>
<td>546</td>
<td>—</td>
</tr>
<tr>
<td>Water B</td>
<td>3</td>
<td>77.6</td>
<td>—</td>
</tr>
<tr>
<td>Blood A</td>
<td>1</td>
<td>30.3</td>
<td>57.6</td>
</tr>
<tr>
<td>Blood A</td>
<td>2</td>
<td>29.6</td>
<td>57.9</td>
</tr>
<tr>
<td>Blood A</td>
<td>3</td>
<td>28.3</td>
<td>54.8</td>
</tr>
<tr>
<td>Blood B</td>
<td>1</td>
<td>43.9</td>
<td>85.3</td>
</tr>
<tr>
<td>Blood B</td>
<td>2</td>
<td>43.8</td>
<td>85.2</td>
</tr>
<tr>
<td>Blood B</td>
<td>3</td>
<td>38.9</td>
<td>80.8</td>
</tr>
<tr>
<td>Blood C</td>
<td>1</td>
<td>119.7</td>
<td>98.8</td>
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<tr>
<td>Blood C</td>
<td>2</td>
<td>120.1</td>
<td>98.8</td>
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<tr>
<td>Blood C</td>
<td>3</td>
<td>57.1</td>
<td>94.9</td>
</tr>
<tr>
<td>Blood D</td>
<td>1</td>
<td>672</td>
<td>99.4</td>
</tr>
<tr>
<td>Blood D</td>
<td>2</td>
<td>531</td>
<td>99.6</td>
</tr>
<tr>
<td>Blood D</td>
<td>3</td>
<td>111.6</td>
<td>98.2</td>
</tr>
</tbody>
</table>

a 1. Syringe, before equilibration; 2, syringe, after equilibration; 3, nitrogen-filled tube, after equilibration.

b 1 Torr = 133 Pa.
Equation 6 predicts 12 and 52 Torr for Reading 3 of the water samples. Although not in perfect agreement, the 29.1 and 77.6 Torr values observed are of the same order of magnitude.

Equations 12 and 14 predict that blood samples with an initial percentage HbO of 30-60% will have a 1.5% and 4-Torr drop in percentage HbO and P(O2). This compares to the 2% and 3-Torr drop actually observed for sample A.

Samples with percentage HbO greater than those considered in Equation 12 also lose significant oxygen when equilibrated in nitrogen-filled tubes. The decrease in percentage HbO for samples B and C was similar, being 4 to 5% each. The 5- and 63-Torr decreases in P(O2) observed for samples B and C are, however, remarkably different.

The data presented confirm the existence of a dynamic equilibrium between the phases in a Vacutainer. Mass transfer will occur to eliminate concentration gradients.

The amount of oxygen transfer necessary to equalize the P(O2) of the phases in a Vacutainer will be less if oxygen is already present in the tube. This is a compensating error, and its existence in the data of Fleisher and Schwartz (1) as well as that of Gambino (5) is a distinct possibility. Gambino’s data include only percentage HbO values, with most of the values being greater than 90%. It would be difficult to detect a difference in percentage HbO’s at these high values, especially if oxygen were already present in the gas phase, as it is in the common heparin Vacutainers. The overall tendency when using Vacutainers will be to produce an erroneous P(O2), the value approaching that of the gas originally present in the Vacutainer.

Oxygen transfer between phases will depend on the treatment of the sample. Little transfer will occur if the sample is drawn in a Vacutainer and analyzed immediately, with no mixing. On the other hand, if the sample is drawn in a Vacutainer, inverted several times to dissolve heparin, transported 100 meters to the laboratory, stored 5 to 15 min on ice while the analyst adjusts the instrumentation, mixed again to disperse the red cells, and then analyzed, error is not only possible, but probable. The former approximates an idealized solution, the latter the treatment of blood gas samples in a real hospital environment. This effect is apparent in the clinically significant difference observed in hospitalized patients (2) when comparing “nitrogen-filled Vacutainers” to syringes. The possibility of error can only be eliminated by avoiding contact of the sample with a gas phase, i.e., by using a bubble-free syringe.

The P(CO2) of the liquid and gas phases will also tend to become equal. Consider the blood specimen in a nitrogen-filled, oxygen-free tube described above, and let the equilibrium P(CO2) be 40 Torr. The volume of CO2 in the gas phase with this P(CO2) can be calculated from equation 7 to be about 0.058 ml, which corresponds to a CO2 content of 2.6 μmol at standard temperature and pressure. Since the total CO2 content of 4.5 ml of normal blood is in the range 120 to 140 μmol, the decrease in total CO2 as a result of that lost to the gas phase in a Vacutainer, although real, would be clinically insignificant, as Still and Rodman (8) also concluded.

Changes in pH caused by gas transfer not only involve changes in carbonic acid, but also changes in the Hb/HbO and other buffer systems. It has been demonstrated (1, 5) and confirmed (2) that pH changes attributable to the Vacutainers are insignificant, and samples drawn in these devices are valid for acid-base studies.

The use of Vacutainers, either nitrogen (oxygen-free), nitrogen (oxygen-contaminated), or air-filled to obtain specimens for the determination of P(O2) or percentage HbO must, however, be condemned. Clinically significant error in these parameters resulting from oxygen transfer between phases is a possibility under ideal conditions, and a probability under actual hospital conditions.

References