BD Biosciences

Technical Bulletin #443
Calculating Oxygen Concentration from Fluorescence Data on the BD™ Oxygen Biosensor System

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Introduction

The oxygen-sensitive fluorophore in the BD™ Oxygen Biosensor System (BD™ OBS) is one whose fluorescence is quenched by molecular oxygen in a predictable fashion. For many applications, knowing the relative concentration of oxygen is sufficient. For certain applications, it may be advantageous to be able to calculate actual oxygen concentrations from the fluorescence. Understanding the theory which relates oxygen concentration to signal will allow the user to make such calculations.

Stern-Volmer Theory

For the fluor used in the BD OBS, and others like it, fluorescence is inversely proportional to the concentration of oxygen in the vicinity of the fluor. The behavior of fluorophores quenched this way follows a mathematical model called the Stern-Volmer equation. At its simplest, the Stern-Volmer equation takes the following form:[1]

[1] $I_0 / I = 1 + K_{SV} * [O_2]$

where $[O_2]$ is the oxygen concentration, K_{SV} is the Stern-Volmer constant, I is the fluorescence intensity at $[O_2]$ and I_0 is the fluorescence intensity at the reference condition (zero oxygen).

Definitions

Normalized Relative Fluorescence (NRF)

The ratio of fluorescence at the oxygen concentration of interest to that at the ambient reference condition. NRF = I / I_A, where I indicates fluorescence intensity, and the A subscript represents "ambient".

Dynamic Range (DR)

The maximum possible signal occurs at zero oxygen, so the DR is the ratio of fluorescence at zero oxygen to that at ambient oxygen, or DR = I_0 / I_A. For a given set of experimental conditions (temperature, wavelengths, etc.), DR will be a constant. To convert fluorescence to actual oxygen concentration, it is necessary to know DR.

Use of Ambient as the Reference Condition

Zero oxygen has traditionally been chosen as the reference condition because it yields the simplest form of ***Equation 1***, although the choice is entirely arbitrary. Because it is impractical to collect fluorescence intensities in a zero-oxygen environment, "ambient" oxygen is used as the reference condition. Thus, we normalize fluorescence intensities to those at ambient oxygen concentration instead of zero oxygen (see definition of "NRF" above).

The use of an alternate reference condition changes the form of ***Equation 1*** slightly, but not its fundamental essence, as will be shown in the following discussion. At ambient $[O_2]$, the Stern-Volmer equation takes the form:

[2] $I_0 / I_A = 1 + K_{SV} * [O_2]_A$

where the subscript "A" designates ambient.

I_0 / I_A = DR, so ***Equation 2*** can be rearranged to yield:

[3] $K_{SV} = (DR - 1) / [O_2]_A$

Thus, by measuring DR, it is possible to calculate K_{SV}. It can be seen that DR and K_{SV} are related constants.

Furthermore, it can be shown that the ratio of DR to NRF is equivalent to I_0 / I. Substituting this into ***Equation 1*** yields:

[4] $DR / NRF = 1 + K_{SV} * [O_2]$

Equation 4 is equivalent to ***Equation 1***. Solving for NRF yields:

[5] $NRF = DR / (1 + K_{SV} * [O2])$

Equation 5 is the expression of the Stern-Volmer law pertinent to typical applications of the BD OBS. It relates the NRF signal to oxygen concentration. It can be seen from ***Equation 5*** that fluorescence (NRF) varies inversely with $[O_2]$, and that $[O_2]$ may be calculated from NRF if K_{SV} and DR are known.

continued

How to Determine DR and K_{SV}

DR may be determined by a simple experiment, or from the sodium sulfite positive control well (100 mM sodium sulfite in PBS buffer) we recommend for each plate. Dynamic range will be the ratio between the signal once it has reached its maximum (typically 30-60 minutes) and that same well's fluor-escence at ambient conditions. Stated another way, DR is the NRF of the sulfite well, once the sulfite has removed all the oxygen from the well. DR may then be used to calculate K_{SV}, per ***Equation 3***.

DR is a characteristic property of the sensor and how it is formulated, and should thus be constant for all wells. Hence, it is possible to calculate DR for a plate from a single well. DR does vary slightly with temperature and from reader to reader, however, so it should be calculated on the same reader, by the same method and under the same experimental conditions as the experi-mental data it will be used to analyze.

A typical value of DR is 6, which yields a K_{SV} of 23.9 atm^{-1} (i.e., for $[O_2]_A$ = 0.209 atm) or 0.031 mmHg^{-1} (for $[O_2]_A$ = 159 mmHg).

Calculating Oxygen Concentrations

Using ***Equation 5***, it is possible to convert fluorescence values into actual oxygen concentrations as shown in ***Figure 1***. To solve for $[O_2]$, simply rearrange ***Equation 5*** to yield:

[6] $[O_2] = (DR / NRF - 1) / K_{SV}$

Thus, it is possible to calculate $[O_2]$ from NRF, provided one knows the values of DR and K_{SV}. These two constants (defined above) may be determined experimentally from a single experiment.

Because BD™ OBS is in equilibrium with the atmosphere, it should be noted that the concentration of oxygen in the well depends on the rate at which it is being consumed by the content of the well (e.g., the cells), as well as the rate at which oxygen diffuses in from the atmosphere. We are still developing a simple method that will allow one to factor out the diffusion term and thereby determine the rate of oxygen consumption in the well.

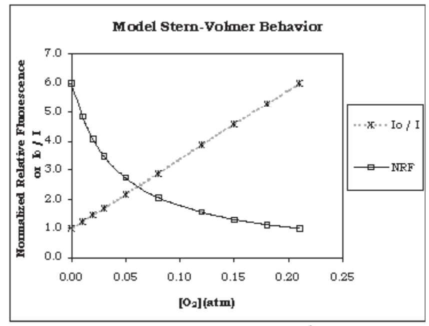

Figure 1*: Theoretical response of a Stern-Volmer fluorophore (such as that utilized in the BD Oxygen Biosensor System) to varying concentrations of oxygen. It can be seen that signal varies inversely with oxygen concentra-tion. This example was calculated using the equations outlined in the text, assuming DR = 6.*

Reference

1. Stern, O. and Volmer, M., Phys. Z., **20**:183 (1919).

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S01F063R2 - Rev. 3