

Configuration of the hemoglobin oxygen dissociation curve demystified: a basic mathematical proof for medical and biological sciences undergraduates

Melvin Khee-Shing Leow

Department of Endocrinology, Division of Medicine, Tan Tock Seng Hospital,
and Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Submitted 16 February 2007; accepted in final form 18 April 2007

Leow MK. Configuration of the hemoglobin oxygen dissociation curve demystified: a basic mathematical proof for medical and biological sciences undergraduates. *Adv Physiol Educ* 31: 198–201, 2007; doi: 10.1152/advan.00012.2007.—The oxygen dissociation curve (ODC) of hemoglobin (Hb) has been widely studied and mathematically described for nearly a century. Numerous mathematical models have been designed to predict with ever-increasing accuracy the behavior of oxygen transport by Hb in differing conditions of pH, carbon dioxide, temperature, Hb levels, and 2,3-diphosphoglycerate concentrations that enable their applications in various clinical situations. The modeling techniques employed in many existing models are notably borrowed from advanced and highly sophisticated mathematics that are likely to surpass the comprehensibility of many medical and bioscience students due to the high level of “mathematical maturity” required. It is, however, a worthy teaching point in physiology lectures to illustrate in simple mathematics the fundamental reason for the crucial sigmoidal configuration of the ODC such that the medical and bioscience undergraduates can readily appreciate it, which is the objective of this basic dissertation.

sigmoidal curve; horizontal asymptote; point of inflexion

THE OXYGEN DISSOCIATION CURVE (ODC) of hemoglobin (Hb) as it is taught in physiology lectures has profound clinical importance, being applicable in numerous situations of health and disease, such as in the neonatal period (16), aging (10), hemorrhage (13), hemoglobinopathies (9), septic shock (22), diabetes mellitus (5), oxygenation of tumor tissues (21), sickle cell disease (6), carbon monoxide intoxication (4), anesthesia (12), open heart surgery (7), and respiratory failure (20), just to highlight a few. A close observation of its sigmoidal shape (Fig. 1) quickly reveals a couple of unique properties, namely, that oxygen saturation (Sa_{O_2}) approaches a horizontal asymptote as the oxygen tension exceeds 70 mmHg, while it declines precipitously down the steep slope toward a point of inflexion when the oxygen tension falls off the “shoulder” of the ODC below ~60 mmHg.

Such clinical implications are undeniably well known to many students and health professionals alike. Because the majority of students can see how the shape of the ODC accounts for the oxygenation behavior of Hb and grasp the physiological and clinical implications of a shift to the left or right of the ODC depending on circumstances, it is dubious if there is any extra value or relevance in trying to impart a deeper understanding into the “mathematical mechanisms” surrounding the ODC. Arguably, though, if our philosophy as

educators has always been that of actively promoting academic excellence, then every devoted and ardent teacher should serve the interest of students to advance in higher learning by delving further into abstruse topics through “made simple” pedagogical approaches using elementary yet enlightening alternative explanations with the objective of fostering academic curiosity and a perpetual penetrating passion for challenging subjects in these young and maturing minds. So, it could be beneficial to illustrate with rudimentary mathematical concepts that capture the plain sigmoidal feature of the ODC without venturing into complicated and confusing models for educational purposes.

The present-day mathematical models that describe the ODC of Hb employ highly sophisticated mathematics with resulting complexity that frequently eludes complete understanding by medical students and biological sciences undergraduates. This usually results in little or superficial comprehension of the conundrum behind the crucial sigmoidal shape of the ODC. Hence, the shape of the ODC is frequently assumed as a fact without further question. Nevertheless, it is worthy to simplify and strip down the complex models of the ODC so as to distill only the most basic mathematical concepts from fundamental thermodynamic and biochemical kinetics principles that would still allow any student to grasp the derivation of the sigmoidal essence of the ODC.

My personal experience lecturing medical students, interns, residents, and fellows over the years using only high school mathematics to explain the shape of the Hb ODC was very instructive in itself, as it showed me that many who followed the mathematical reasoning were overwhelmed with a deep sense of satisfaction that came with renewed understanding, and thus well rekindled their appreciation of the ODC much better than they ever did before. The notion that many medical students, perhaps, have chosen to enter medical schools to escape mathematics and the harder sciences remains debatable and yet might have some element of truth. However, in my contacts with students, I found it equally undeniable that a good proportion of them had commented that they could finally figure out how the basic oxygenation process of Hb should naturally lead to an equation of the form that bears a sigmoidal curve following a demonstration of a straightforward mathematical derivation. Interestingly, there had even been anecdotal instances in which students who never truly enjoyed mathematics had paradoxically remarked how they were positively marveled by the mathematical illustration that allowed them to finally “see” why the ODC should be sigmoidal and how that experience itself had inspired them and fueled their interest to consider such approaches for other physiological processes as well.

Address for reprint requests and other correspondence: M. K.-S. Leow, Dept. of Endocrinology, Div. of Medicine, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433 (e-mail: mleowsj@massmed.org).

Hemoglobin-Oxygen Dissociation Curves at 3 different pH levels

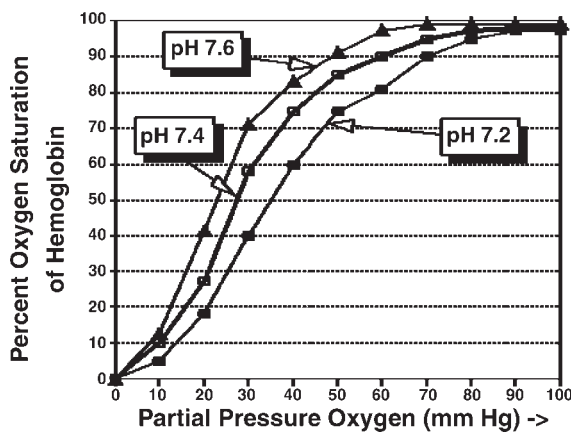


Fig. 1. Oxygen dissociation curves (ODCs) for human hemoglobin (Hb) at 3 different pH levels. The “S” shape of the curves is due to the fact that Hb begins to absorb O₂ rapidly when O₂ levels are between 20 and 40 mmHg. The Bohr effect is illustrated here by the shift of the curve to the right as pH decreases. [Reproduced, with kind permission, from Prof. Dave McShaffrey (<http://www.marietta.edu/~mcshaffd>).]

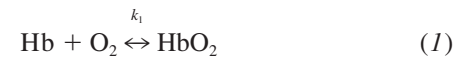
A Brief History of Mathematical Models of the ODC of Hb

One of the earliest mathematical descriptions of the ODC of Hb is the well-known Hill's equation, a simple equation proposed in 1910 by Archibald Vivian Hill (1886–1977), a British physiologist and Nobel laureate in Physiology or Medicine (8) (Fig. 2). Following this, other models, such as Adair's equation and Margaria's equation, which took into account the affinity of the heme ferrous moiety for the fourth molecule of oxygen being some 125 times that of the first 3 reactions, emerged and remain very useful today but may appear somewhat unwieldy to nonmathematicians (2,14). Soon after, the original Hill's equation was revisited and modified more accurately by John Severinghaus, who incorporated additional terms that reflected the cooperativity kinetics of virtually simultaneous binding of the last three oxygen molecules through favorable steric conformational changes (17).

Over time, the modeling of the Hb-oxygen interaction has increasingly relied on advanced mathematics to describe the ODC with yet greater accuracy. These include models that employed partial differential equations (15), hyperbolic trigonometrical functions based on known dynamic structural changes during oxygenation and deoxygenation (3,18), and the equations of Ackers and Halvorson (1). Still other formulas, such as the Kelman's equation, have been devised that are also highly precise in the prediction of Sa_{O₂} for any given oxygen tension, but which are totally empirical and not based on any physiological principles, and would therefore not offer any additional insights to its special configuration (11). These models are unquestionably more complex, and their comprehensibility by students is correspondingly diminished. As such, it is helpful to introduce students of physiology and biomedicine to the ODC by using only the simplest mathematical principles to achieve better understanding, from which the more discerning and interested students with greater mathematical aptitude may subsequently delve further to more complex models if they so desire.

Derivation of the Oxyhemoglobin ODC

Consider the oxygenation of a Hb molecule as four sequential steps, given that each of the four heme groups within the two α-globin and two β-globin chains binds to a molecule of oxygen. Thus,



From both a thermodynamic and kinetic perspective, the association constants (k_1 , k_2 , k_3 , and k_4 , respectively) can be represented by Eqs. 5–8 according to the law of mass action as follows:

$$k_1 = \frac{[\text{HbO}_2]}{[\text{Hb}][\text{O}_2]} \quad (5)$$

$$k_2 = \frac{[\text{HbO}_4]}{[\text{HbO}_2][\text{O}_2]} = \frac{[\text{HbO}_4]}{k_1[\text{Hb}][\text{O}_2]^2} \quad (6)$$

$$k_3 = \frac{[\text{HbO}_6]}{[\text{HbO}_4][\text{O}_2]} = \frac{[\text{HbO}_6]}{k_1 k_2 [\text{Hb}][\text{O}_2]^3} \quad (7)$$

$$k_4 = \frac{[\text{HbO}_8]}{[\text{HbO}_6][\text{O}_2]} = \frac{[\text{HbO}_8]}{k_1 k_2 k_3 [\text{Hb}][\text{O}_2]^4} \quad (8)$$

and, therefore,

$$K = k_1 k_2 k_3 k_4 = \frac{[\text{HbO}_8]}{[\text{Hb}][\text{O}_2]^4} \quad (9)$$

where K is the net association constant for the overall reversible reaction of oxygenation of Hb.



Hill's equation

$$S_{\text{HbO}_2} = \frac{[\text{HbO}_2]}{[\text{Hb}]}$$

$$= \frac{K_{\text{HbO}_2} [\text{O}_2]^n}{1 + K_{\text{HbO}_2} [\text{O}_2]^n}$$

Fig. 2. Archibald Vivian Hill (1886–1977). S_{HbO_2} , saturation of HbO₂; K_{HbO_2} , net association constant of HbO₂; n , Hill coefficient. [Reproduced, with permission, from the Nobel Foundation (<http://nobelprize.org/>).]

$$\Rightarrow [\text{HbO}_8] = K [\text{Hb}] [\text{O}_2]^4 \quad (10)$$

where K is the association constant of the reaction.

For simplicity, let us assume that all oxyhemoglobin in the circulation at any point in time is predominantly in the form of HbO_8 molecules without any significant contributions from intermediate species of partially oxygenated Hb molecules.

Thus,

$$\begin{aligned} \text{Total Hb} &= \text{Total deoxygenated Hb} + \text{total oxyhemoglobin} \\ &= [\text{Hb}] + [\text{HbO}_8] \\ &= [\text{Hb}] + K[\text{Hb}] [\text{O}_2]^4 \end{aligned} \quad (11)$$

$$\begin{aligned} \text{Let } \text{Sa}_{\text{O}_2} (\%) &= \frac{\text{total oxyhemoglobin}}{\text{total Hb}} \\ &= \frac{[\text{HbO}_8]}{[\text{Hb}] + K[\text{Hb}] [\text{O}_2]^4} \\ &= \frac{K [\text{Hb}] [\text{O}_2]^4}{[\text{Hb}] (1 + K [\text{O}_2]^4)} \end{aligned} \quad (12)$$

$$\Rightarrow \text{Sa}_{\text{O}_2} = \frac{K [\text{O}_2]^4}{1 + K [\text{O}_2]^4}$$

This expression is readily identifiable as having a form similar to the Hill's equation.

Let $x = [\text{O}_2] = \text{Po}_2$, where Po_2 is the partial pressure of oxygen, and let $y = \text{Sa}_{\text{O}_2}$, such that we can then represent Eq. 12 algebraically with y as a function of x as follows:

$$\therefore y = f(x) = \frac{Kx^4}{1 + Kx^4} \quad (13)$$

The configuration of this mathematical relation can be solved by determining the presence of any turning points, points of inflexion, and horizontal or vertical asymptotes.

Hence, differentiating y with respect to x yields:

$$\frac{dy}{dx} = \frac{4Kx^3}{(1 + Kx^4)^2}$$

$$\Rightarrow \text{if } x > 0, \quad \frac{dy}{dx} > 0$$

Also, when $x = 0$, $dx/dy = 0$, which indicates that a minimum extremum (i.e., minimum turning point) exists at $x = 0$.

When $x \rightarrow \infty$, $dx/dy \rightarrow 0$, indicating that the curve approaches a horizontal asymptote at large values of x . In the actual situation, this horizontal asymptote corresponds to a Sa_{O_2} of 100% at standard conditions of temperature, pH, and pressure.

Thus,

$$y = \lim_{x \rightarrow \infty} f(x) = 100\% = \text{horizontal asymptote}$$

We next examine the second derivative of x to determine the existence of any points of inflexion along the curve. Hence, differentiating dy/dx with respect to x yields:

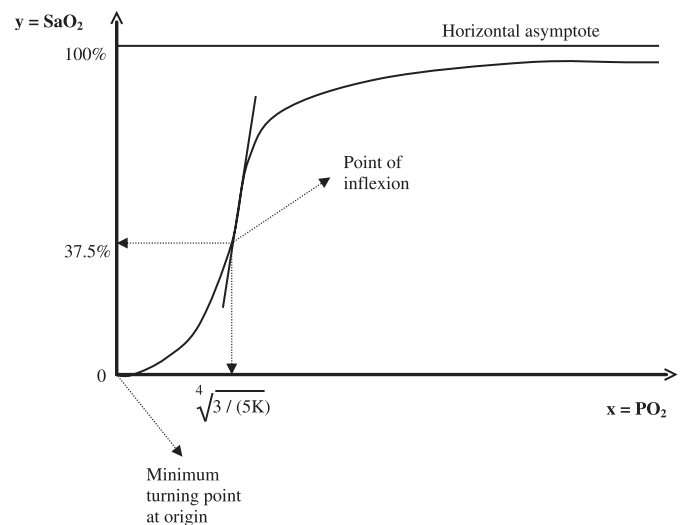


Fig. 3. Illustration of the sigmoidal configuration of the ODC as constructed by sketching based on the mathematical deductions as highlighted in the text using only basic algebra and differential calculus techniques. Sa_{O_2} , oxygen saturation.

$$\frac{d^2y}{dx^2} = \frac{12Kx^2(1 + Kx^4)^2 - 32K^2x^6(1 + Kx^4)}{(1 + Kx^4)^4}$$

$$\text{When } \frac{d^2y}{dx^2} = 0, \quad 12Kx^2(1 + Kx^4)^2 = 32K^2x^6(1 + Kx^4)$$

$$\Rightarrow 1 + Kx^4 = (8/3)(Kx^4) \quad (14)$$

$$\Rightarrow x^4 = 3/(5K)$$

$$\therefore x = \sqrt[4]{3/(5K)}$$

Since K is positive, x is positive when $d^2y/dx^2 = 0$. Thus, when

$$x = \sqrt[4]{3/(5K)}$$

$$\Rightarrow \frac{dy}{dx} = \frac{4(3/5)^{3/4}(K)^{1/4}}{(64/25)} = (1.07)(K)^{1/4} \cong (K)^{1/4}$$

Therefore, when $d^2y/dx^2 = 0$, the gradient [i.e., $dy/dx \cong (K)^{1/4}$] at this point of inflexion is positive.

Because $dy/dx > 0$ when $x > 0$, this implies that when $x = 3/(5K)$, a nonstationary rising point of inflexion occurs with the derivative at that point having a positive gradient. In addition, the derivative of y is also positive on both sides of this point.

At this point of inflexion, the value of y can be shown to be $\sim 40\%$, as follows:

$$y = \frac{K(3/5K)}{1 + K(3/5K)}$$

$$\Rightarrow y = (3/5)(5/8) = 0.375 \cong 0.4$$

$$\Rightarrow \text{Sa}_{\text{O}_2} \cong 40\% \text{ at the predicted point of inflexion.}$$

Also, based on actual data on normal human Hb (HbA), the association constants of oxygen are $2.0 \pm 1.1 \times 10^{-6}$ and $2.9 \pm 1.4 \times 10^{-6} \text{ M}^{-1}$ for the α -chain ($K\alpha$) and the β -chain ($K\beta$) of

Hb, respectively (19). The overall association constant is taken as the geometric mean of $K\alpha$ and $K\beta$, that is,

$$K = \sqrt{(K\alpha \times K\beta)} = 2.4 \times 10^{-6} \text{ M}^{-1}$$

Substituting this value of K into Eq. 14, it can be predicted from this model that the PO_2 at the point of inflexion is as follows:

$$x = \sqrt[4]{(3/1.2 \times 10^{-5})} = 22.4 \text{ mmHg (Torr)}$$

In human experimental ODC data such as those shown in Fig. 1, the SaO_2 when the point of inflexion occurs at a pH of 7.4 lies in the region between ~30% and 50%, which comes fairly close to our calculated value. Also, the value of the oxygen tension at that point of inflexion falls between ~20 and 25 mmHg, which agrees quite well with our calculated value of 22.4 mmHg. It is crucial to distinguish the point of inflexion of the ODC from the point at which the oxygen tension corresponds to a SaO_2 of 50%. Figure 3 illustrates the shape of the ODC as predicted from the mathematical reasoning and calculations as depicted above.

Conclusions

Although it is clear that only the most accurate mathematical models that predict the variables of the ODC robustly will find their ways into actual medical applications, such as being utilized clinically in commercial blood gas analyzers for automated computerized calculations of the various blood gas parameters within the austere environments of intensive care units that manage the critically ill, it is still worthwhile exploring the first principles and simple concepts that lead to the derivation of an “oversimplified” model that still reveals the basic sigmoidal shape of the ODC for educational purposes, which is the discourse of this article.

For didactic purposes in this article, meant to illumine students of physiology, medicine, and biological sciences, as many of the intermediate steps of calculations as possible are deliberately shown in this minitreatise to take the students through the process of deriving the equations of interest, as it is a general observation that many students often face difficulties, albeit to varying degrees, in deciphering how an initial mathematical axiom leads to the final concluding formulas without illustrating clearly the process of derivation. Thus, unlike a paper targeted at a mathematical audience, this article is pitched to students of biomedicine who do not major in mathematics and therefore makes no assumptions that such intermediate steps should be understood and simply omitted in the interest of space and time. It is hoped that this basic mathematical proof of the sigmoidal shape of the ODC can be useful to both students and teachers of physiology alike as they embark on their journey in deeper appreciation of the nature of Hb-oxygen interactions.

ACKNOWLEDGMENTS

The author is grateful to Sim Joo Tan for the patient secretarial assistance, to Rachel May-Wern Leow for discussion of the outline and invaluable

pointers with regard to simplifying the mathematical derivation to maximize facilitation of teaching, and to Veronica May-Gwen Leow and Abigail May-Shan Leow for the wonderful inputs and encouragements.

REFERENCES

1. Ackers GK, Halvorson HR. The linkage between oxygenation and subunit dissociation in human hemoglobin. *Proc Natl Acad Sci USA* 71: 4312–4316, 1974.
2. Adair GS. The hemoglobin system. VI. The oxygen dissociation curve of hemoglobin. *J Biol Chem* 63: 529, 1925.
3. Anstey C. A new model for the oxyhemoglobin dissociation curve. *Anaesth Intensive Care* 31: 376–387, 2003.
4. Bruce EN, Bruce MC. A multicompartment model of carboxyhemoglobin and carboxymyoglobin responses to inhalation of carbon monoxide. *J Appl Physiol* 95: 1235–47, 2003.
5. Ditzel J, Standl E. The problem of tissue oxygenation in diabetes mellitus. *Acta Med Scand Suppl* 578: 59–68, 1975.
6. Gill SJ, Skold R, Fall L, Shaeffer T, Spokane P, Wyman J. Aggregation effects on oxygen binding of sickle cell hemoglobin. *Science* 201: 362–364, 1978.
7. Glynn MF, Cornhill F. Studies on the relationship between the equilibrium curve of oxyhemoglobin and 2,3-diphosphoglycerate in open-heart surgery. *Can J Surg* 18: 73–76, 1975.
8. Hill AV. The possible effects of the aggregation of molecules of hemoglobin on its dissociation curve. *J Physiol (Lond)* 40: 4, 1910.
9. Imai K, Tientadakul P, Opartkiattikul N, Luene P, Winichagoon P, Svasti J, Fucharoen S. Detection of haemoglobin variants and inference of their functional properties using complete oxygen dissociation curve measurements. *Br J Haematol* 112: 483–487, 2001.
10. Ivanov LA, Chebotarev ND. Effect of hyperoxia on the oxyhemoglobin dissociation curve in various periods of aging. *Biull Eksp Biol Med* 98: 156–158, 1984.
11. Kelman GR. Digital computer subroutine for the conversion of oxygen tension into saturation. *J Appl Physiol* 21: 1375–1376, 1966.
12. Lanza V, Mercadante S, Pignataro A. Effects of halothane, enflurane, and nitrous oxide on oxyhemoglobin affinity. *Anesthesiology* 68: 591–594, 1988.
13. Malmberg PO, Hlastala MP, Woodson RD. Effect of increased blood-oxygen affinity on oxygen transport in hemorrhagic shock. *J Appl Physiol* 47: 889–895, 1979.
14. Margaria R. A mathematical treatment of the blood dissociation curve for oxygen. *Clin Chem* 11: 745–762, 1963.
15. Mochizuki M, Kagawa T. Numerical solution of partial differential equations describing the simultaneous O_2 and CO_2 diffusions in the red blood cell. *Jpn J Physiol* 36: 43–63, 1986.
16. Oski FA. Clinical implications of the oxyhemoglobin dissociation curve in the neonatal period. *Crit Care Med* 7: 412–418, 1979.
17. Severinghaus JW. Simple, accurate equations for human blood O_2 dissociation computations. *J Appl Physiol* 36: 599–602, 1979.
18. Siggaard-Anderson O, Wimberley PD, Gothgen I, Siggaard-Anderson M. A mathematical model of the hemoglobin-oxygen dissociation curve of human blood and of the oxygen partial pressure as a function of temperature. *Clin Chem* 30: 1646–1651, 1984.
19. Vandegriff KD, Bellelli A, Samaja M, Malavalli A, Brunori M, Winslow RM. Kinetics of NO and O_2 binding to a maleimide poly(ethylene glycol)-conjugated human hemoglobin. *Biochem J* 382: 183–189, 2004.
20. van der Elst AM, van der Werf T. Some circulatory aspects of the oxygen transport in patients with emphysema. *Respiration* 48: 310–320, 1985.
21. Wang HW, Putt ME, Emanuele MJ, Shin DB, Glatstein E, Yodh AG, Busch TM. Treatment-induced changes in tumor oxygenation predict photodynamic therapy outcome. *Cancer Res* 64: 7553–7561, 2004.
22. Watkins GM. The left shifted oxyhemoglobin curve in sepsis: a preventable defect. *Ann Surg* 180: 213–220, 1974.