Effect of small air bubbles on changes in blood pO_2 and blood gas parameters: calculated vs. measured effects

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When collecting blood for blood gas analysis, it is important to remove air bubbles from syringes to avoid erroneous results, especially for pO_2, with a number of factors affecting the potential magnitude of the interference to pO_2 by air bubbles.

In this study, we calculate the expected theoretical changes in pO_2 when either 20 µL or 40 µL increments of air are added, and determine the validity of these calculations by measuring blood gas and CO-oximetry parameters on 19 blood samples after equilibration with similar increments of air.

We conclude that (A) adding air has very different effects on pO_2, depending on the initial pO_2 of blood, with relatively minor effects up to 70 mmHg, larger increases for initial pO_2 values of 80 to 140 mmHg, and decreases for initial pO_2 values of about 180 mmHg and higher; and (B) adding air has very small effects on either pH or pCO_2.

Furthermore, our results support an earlier conclusion that increases in pO_2 by pneumatic tube transport are due solely to equilibration by vigorous mixing of air bubbles entrapped in blood.

It is well known that air bubbles erroneously introduced into blood collected in syringes can affect blood gas results, especially the pO_2 value [1, 2]. This pO_2 interference is greater if the air bubble is vigorously mixed with the blood, such as by intense shaking [2] or by pneumatic tube transport [3].

We wanted to quantitate by both calculations and measurements how increments of smaller air bubbles (20 and 40 µL) added and equilibrated into blood can change pO_2 over a range of initial pO_2 values.

It is simple to calculate the O_2 content of 10 to 100 µL air bubbles at atmospheric pressure (Table I), and relatively easy to calculate the O_2 content of an air bubble at various pO_2 values (Table II for 20 µL and 40 µL air bubbles). However, it is more challenging to estimate the effect this increased O_2 content would have on the pO_2 value of a blood sample for several reasons:

1. The amount of O_2 that can be transferred from air to blood depends on the difference in initial pO_2 between blood and the air bubble.
2. After equilibrium is achieved between air and blood, the only assumption that can be made is that pO_2 will be the same in air and blood. However, the pO_2 of both air and blood would change, and could either increase or decrease, depending on the initial pO_2 of blood (assuming an atmospheric pO_2).
3. Assuming an initial pO_2 in air of 150 mmHg, the pO_2 of blood would usually increase (for persons breathing atmospheric air), but could decrease (for persons breathing oxygen-enriched air), or change very little (if blood has an initial pO_2 of approximately 150 mmHg). This is further complicated because blood is typically exposed to air bubbles at room temperatures (RT; 21-24 °C), but is analyzed in a closed system at 37 °C. Thus, for blood at pO_2 150 mmHg (37 °C), exposure to air at 150 mmHg (RT) should increase the pO_2 in blood to above 150 mmHg.
4. The cooler room temperature relative to 37 °C will also increase the affinity of Hb for O_2. This will increase the transfer of O_2 from air to blood with $pO_2 < 150$ mmHg.
5. The measurement of pO_2 detects the oxygen dissolved in the aqueous phase of blood and not the larger reservoir of oxygen bound to Hb. Oxygen bound to Hb is not detected when pO_2 is measured, although Hb-O_2 does contribute to the equilibrium of O_2 in the aqueous phase of blood.
6. Depending on the initial saturation of Hb (%O_2Hb), the change in pO_2 is not linear as oxygen is added to blood. This is because Hb can bind some of the O_2 absorbed from the air bubble, depending on the initial saturation of Hb with oxygen. For example, at a low sO_2 (i.e., 60 %), when O_2 is added to blood, more oxygen will bind to Hb, with relatively less change in pO_2. If Hb is nearly saturated with oxygen (i.e., 98 %), more of the added oxygen dissolves in the aqueous phase of blood, causing a larger change in pO_2.
7. The concentration of Hb also adds to the complexity. For examples, at an initial pO_2 of 90 mmHg and an Hb of 6 g/dL, an increase in O_2 content of 0.5 mL O_2/dL blood will increase pO_2 from 90 to about 170 mmHg. At Hb = 15 g/dL, the pO_2 will increase from 90 to only 120 mmHg.

Given these challenges, we sought to calculate the expected theoretical change in pO_2 when either 20 µL or 40 µL increments of air are added to blood.

To determine the validity of these calculations, we measured blood gas and CO-oximetry parameters on 19 blood samples to which we added 20 and 40 µL increments of atmospheric air and equilibrated this air with the blood. We also calculated the measured changes in pH and pCO_2 of blood after adding these increments of air.

Methods and calculations

Measurements

We measured blood gases and CO-oximetry parameters with two different standard blood gas/CO-oximetry analyzers. The most relevant measurements for this study were: pO_2, Hb, sO_2, %O_2Hb, O_2 content and calculated sO_2 (from pO_2). pH and pCO_2 were also measured.

Calculations

The calculated O_2 content of air bubbles (10 to 100 µL) at atmospheric pressure is shown in Table I and the calculated O_2 content of an air bubble at various pO_2 values is shown in Table II for 20 µL and 40 µL air bubbles.

To estimate how the change in O content would affect O of blood, several parameters were first calculated. The O was calculated for various values of O using the Severinghaus equation [4] with the aid of an online calculator [5]. With this data and using the equation for

Bubble vol (µL)	O_2 content (mL O_2/dL blood)	O_2 content (mL O_2/mL blood)
10	0.21	0.0021
20	0.42	0.0042
30	0.63	0.0063
50	1.05	0.0105
100	2.09	0.0209

TABLE I: O_2 content of 10-100 µL air bubbles; assuming an atmospheric gas pressure of 710 mmHg = 760 – 47 (47 mmHg is the contribution of water vapor to atmospheric pressure).

pO_2 (mmHg)	O_2 content of 20 µL air bubble (mL O_2)	O_2 content of 40 µL air bubble (mL O_2)
40	0.00113	0.00225
50	0.00141	0.00282
60	0.00169	0.00338
70	0.0020	0.0040
80	0.00225	0.00451
85	0.0024	0.0048
90	0.00253	0.00507
95	0.0027	0.0054
100	0.00282	0.00563
110	0.0031	0.0062
120	0.00338	0.00676
150	0.00423	0.00845
200	0.00563	0.01127
250	0.00704	0.01408
300	0.00845	0.01690
400	0.0113	0.0226

TABLE II: O_2 content vs. pO_2 for 20 and 40 µL air bubbles at atmospheric gas pressure of 710 mmHg (760 – 47).

O content of blood, the O content was calculated vs. O for Hb levels, with the calculations for 10 g/dL Hb shown in Table III.

Although we initially calculated this with an online Arterial Oxygen Content Calculator [6], we had to manually calculate the O_2 content to 3 decimal places because small changes in O_2 content have a large effect on pO_2. The equation used in the calculation was:

O_2 content (mL/dL blood) =1.36 × Hb × sO_2 + 0.0031 × pO_2; with Hb in g/dL; sO_2 as decimal percent; and pO_2 in mmHg.

Using an Hb of 10 g/dL as an example, we calculated the O_2 content of blood at pO_2s from 20 to 250 mmHg (Table IV). However, we had to estimate the percent of oxygen that would be extracted from both a 20 µL (Table IVa) and a 40 µL air bubble (Table IVb).

We reasoned that blood at a low pO_2, such as 40 mmHg, would extract more O_2 from the bubble (~80 %) than if the pO_2 were 100 mmHg (~32 %). For blood at pO_2 higher than 150 mmHg, the O_2 would transfer from blood into the air bubble. However, the amount transferred would be limited because the pO_2 of the air and blood must both remain above 150 mmHg.

pO_2 (mmHg)	sO_2 (% O_2Hb)	O_2 content (mL O_2/dL blood)	O_2 content (mL O_2/mL blood)
20	32.0	4.414	0.0441
30	57.4	7.899	0.0790
40	74.9	10.310	0.1031
50	85.0	11.715	0.1172
60	90.6	12.508	0.1251
70	93.8	12.974	0.1297
80	95.7	13.263	0.1326
90	96.9	13.457	0.1346
100	97.7	13.597	0.1360
110	98.3	13.710	0.1371
120	98.7	13.795	0.1380
150	99.3	13.970	0.1397
200	99.7	14.179	0.1418
250	99.9	14.361	0.1436
300	99.9	14.516	0.1452
400	100.0	14.840	0.1484

TABLE III: Calculation of O_2 content vs. pO_2 in blood with [Hb] = 10 g/dL.

Initial pO_2 (mmHg)	Initial O_2 content[a]	Assumed % extraction of O_2 from bubble	Estimated change in O_2 content[a]	New O_2 content of blood[a]	Estimated New pO_2 (mmHg)[c]	Change in blood pO_2 (mmHg)
20	0.0441	90	0.00378	0.0479	21	+1
40	0.1031	80	0.00336	0.1065	42	+2
60	0.1251	60	0.00252	0.1276	66	+6
80	0.1326	45	0.00189	0.1345	90	+10
90	0.1346	40	0.00168	0.1363	103	+13
100	0.1360	32	0.00134	0.1373	113	+13
120	0.1380	20	0.00084	0.1388	135	+15
150	0.1397	10	0.00042	0.1401	160	+10
200	0.1418	-10	-0.0004 [b]	0.1414	190	-10
250	0.1436	-30	-0.0012 [b]	0.1424	215	-35

TABLE IVa: Estimates of pO_2 changes when 20 µL air bubble is added to blood with Hb = 10 g/dL.

From Table I, a 20 µL air bubble contains 0.0042 mL O_2/mL blood.

[a] Units for O_2 content are mL O_2/mL of blood.

[b] For blood at pO_2 250 mmHg, the maximum volume of O_2 that can be lost from blood to an air bubble is 0.0039 mL O_2/mL blood (0.1436 – 0.1397), since the blood pO_2 must stay above 150 mmHg (from Table III).

[c] The pO_2 was estimated from the O_2 content vs. pO_2 data in Table III.

Initial pO_2 (mmHg)	Initial O_2 content[a]	Assumed % extraction of O_2 from bubble	Estimated change in O_2 content[a]	New O_2 content of blood[a]	Estimated New pO_2 (mmHg)[c]	Change in blood pO_2 (mmHg)
20	0.0441	90	0.00756	0.0517	22	+2
40	0.1031	80	0.00672	0.1098	44	+4
60	0.1251	60	0.00504	0.1301	72	+12
80	0.1326	45	0.00378	0.1364	104	+24
90	0.1346	40	0.00336	0.1380	120	+30
100	0.1360	32	0.00269	0.1387	132	+32
120	0.1380	20	0.00168	0.1397	150	+30
150	0.1397	10	0.00084	0.1405	171	+21
200	0.1418	-15	-0.0006 [b]	0.1412	185	-15
250	0.1436	-40	-0.0016 [b]	0.1420	205	-45

TABLE IVb: Estimates of pO_2 changes when 40 µL air bubble is added to blood with Hb = 10 g/dL.

From Table I, a 40 µL air bubble contains 0.0084 mL O_2/mL blood.

[a] Units for O_2 content are mL O_2/mL of blood.

[b] For a blood pO_2 of 250 mmHg, the maximum volume of O_2 that can be lost from blood into an air bubble is 0.0039 mL O_2/mL blood (0.1436 – 0.1397), since the blood pO_2 must stay above 150 mmHg (from Table III).

[c] The pO_2 was estimated from the O_2 content vs. pO_2 data in Table III.

Therefore, if blood at a pO_2 of 250 mmHg has 0.1436 mL O_2/mL blood (Table III and IV) and if the O_2 content can go no lower than 0.1397 mL O2/mL blood (O_2 content of blood at 150 mmHg), then the maximum amount of O_2 that could be lost from blood is 0.0039 mL/mL blood (0.1436 – 0.1397).

From this, we calculated the estimated change in O_2 content, the new O_2 content of blood, the new pO_2 of blood, and finally the change in blood pO_2 at each initial pO_2 of blood, which are shown in the last four columns of Tables IVa and IVb.

Preparation of samples

Using approximately 1 to 1.6 mL of blood from 19 discarded de-identified heparinized blood samples after blood gas analysis, we removed all visible air bubbles, mixed by rotation, then analyzed on blood gas analyzers for blood gas parameters, including pH, pCO_2, pO_2, total Hb, %O_2Hb, sO_2 and O_2 content.

Following this, we successively added 20 µL or 40 µL of atmospheric air to the sample, with thorough equilibration of blood and air before analysis. Thus, we obtained data with either 40, 80, 120, 160 µL, etc. air added, or 20, 40, 60, 80 µL, etc. air added to each sample.

The volume of air added was approximated by visually pulling back on the syringe plunger until the syringe tip was either half full of air (~20 µL) or full of air (~40 µL). While this will not give a highly accurate volume of air, it has the advantage of minimally exposing the blood to extraneous air. Samples were analyzed on either of two different blood gas analyzers, with six on one analyzer and 13 on the other analyzer.

Results and conclusions

While the data in Table IV shows the theoretical changes in pO_2 as increments of air were added to blood, to determine if our calculations are valid, we measured blood gas and CO-oximetry parameters on 19 blood samples before and after various volumes of air were added and mixed into the blood.

Blood gas/CO-oximetry results from a representative blood sample with 40 µL increments of air added are shown in Table V, which also includes the calculated difference in pO_2 results (ΔpO_2) as each increment of air was added.

Fig. 1 shows both the theoretical changes in pO_2 as either 20 or 40 µL air bubbles are added to blood and the actual changes in pO_2 measured in the 19 blood samples. The measured changes for pO_2 in Fig. 1 confirm the general trends of the calculated changes. The other conclusions from this figure follow.

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1. Adding air has very different effects on the pO_2 of blood, depending on the initial pO_2 of the blood. Adding air has minimal effect at low pO_2 values (20-40 mmHg), and moderate effects up to about 60 mmHg. At 80-150 mmHg, the increase in pO_2 can be quite large, ranging from 20 to 30 mmHg.

We also note that, despite blood at low initial pO_2 extracting more O_2 from the air bubbles, the changes in blood pO_2 were much less at lower initial pO_2 values. This is because Hb is less saturated and has a greater capacity to bind oxygen, such that less oxygen is added to the aqueous phase of blood, which determines the pO_2.

In effect, if Hb is about 90 % or less saturated with oxygen, it is able to buffer the increase in pO_2 when air is added. At saturations of 95 % and above, Hb apparently has less ability to buffer changes in pO_2 if air is added to blood.

2. Although we added oxygen in air at atmospheric pressure (pO_2 of ~150 mmHg), the amount of O_2 absorbed by blood will depend on the difference

Time	10:20	10:24	10:28	10:32	10:36	10:40
Bubble vol (µL)	0	40	80	120	160	200
pH	7.243	7.241	7.241	7.244	7.248	7.255
pCO_2 (mmHg)	34.5	34.1	33.7	32.9	32.0	30.9
pO_2 (mmHg)	69.4	85.1	105	127	159	183
ΔpO_2 (mmHg)	16	20	22	32	24	----
Hb (g/dL)	13.7	13.6	13.5	13.6	13.6	13.6
$\%O_2Hb$	89.7	93.3	95.0	95.8	96.1	96.2
sO_2 %	92.6	96.2	97.8	98.8	99.1	99.3
O_2 ct (mL/mL)	0.173	0.179	0.182	0.185	0.187	0.187

TABLE V: Blood gas and CO-oximetry measurements on a representative arterial blood sample as 40 µL increments of room air were added to 1.2 mL blood. The ΔpO_2 is the difference between the pO_2 values before and after addition of each 40 µL increment of air. O_2 ct = oxygen content.

between 150 mmHg and the actual pO_2 of blood. Thus, for blood at a low pO_2 (i.e., 60 mmHg), more O_2 from the gas bubble would be absorbed.

For blood at 150 mmHg, essentially no O_2 would be absorbed by the blood (assuming no temperature effects), and for blood at high pO_2, O_2 would be lost from the blood into the air bubble. Being unaware of an equation to calculate the percent of O_2 absorbed by blood from air at differing pO_2 values, we estimated such percentages.

In Tables IVa and IVb, we show the percent of O_2 in 20 and 40 µL air bubbles that would be absorbed by blood at pO_2 values ranging from 20 to 250 mmHg. For example, in blood at pO_2 40 mmHg, we estimate that a large proportion of O_2 in the air bubble would be absorbed by blood (~80 %) at an Hb concentration of 10 g/dL.

At a pO_2 of 250 mmHg, oxygen would move from blood into the air bubble. Furthermore, since the blood pO_2 could not go below 150 mmHg, we estimated that the maximum O_2 lost from blood into the air bubble would be 0.0039 mL/mL blood (0.1436 – 0.1397 from Table III).

With these assumptions, we could then calculate the change in O_2 content, the new O_2 content of blood, and (using data from Table III) the new pO_2 of blood (Table IV). The last column in Tables IVa and IVb shows the change in blood pO_2 for the initial pO_2 of blood.

3. Because of the aforementioned temperature effects, adding air has almost no effect on blood with an initial pO_2 of 170-190 mmHg. It became clear that adding air to blood could increase pO_2 to well above 150 mmHg, typically to 170-180 mmHg.

This was readily explained because the equilibration with the air bubble was done at room temperature (approx. 22 °C), while the analysis was at 37 °C. At cooler temperatures, Hb increases its affinity for O_2 and O_2 becomes more soluble in the aqueous phase of blood.

So while more O_2 would be absorbed by blood at the cooler room temperature, this O_2 would be released during analysis at 37 °C. After slightly modifying the data in Table IV to account for this effect, we plotted the theoretical changes in pO_2 vs. the initial pO_2 when either 20 or 40 µL increments of oxygen were added (Fig. 1), along with the pO_2 data from individual blood samples.

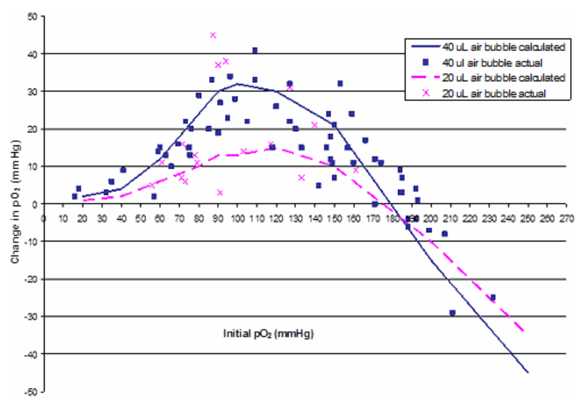

FIG. 1: Calculated and measured changes in blood pO_2 when 20 or 40 µL air (atmospheric pO_2) was added to blood. Data points are based on changes in pO_2 as measured on 19 blood specimens as air was sequentially introduced and equilibrated with the blood in a syringe.

The results in this study confirm the changes reported previously in pO_2 values during pneumatic transport caused by air contamination of blood gas specimens [3].

Our results suggest that for air bubbles of 20 µL or less, the effect on pO_2 is less than 10 mmHg up to about 70 mmHg. At initial pO_2 values of 80 to 140 mmHg, 20 µL air can have a wide range of effects, ranging from 5 to 40 mmHg. In fact, at initial pO_2 values of 90 mmHg, 20 µL air bubbles often had as much effect as 40 µL air bubbles.

These results support the conclusion of an earlier report that increases in pO_2 are due solely to equilibration of air and blood due to vigorous mixing during pneumatic transport [3]. We note this earlier study also determined that a 50 % reduction in the speed of pneumatic transport significantly decreased the effect of air bubbles introduced in the blood specimen.

We believe that the effect of pneumatic transport is due to the volume of air added to blood and the extreme degree of agitation during transport. While another study suggested that sending blood specimens in pressure-sealed vessels overcame this effect [7], we conclude that these vessels must have minimized the agitation of the specimen. Since the Hb level should affect these changes, we calculated the theoretical changes at different Hb levels of 8, 10 and 12 g/dL. However, the effect was fairly minimal (data not shown).

The changes in pH and pCO_2 were more straightforward. As air was added, changes to these parameters were small compared to changes in pO_2. The mean change in pH was +0.02 for 120 μL air added. For pCO_2, the mean change was –1 mmHg for 40 μL air added and about –3 mmHg for 120 μL air added (see Table VI). Clearly, air has much less effect on the pH and pCO_2 than on the pO_2.

	Air volume added		
	40μL	80μL	120μL
Mean ΔpH	0.004	0.006	0.014
min	–0.01	–0.01	0.00
max	0.04	0.04	0.05
Mean ΔpCO_2	–0.76	–1.41	–2.84
min	–3.0	–4.0	–9.0
max	1.0	0.2	–0.9

TABLE VI: Mean changes in pH and pCO_2 in the 19 blood samples tested as 40 μL increments of room air were added to blood. The mean initial pH of the samples was 7.32 (range 6.89-7.53) and the initial pCO_2 of the samples was 47.9 mmHg (range 31-84 mmHg).

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