

THE pH-log P_{CO_2} DIAGRAM OF SEPARATED HUMAN BLOOD PLASMA

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If blood plasma is rapidly separated under anaerobic conditions from the blood cells at 38°, the pH and P_{CO_2} of this "true" plasma are the same as those of the originating blood, because the pH of whole blood is defined as that of its plasma and because an equilibrium for P_{CO_2} may be assumed between cells and plasma in the centrifuging tube.

If plasma is equilibrated with a gas mixture having another P_{CO_2} changes take place in the concentrations of H^+ , OH^- and HCO_3^- ions and in the number of negative charges of the protein molecules. As the final state differs from that obtained after first equilibrating whole blood (chloride shift!) and then centrifuging, the equilibrated plasma is called "separated" plasma. When the simultaneous changes of pH and P_{CO_2} are plotted in a pH-log P_{CO_2} diagram an approximately straight line is obtained for each sample of plasma.

This paper deals with the physico-chemical basis of the pH-log P_{CO_2} diagram of separated human blood plasma.

According to VAN SLIJKE *et al.*¹ the base bound by serum albumin and serum globulin at 38° is a linear function of pH between pH 6.8 and pH 7.8.

$$BP_s = 1.25 A (\text{pH} - 5.16) + 0.77 G (\text{pH} - 4.89) \quad (1)$$

where A and G are the concentrations of serum albumin and globulin respectively in grams per 100 ml. Equation (1) may be worked out for different A/G ratios between 1.0 and 2.0 with $A + G = Pr$ (total protein concentration).

| A/G | |
|-------|-------------------------------------|
| 1.0 | $BP_s = 1.01 Pr (\text{pH} - 5.06)$ |
| 1.2 | $BP_s = 1.03 Pr (\text{pH} - 5.07)$ |
| 1.4 | $BP_s = 1.05 Pr (\text{pH} - 5.08)$ |
| 1.6 | $BP_s = 1.07 Pr (\text{pH} - 5.09)$ |
| 1.8 | $BP_s = 1.08 Pr (\text{pH} - 5.09)$ |
| 2.0 | $BP_s = 1.09 Pr (\text{pH} - 5.10)$ |

Within these limits

$$[P^-] = BP_s = 1.05 (1 \pm 0.04) Pr (\text{pH} - 5.08 \pm 0.02) \quad (2)$$

Differentiating equation (2) with respect to pH, we obtain

$$d[P^-]/d\text{pH} = 1.05 (1 \pm 0.04) Pr \quad (3)$$

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In separated plasma

$$[P^-] + [HCO_3^-] = \text{constant} \quad (4)$$

or

$$d[P^-]/d\text{pH} = -d[HCO_3^-]/d\text{pH} \quad (5)$$

hence

$$d[HCO_3^-]/d\text{pH} = -1.05(1 \pm 0.04)Pr \quad (6)$$

A second relationship between $[HCO_3^-]$ and pH is the Henderson-Hasselbalch equation

$$\text{pH} - \text{p}K' = \log [HCO_3^-] - \log P_{CO_2} - \log \alpha \quad (7)$$

which we will use in the differentiated form

$$1 - \frac{d\text{p}K'}{d\text{pH}} = \frac{1}{2.3[HCO_3^-]} \frac{d[HCO_3^-]}{d\text{pH}} - \frac{d \log P_{CO_2}}{d\text{pH}} \quad (8)$$

In this equation for $d\text{p}K'/d\text{pH}$ the value -0.044 (SEVERINGHAUS *et al.*²) is substituted and $d[HCO_3^-]/d\text{pH}$ is substituted from equation (6). After rearrangement the resulting equation is:

$$- \frac{d \log P_{CO_2}}{d\text{pH}} = 1.044 + \frac{1.05(1 \pm 0.04)Pr}{2.3[HCO_3^-]} \quad (9)$$

For a normal $[HCO_3^-]$ of about 25 mequiv./l the second term of the right hand term of equation (9) equals

$$0.018(1 \pm 0.04)Pr$$

If $Pr = 7 \pm 1$ this value becomes

$$0.128(1 \pm 0.04)(1 \pm 0.14) = 0.128(1 \pm 0.18) = 0.128 \pm 0.023$$

and

$$- \left[\frac{d \log P_{CO_2}}{d\text{pH}} \right]_{(HCO_3^- = 25)} = 1.044 + 0.128 \pm 0.023 = 1.172(1 \pm 0.02)$$

It is seen that the 18% relative error of the second term of equation (9), due to variations of the A/G ratio and the total protein content, corresponds to an absolute error of 0.023, or a relative error of only 2% in the value of $-d \log P_{CO_2}/d\text{pH}$ in this case. A deviation of 2% in the value of $-d \log P_{CO_2}/d\text{pH}$ would be difficult to verify experimentally, as two P_{CO_2} and two pH determinations each with an error of no more than 1% would be necessary. For a $P_{CO_2} = 40$ mm Hg this means an accuracy of 0.4 mm Hg, or 0.06 vol% and for pH, as $\log 1.01 = 0.004$, an accuracy of 0.004 pH unit. These accuracies are on the limit of experimental possibilities. For bicarbonate concentrations above 25 mequiv./l the influence of A/G and Pr is even less; for lower $[HCO_3^-]$ the uncertainty increases. We may, therefore, neglect the influence of different A/G ratios and we may take a mean value for Pr , e.g. 7.0, if $[HCO_3^-]$ is not too low. The resulting approximation of equation (9) is

$$- d \log P_{CO_2}/d\text{pH} = 1.044 + 3.2/[HCO_3^-] \quad (10)$$

In Fig. 1, a pH-log P_{CO_2} diagram, lines have been drawn for several values of $[HCO_3^-]$ at $P_{CO_2} = 40$. The slope of each line equals the value of $-d \log P_{CO_2}/d\text{pH}$ calculated from equation (10) after substitution of the appropriate value of $[HCO_3^-]$, the position of each line is such that at $P_{CO_2} = 40$ the values of P_{CO_2} , $[HCO_3^-]$ and pH agree with the Henderson-Hasselbalch equation (7) with

$$\text{p}K' = 6.090 - 0.044(\text{pH} - 7.4) \quad (11)$$

and $\alpha = 0.0301 \text{ mM/mm Hg}$. The diagram is approximately the same as ASTRUP's³ who based his on experimental data and did not give any particulars concerning the construction. The lines drawn in the diagram are called standard bicarbonate lines by ASTRUP. It must be noted that these lines do not represent iso- $[\text{HCO}_3^-]$ lines. Differentiating equation (7) with respect to pH at constant $[\text{HCO}_3^-]$ yields

$$1.044 = - [d \log P_{\text{CO}_2}/d\text{pH}]_{[\text{HCO}_3^-]}$$

the value of the slope of the iso- $[\text{HCO}_3^-]$ lines. A numerical example will show that a negligible error is made if one assumes that the value of $d \log P_{\text{CO}_2}/d\text{pH}$ at any particular point in the diagram equals the slope of the standard bicarbonate line through that point.

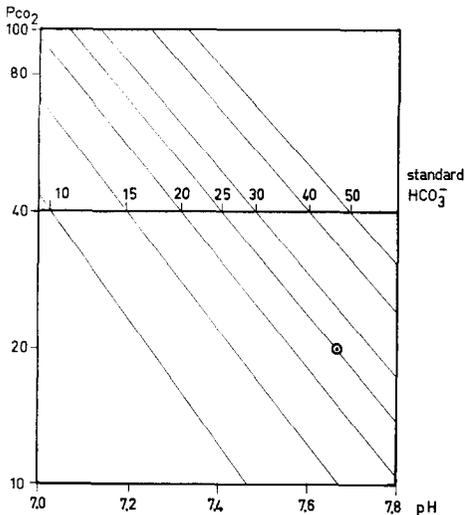


Fig. 1. pH-log P_{CO_2} diagram of separated human blood plasma. At $P_{\text{CO}_2} = 40 \text{ mm Hg}$ the true bicarbonate concentration equals the standard bicarbonate concentration by definition.

Let us take the standard bicarbonate line for $[\text{HCO}_3^-] = 25 \text{ mequiv./l}$. The slope of this line is $-1.044 - 3.2/25 = -1.172$. This standard bicarbonate line passes through the point $\text{pH} = 7.407$, $P_{\text{CO}_2} = 40 \text{ mm Hg}$ at which point the true bicarbonate concentration is defined as the standard bicarbonate concentration. The line also passes through the point $\text{pH} = 7.664$, $P_{\text{CO}_2} = 20 \text{ mm Hg}$. From the Henderson-Hasselbalch equation (7) the true bicarbonate concentration at this point is calculated as 23.2 mequiv./l , and from equation (10) $d \log P_{\text{CO}_2}/d\text{pH} = -1.044 - 3.2/23.2 = -1.182$. So the difference from the slope of the standard bicarbonate line amounts to only 1%.

SUMMARY

In a pH-log P_{CO_2} diagram separated plasma lines have a slope which is practically independent of the albumin/globulin ratio and the total protein content. The bicarbonate concentration has an effect but it makes a negligible difference if the standard bicarbonate concentration is taken instead of the true bicarbonate concentration.

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