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Algorithm for an Implantable Fluorescence Based Glucose Sensor

Xiaolin Wang, Colleen Mdingi, Andrew DeHennis, Arthur E. Colvin

Abstract— **This article describes the algorithm for a continuous glucose monitor (CGM). The CGM system consists of an external reader and an insertable fluorescence based sensor. The sensor consists of a miniaturized optical sensor that incorporates a biocompatible macromolecular indicator that selectively binds glucose. It is designed to be subcutaneously inserted and allows for the direct measurement of interstitial fluid (ISF) glucose.**

I. Introduction

A fluorescent, glucose sensing copolymer material integrated with a wireless optical spectroscopy interface has been developed. This biocompatible and stable polymer, grafted onto the surface of our miniaturized optical sensor platform, is designed to allow for the direct measurement of ISF glucose after subcutaneous implantation. Herein, we describe a proprietary preliminary algorithm that converts the signal received by the photodetector into glucose concentration. The algorithm incorporates the optics, electronics and the chemistry of the sensor. The paper also describes *in vivo* feasibility analysis of the performance of the algorithm.

II. Basis of The Algorithm

The inputs to the algorithm are the raw signal, parameters measured during manufacturing of each sensor, and parameters characterized using *in vitro* and *in vivo* tests. The intermediate output from the algorithm is a much purified signal derived from glucose modulated indicator fluorescence that is normalized (Sn) and directly proportional to glucose concentration. The final output from the algorithm is the glucose concentration calculated using Sn and an interpretive algorithm.

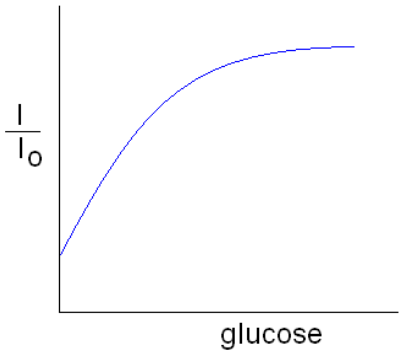

Figure 1. Indicator normalized modulation versus glucose concentration

X. Wang, C. Mdingi, A. Dehennis and A. Colvin are with Sensors for Medicine and Science, Inc., Germantown, MD 20876 USA (corresponding author phone: 301-556-1614; fax: 301-515-0988; e-mail: xwang@s4ms.com).

The entire signal processing cascade is based on the well-known relationship of Fig. 1, I is the fluorescence from the glucose sensor indicator and I_0 is baseline fluorescence at zero glucose concentration. The percent modulation I/I_0 versus glucose concentration is constant throughout the life of the glucose sensor (indicator). The end of life of the glucose sensor arises when the signal to noise ratio declines over time to a point where the error specification can no longer be maintained. The raw signals from the sensor, as captured, contain noise, offset, and distortions, which are not related to actual glucose modulation of the indicator. The fluorescent amplitude of the indicator, as well as some elements of the electronic circuitry within the sensor, are also temperature sensitive. The Sn equation purifies and normalizes the signal by removing the non-glucose-modulated offset/distortion of the signal, and corrects for temperature sensitivity.

Once Sn is calculated, an interpretive algorithm is used to convert Sn into glucose concentration. The interpretive algorithm is derived through a standard curve, which is the basis for the overall calibration design. This standard curve is based on the reaction:

$$A + B \longleftrightarrow BA \tag{1}$$

Where, A is glucose indicator, B is glucose and BA is glucose-indicator complex. The fluorescence of the indicator increases upon binding glucose.

The following sections will describe Sn algorithm and the interpretive algorithm in details.

III. Sn Equation

The simplest form of Sn equation is that

$$Sn = \frac{I}{I_0} \tag{2}$$

Where I is the fluorescence from the glucose indicator and I_0 is baseline fluorescence at zero glucose concentration. The raw signal measurement data contains offset and all the distortion producing subspecies of Fig. 2.

$$Signal = I + Z + I_{distortion} \tag{3}$$

where I is the fluorescence from the glucose indicator, Z is an offset and $I_{distortion}$ is a distortion to the glucose indicator. In order to calculate I, Z and $I_{distortion}$ must be removed from the raw signal.

The Offset (*Z*) – the offset is hardware based. More specifically, it is due to the LED peak wavelength or optical band-pass filter tolerance. For LED light that is not absorbed by the indicator, mostly because it did not encounter an indicator molecule, that light is partially passed into the photodetector. The light (offset) is removed by Sn equation

since it is convoluted in the total light arriving at the photodetector. The offset is measured during the manufacturing of the sensor and it increases due to photobleaching. The Sn equation dynamically tracks the offset. The offset (Z) is used by the Sn equation to normalize each sensor's output to a numerical value equal to one at zero glucose concentration (Sn_{min}).

The Distortion ($I_{distortion}$) – the system distortion is chemistry (photochemistry) and kinetics based. The distortion is any non-glucose-modulated light arriving at the photodetector. Photo, thermal, and oxidative decay species emit fluorescent light that is not modulated by glucose. Most of the system distortion is due to various matrix species kinetically related to the parent indicator [BA] as shown in Fig. 2.

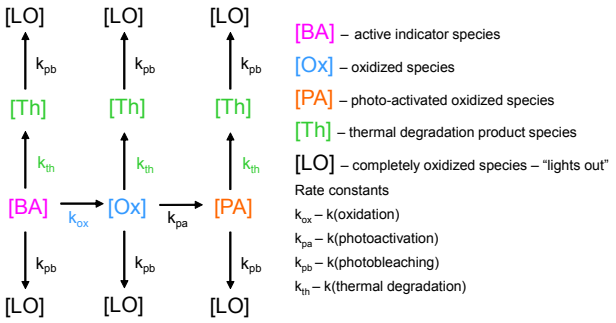

Figure 2. Reactions and kinetics

All colored species shown within Fig. 2 are fluorescent derivatives of the base indicator [BA], however, only [BA], the glucose-indicator complex, is a glucose modulated species, and therefore carries the important glucose concentration information that must be de-convoluted from the matrix. [Ox], [PA] and [Th] are all distortion-producing species. The non-glucose-modulated light from these species must be removed from the raw signal. The Sn equation tracks each of these distortion-producing species and removes them from the final signal that is ultimately provided to the interpretive algorithm.

Glucose indicator (BA): Within an in-vivo environment, the glucose indicator molecule undergoes a steady loss of signal amplitude over time. It is temperature sensitive like all fluorescent molecules [1]. Oxidation, thermal degradation, and photobleaching are the dominant mechanisms of the signal degradation. In addition, they are all chronic and predictable under a first order decay function on the loss of signal amplitude. Ultimately, this decay establishes the end of useful life for the overall sensor product. As a result of all three first order decay, set to the same measured value obtained for the glucose indicator within the in-vivo environment, the glucose indicator would be degraded by the superimpose of the 3 decay mechanisms.

Oxidized Indicator (Ox): under in-vivo oxidation pressure from ambient and normal reactive oxidation species (ROS), the glucose indicator progressively undergoes a highly specific oxidative de-boronation. This reaction removes the boronate recognition moiety of the indicator molecule. The resulting deboronated indicator is also fluorescent at a lower quantum efficiency and does not modulate. It is temperature sensitive [1] and decays due to photo activation, photobleaching, and thermal degradation.

Photo activated Indicator (PA): When the oxidized indicator Ox is photo activated, it produces a major product 'PA'. PA is fluorescent at a higher quantum efficiency than Ox and does not modulate. Similar to Ox, PA is temperature sensitive [1] and decays due to photobleaching and thermal degradation.

Thermally degraded Indicator (Th): BA, Ox and PA all thermally degrade. Similar to Ox and PA, the resulting thermally degraded indicator Th is also fluorescent at a lower quantum efficiency and does not modulate. Th is also temperature sensitive [1] and decays due to photobleaching.

Only modulated fluorescence, I, carries glucose concentration information within the system. The Sn equation must remove the offset (Z) and the un-modulated signal ($I_{distortion}$) from modulated signal (I). The effect of not removing these noise sources would compress the modulation shown in Fig. 1 (i.e., the Y-axis displacement from zero to infinite glucose). The Sn equation removes or compensates distortion inducing factors such that the processed signal is normalized and a constant value at infinite glucose concentration (Sn_{max} - asymptote as shown in Fig. 1). The simple form of Sn equation is therefore:

$$Sn = \frac{[Signal]_T - Z - I_{distortion}}{I_0} \qquad (4)$$

The expanded terms of the Sn Equation are as listed below.

$$I_0 = I_{0,QC} e^{-k_{ox} t_{ox}} e^{-k_{th} t_{th}} e^{-k_{pb} t_{pb}} \left[1 - (T - 37) c_f\right] \qquad (5)$$

Where I_0 is the fluorescence intensity of the glucose indicator at zero glucose concentration. $I_{0,QC}$ is I_0 obtained from manufacturing quality control (QC). k_{ox}, k_{th}, and k_{pb} are rates for oxidation, thermal degradation and photobleaching, respectively. t_{ox}, t_{th} and t_{pb} are oxidation time, thermal degradation time and photobleaching time, respectively. c_f is the temperature correction coefficient of glucose indicator. In actual execution, t_{pb} is tracked by using the cumulative LED-On time, t_{ox} and t_{ox} are the time since the sensor is implanted. $e^{-k_{ox} t_{ox}} e^{-k_{th} t_{th}} e^{-k_{pb} t_{pb}}$ is the glucose indicator decay due to the superimpose of oxidation, thermal degradation and photobleaching [2]. It is configured within the algorithm to kinetically track the first order decay loss of signal that occurs over time. The temperature, T, is recorded by the temperature sensor embedded inside the sensor.

$$[Signal]_T = Signal\left(1 + (T - 37) c_z\right) \qquad (6)$$

The Signal value is taken from the photodetector, this value is temperature corrected and becomes $[Signal]_T$ to compensate for the temperature sensitivity (c_z) of the LED (excitation) light.

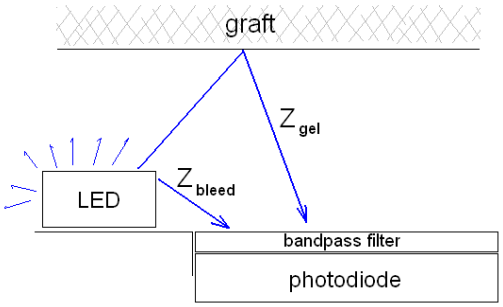

Figure 3. The offset Z

$$[Z] = Z_{gel}\left(1+\phi_Z\left(1-e^{-k_{pb}t_{pb}}\right)\right)+Z_{bleed} \quad (7)$$

Z_{gel} is the LED spillover component which is reflected from the gel (graft) to the photodetector (see Fig. 3). As the indicator is photo-bleached, the overall absorbance of the gel decreases, which increases the reflectance. ϕ_z is the percent increase of Z_{gel} when the indicator is fully photo-bleached.

The fluorescence from all the distortion-producing species is:

$$[I_{distortion}] = [Ox]+[Th]+[PA] \quad (8)$$

Where [Ox], [PA] and [Th] are fluorescence from Ox, PA and Th, respectively.

$$[OX] = I_{0,QC}\%F_{Ox}\left[\left(1-e^{-k_{ox}t_{ox}}\right)e^{-k_{th}t_{th}}e^{-k_{pb}t_{pb}}e^{-k_{pa}t_{pb}}\right]\left[1-(T-37)c_{OX}\right] \quad (9)$$

$$[Th] = I_{0,QC}\%F_{Th}\left[\left(1-e^{-k_{th}t_{th}}\right)e^{-k_{pb}t_{pb}}\right]\left[1-(T-37)c_{Th}\right] \quad (10)$$

$$[PA] = I_{0,QC}\%F_{PA}\left[\left(1-e^{-k_{ox}t_{ox}}\right)e^{-k_{th}t_{th}}e^{-k_{pb}t_{pb}}\left(1-e^{-k_{pa}t_{pb})}\right)\right]\left[1-(T-37)c_{PA}\right] \quad (11)$$

Where c_{ox}, c_{Th}, c_{PA} are the temperature correction coefficients of Ox, Th and PA respectively. $\%F_{Ox}$, $\%F_{Th}$ and $\%F_{PA}$ are the relative quantum efficiencies of Ox, Th and PA to the glucose indicator BA.

As seen from inspection of the Sn equation, at manufacturing when the sensor is new, the distortion producing subspecies (Ox, Th and PA) have not yet formed and contribute nothing significant to the initial signal at turn-on. Once the sensor is inserted *in vivo*, they will form progressively and the algorithm kinetically tracks the fluorescence from each subspecies.

IV. The Proprietary Interpretive Algorithm

The proprietary interpretive algorithm is a fairly simple and familiar relationship. It is taken from the first principles constant base relationship between percent modulation and glucose as shown in Fig. 4. It is based on the reaction in Equation (1) and converts Sn into glucose concentration.

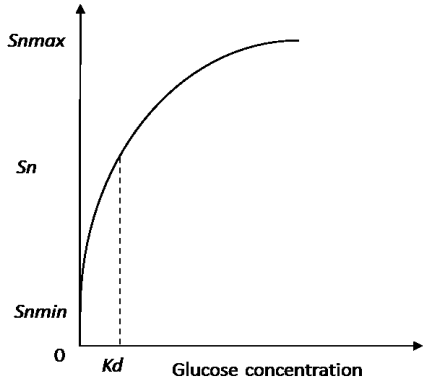

Figure 4. Illustration of the SMSI Interpretive Algorithm

The equilibrium expression for the dissociation defining *Kd* is

$$K_d = \frac{[A][B]}{[AB]} \quad (12)$$

The glucose concentration $[A]$ is

$$[A] = K_d\frac{[AB]}{[B]} \quad (13)$$

K_d is constant, $[AB]$ and $[B]$ terms must be determined from measurement – derivation that follows is to fulfill these two terms in order to calculate glucose concentration at any one measurement.

Define total fluorescent signal (fluorescence)

$$F = F_B + F_{AB} \quad (14)$$

Which states that the total fluorescence from the unbound indicator (F_B) and the glucose indicator complex (F_{AB}).

Using Beer's law [1] ,

$$F = I_e ebc\phi \quad (15)$$

Where F is fluorescence of the species, b is path length, I_e is excitation light, c is concentration of the fluorescer, e is molar extinction coefficient and Φ is quantum efficiency. By substituting specifically for the concentration terms for each A and AB

$$F = I_0 eb[B]\phi_B + I_0 eb[AB]\phi_{AB} \quad (16)$$

By defining

$$q_B = \phi_B([B]+[AB]) \quad (17)$$

$$q_{AB} = \phi_{AB}([B]+[AB]) \quad (18)$$

$$f_B = \frac{[B]}{[B]+[AB]} \quad (19)$$

$$f_{AB} = \frac{[AB]}{[B]+[AB]} \quad (20)$$

Equation (16) becomes,

$$F = I_e eb\left(f_B q_B + f_{AB} q_{AB}\right) \quad (21)$$

Since the zero glucose concentration condition is the lowest fluorescent signal value from the sensor, we define this point as F_{min}.

$$F_{\min} = I_e ebq_B \quad (22)$$

The opposite boundary condition is where glucose concentration is very high such that 99.99% of fluorescence signal is from the glucose indicator complex AB and no (approaching zero) signal from unbound indicator B. At

glucose saturation, the highest possible value of fluorescence is output from the sensor and defined as F_{max}

$$F_{\max} = I_e e b q_{AB} \tag{23}$$

By incorporating (22) (23) into (21), (21) becomes

$$F = F_{\min} f_B + F_{\max} f_{AB} = F_{\min} f_B + F_{\max}(1 - f_B) \tag{24}$$

Therefore,

$$f_B = \frac{F_{\max} - F}{F_{\max} - F_{\min}} \tag{25}$$

and

$$f_{AB} = 1 - f_B = \frac{F - F_{\min}}{F_{\max} - F_{\min}} \tag{26}$$

The glucose concentration $[A]$ is

$$[A] = K_d \frac{[AB]}{[B]} = K_d \frac{f_{AB}}{f_B} = K_d \frac{F - F_{\min}}{F_{\max} - F} \tag{27}$$

Since Sn is the normalized fluorescence, the glucose concentration $[A]$ is

$$[A] = K_d \frac{Sn - Sn_{\min}}{Sn_{\max} - Sn} \tag{28}$$

During sensor manufacturing, each sensor is cycled through a computer automated quality control measurement rig. This system measures key parameters (c_z, K_d, Sn_{max}, Z_{gel}, Z_{bleed}). The cycle includes operating newly manufactured sensors at two different temperatures (32C and 37C) at three different glucose concentrations – 0mM, 4.0mM and 18.0mM glucose. The automated system tracks the performance of each sensor manufactured under these changing conditions and makes specific measurements for each sequential temperature and concentration test. The other key parameters are developed from designed and controlled *in vitro* experiments (K_{pb}, K_{pa}, K_{th}, ϕ_z, c_f, c_{Th}, c_{ox}, c_{PA}, $\%F_{Ox}$, $\%F_{PA}$ and $\%F_{Th}$) and in vivo tests (K_{Ox}). These values are used by the Sn algorithm and the interpretive algorithm for each sensor according to their corresponding serial number.

V. The In Vivo Performance

Under an Institutional Review Board (IRB) approved Non-Significant Risk (NSR) study, 18 sensors were implanted into type-I diabetic subjects. Data was collected during 6 in-clinic sessions in a feasibility study to evaluate initial sensor performance and the accuracy of the algorithm *in vivo*. The sensors were removed 28 days after insertion. The Mean Absolute Relative Difference (MARD) for all the 18 sensors from day 3 data collection through day 28 is 13.7%. Day 0 data collection is excluded as the sensor is not fully responsive to glucose during the heal-up period. A total of 3466 paired data points were obtained to evaluate sensor performance. The reference is blood glucose measured by YSI machine. The Clarke Error Grid, Fig. 5, shows 3328 data points (96.02%) in the A & B range. Fig. 6 shows the performance of the algorithm on one sensor, it clearly shows that the sensor tracks the blood glucose well. The MARD for this sensor is 13.0%.

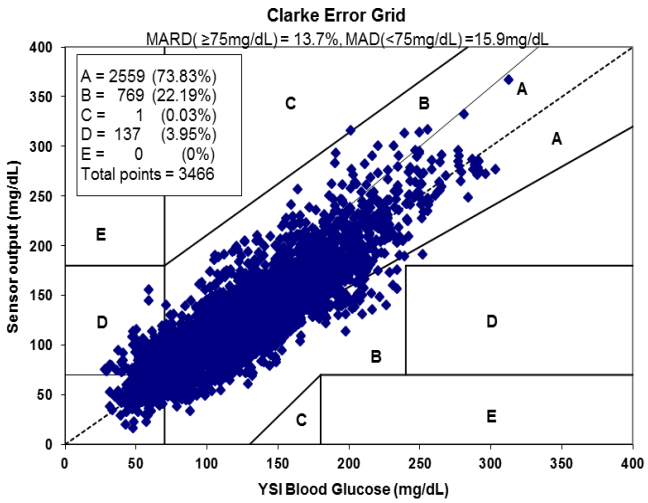

Figure 5. Clarke error grid of the 18 sensors implanted in Type I diabetic subjects

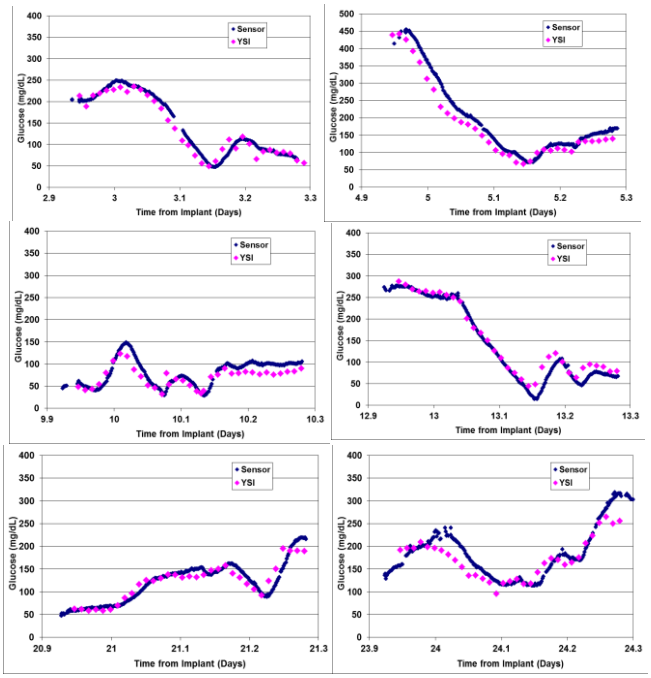

Figure 6. Performance of the algorithm on one sensor during 6 read sessions

Acknowledgment

The authors would like to thank our colleagues in the chemistry group for performing experiments to determine the reactions and kinetics, and to develop key parameters for the algorithm.

The authors would like to acknowledge that the study device is an investigational device limited to investigational use only.

References

[1] J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*. Kluwer Academic/Plenum Publishers, New York, 1999.

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