

### 39. A New Analytical Formula for Hill's Equation

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The oxygen equilibrium of myoglobin<sup>1)</sup> accords completely with the mass action considerations for the reaction,  $\text{Mb} + \text{O}_2 = \text{MbO}_2$ , i.e., if we plot the percentage oxygenation against the partial pressure of oxygen we obtain the theoretical rectangular hyperbola. Whereas in the case of vertebrate hemoglobin, of which that from mammals has been chiefly studied, and other respiratory pigments such as hemerythrin<sup>2)</sup>, the curve is sigmoid in shape and can be described with a fair degree of accuracy by Hill's empirical equation<sup>3)</sup>,

$$Y = \frac{Kp^n}{1 + Kp^n} \quad (1)$$

Here  $Y$  denotes the partial degree of saturation with oxygen,  $p$  the partial pressure of the gas in equilibrium with the solution of the respiratory pigment.  $K$  is a constant characteristic to the individual respiratory pigment and depends upon the affinity of the pigment for oxygen. Another constant  $n$  expresses to a certain extent the facilitating interaction between the oxygen-combining centers. By this we mean that when an oxygen molecule combines with one center it increases the likelihood that a second oxygen will be attracted to another. When  $n=1$  the formula (1) reduced to a formula to be represented by a rectangular hyperbola signifying no appreciable facilitating interaction. Usually  $n$  is greater than unity as, for example, in purified horse hemoglobin, for which  $n=2.8$ .<sup>4)</sup>

From the experimental point of view, it is reasonable to plot  $Y$  against  $\log p$  instead of against the pressure itself, since the percentage error  $\delta p/p$  rather than the absolute error  $\delta p$  is nearly uniform over the whole pressure range. In fact many workers<sup>5)</sup> prefer  $\log p$  to  $p$  as the abscissa, though the corresponding analytical formula has not been given as yet. The present writer wishes to propose a new analytical formula which is mathematically equivalent to the equation (1) and meets this experimental requirements. Namely,

$$Y = \frac{1}{2} + \frac{1}{2} \tanh\left(\frac{n}{2} \ln \frac{p}{p_{1/2}}\right), \quad (2)$$

where  $p_{1/2}$  is the partial pressure of oxygen in equilibrium with the solution in which the amount of oxygenated hemoglobin is just the same as that of the deoxygenated form. The equation (2) follows a hyperbolic tangent curve, irrespective of the value of  $n$ . Hence any

curve obeying the Hill's equation can be brought into coincidence with each other by shifting the origin of the abscissa and by the change of its scale. The apparent difference between the arc of a rectangular hyperbola for myoglobin and the sigmoid curve for hemoglobin disappears completely.

The hyperbolic tangent curve has a center of symmetry and the deviation from this symmetry reveals very clearly the denaturation of hemoglobin. Wyman and others<sup>6)</sup> demonstrated that in the case of a fresh hemoglobin solution  $Y$  versus  $\log p$  curve has a center of symmetry, whereas for denatured hemoglobin solution the part of the curve at the lower left branch is higher than the value predicted by the equation (2). The sigmoid curve of the original Hill's equation has no center of symmetry and the denaturation is not to be seen so clearly as in the present equation (2). It will be pointed out that the theoretical formula proposed by Pauling<sup>7)</sup> has a center of symmetry and does not apply strictly to denatured specimens.

The equation (1) can be derived very easily from the equation (2). From the latter equation we have

$$\ln \frac{Y}{1-Y} = n(\ln p - \ln p_{1/2}). \quad (3)$$

Hence

$$\frac{Y}{1-Y} = \left( \frac{p}{p_{1/2}} \right)^n. \quad (4)$$

If we identify  $p_{1/2}^n$  with  $K$ , the equation gives directly the original Hill's equation.

It is very interesting to note that some of the equations in this derivation are similar in form to those employed in various other chemical phenomena, and this similarity enables us to unify and correlate these apparently independent phenomena from the common theoretical standpoint.

First let us consider for example a platinum electrode immersed in the solution of ferrous and ferric ions. The single electrode potential is given by the well-known formula,

$$E = E_0 + \frac{RT}{nF} \ln \frac{(\text{Fe}^{+++})}{(\text{Fe}^{++})} \quad (5)$$

If we rewrite it in the following form,

$$\ln \frac{(\text{Fe}^{++})}{(\text{Fe}^{+++})} = \ln \frac{(\text{Fe}^{++})/(\text{Fe})}{1 - (\text{Fe}^{++})/(\text{Fe})} = n \left( \frac{-EF}{RT} - \frac{-E_0F}{RT} \right), \quad (6)$$

where  $(\text{Fe})$  denotes the total concentration of iron ions, the similarity with the equation (3) is obvious.  $(\text{Fe}^{++})/(\text{Fe})$  is nothing but the partial degree of saturation of ferric ions with electrons.

Secondly we take up, as an example of the acid-base titration, the case of the addition of hydrochloric acid to the equivalent amount of

sodium acetate solution. Usually we plot the  $pH$  value as ordinate against the volume of the titer as abscissa. If we make the plot of the volume of the titer against minus  $pH$ , the titration curve can well be described by the equation (2), at least in the range near the inflection point, since we are tracing the whole process in which the acetate ions are saturated with hydrated protons to be converted into neutral acetate molecules. The analogy with the equation (3) is complete if we rewrite the formula for the  $pH$  value of the buffer solution,

$$pH = pK + \log \frac{(\text{Ac}^-)}{(\text{AcH})} \quad (7)$$

as

$$\log \frac{(\text{AcH})}{(\text{Ac}^-)} = (-pH) - (-pK). \quad (8)$$

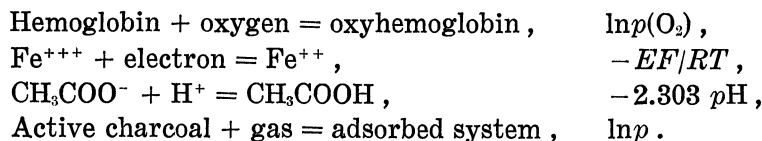
Lastly in the Langmuir's adsorption isotherm,

$$x = \frac{abp}{1 + bp}, \quad (9)$$

$a$  corresponds to the amount of adsorption at the limit of an infinitely high pressure and  $x/a$  is the degree of partial saturation. Therefore the equation represents the special case of the relation (1) with  $n=1$ . In fact it is known that in many cases the experimental data on adsorption can be more adequately described if we substitute  $p$  by  $p^n$ . This suggests that there are some facilitating interactions between adsorbing centers.

Many other phenomena belong to the same category, for example the absorption of oxygen by inorganic complex salts and the inhibition of enzyme action or bacterial growth by poisons, for which the Hill's equation or its equivalent form has already been employed.

These prevailing analogy is not surprising since all the phenomena considered above are saturation processes in addition reactions. The correspondence will be seen clearly in the following table.



Therefore all these phenomena can be treated in the same manner with the use of any of the equations from (1) to (4). The fact is that usually different equation is used for each of these phenomena, obscuring the intimate correlation underlying them. Hence the new analytical formula proposed here can be applied not only to the problem of respiratory pigments but also to the saturation phenomena of addition reactions in general.

### References

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