Dual-wavelength optical fluidic glucose sensor using time series analysis of α(+)-glucose measurement

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This paper presents a rising-edge time-series analysis (TSA) method that can be applied to a dual-wavelength optical fluidic glucose sensor (DOWFGS). In the experiment, the concentration of glucose in phosphate buffered saline (PBS) was determined by measuring the absorbance of the solution as determined by variation in the rising edge of the photodiode (PD) voltage response waveform. The DOWFGS principle is based on near-infrared (NIR) absorption spectroscopy at selected dual wavelengths (1450 and 1650 nm) in the first overtone band. The DOWFGS comprises two light-emitting diodes (LEDs) and two PD detectors. No additional fibers or lenses are required in our device. The output light level of the LEDs is adjusted to a light intensity suitable to the glucose absorption rate in an electronic circuit. Four light absorbance paths enable detection of α(+)-glucose concentrations from 0 to 20 wt % in steps of 5 wt %. The glucose light absorbance process was calculated based on the rising edge of the PD waveform under a low-intensity light source using TSA. The TSA method can be used to obtain the glucose level in PBS and reduce measurement background noise. The application of the rising-edge TSA method improves sensor sensitivity, increases the accuracy of the data analysis, and lowers measurement equipment costs. © 2016 The Japan Society of Applied Physics

1. Introduction

According to the International Diabetes Federation, more than 382 million people worldwide had diabetes in 2013,¹⁻³ and this number is expected to increase to 366 million by 2030.⁴⁻⁵ Studies have shown that the majority of patients with type 1 diabetes only measure their blood glucose levels once per day, which is insufficient to maintain tight control of blood glucose levels.⁶ Such patients should monitor their blood glucose levels on a regular basis to control their condition and detect anomalies. Continuous glucose monitoring (CGM) of blood glucose levels is important in diabetes patients so that patients can adjust their blood glucose levels using oral medication, diet, or insulin injections.⁷ The aim of CGM is to aid self-management of diabetes. CGM used in conventional diabetes treatment has become increasingly compact, accurate, inexpensive, and practical. In recent years, CGM time-series mathematical analysis methods have emerged for exploring how blood glucose levels vary with time.⁸⁻¹⁰ Various studies have investigated ways of increasing the reliability of blood glucose concentration (BGC) measurements. Trajanoski proposed the use of a radial-basis function neural network model to predict and control variability in subcutaneous glucose concentration measurements.¹¹ Bremer and Gough suggested using time-series signals of glucose concentrations measured with CGM devices to analyze changes in glucose levels in real time.¹² Skrovseth and Godtfiebsen described a scale-space method that revealed important effects of uneven data on blood glucose values.¹³ The underlying physiology of glucose regulation, coupled with the nonlinear dynamics of insulin action and glucose kinetics, remains unclear and is an active area of research. Autoregressive (AR) models are simpler than the aforementioned methods and possess a number of other advantages.¹⁴ Sparacino et al. used AR model analysis in which the coefficient was dynamically computed at each time step using weighted least squares.¹⁵ They used a Butterworth filter to remove noise from the raw CGM data and found fixed coefficients in the AR model. Original time-series analysis (TSA) usually uses mathematical model-matching data or calculated peak amplitude.

Various optical glucose sensing approaches have also been proposed.¹⁶⁻¹⁹ Absorption spectroscopy in the near-infrared (NIR) spectral region is one of the most popular analytical methods for measuring glucose concentrations.²⁰⁻²⁵ The appropriate NIR wavelength for measurement is based on the wavelength of glucose absorption detected by photodiodes (PDs). The concentration of glucose affects the rate of absorption of NIR light, and thus the concentration can be calculated from various waveforms detected by the PDs. The NIR spectrum ranges from 800 to 2500 nm. NIR light passes easily through biological tissue and is absorbed by only a few biological chromophores. Thus, NIR absorption spectroscopy is frequently applied in biology and medical science.²⁶,²⁷ Based on NIR absorption spectroscopy, a compact dual-wavelength optical fluidic glucose sensor (DOWFGS) was developed, and the PD voltage response waveform was used to analyze glucose concentration.

This paper describes a rising-edge method that can be used for TSA of measurements obtained from a DOWFGS. In the DOWFGS, the output light level of the light-emitting diodes (LEDs) is adjusted to a light intensity suitable for absorbance saturation. The DOWFGS has four light absorbance paths, enabling α(+)-glucose to be detected using the TSA method to analyze the photodiode response. The DOWFGS voltage response waveforms show peak amplitude variation of 10 to 90% at the rising edge based on the specific amount of light absorbed by glucose. The application of the TSA method improves the sensor sensitivity, increases the accuracy of the data analysis, and lowers measurement equipment costs. The DOWFGS combined with the TSA method can be used to detect glucose concentrations from 0 to 20 wt % in phosphate buffered saline (PBS).
2. Theory

2.1 Time-series analysis method
The TSA method analyzes physical signals or time-series data over a specific period of time. An electronic PD voltage response capable of measuring the light absorption by glucose in a PBS solution is described, and a description of the application to the detection of a small light signal is presented. Time-series methods primarily include AR, moving average (MA), and autoregressive moving average (ARMA) models. In the AR (p) model below:

\[ X_t = \sum_{i=1}^{p} \psi_i X_{t-i} + \varepsilon_t + C, \]

where \( \psi_0, \psi_1, \ldots, \psi_p \) are parameters, \( C \) is a constant, and the random variable \( \varepsilon_t \) is white noise. In the MA (q) model:

\[ X_t = \sum_{i=1}^{q} \theta_i \varepsilon_{t-i} + \mu + \varepsilon_t, \]

where \( \theta_0, \theta_1, \ldots, \theta_q \) are parameters, \( \mu \) is the expectation value of \( X_t \), and \( \varepsilon_{t-1}, \ldots, \) are white noise error terms. The ARMA (p, q) model contains the AR (p) and MA (q) models with p autoregressive terms and q moving-average terms. Substituting Eq. (1) with Eq. (2) yields:

\[ X_t = \sum_{i=1}^{p} \psi_i X_{t-i} + \sum_{i=1}^{q} \theta_i \varepsilon_{t-i} + \varepsilon_t + C. \]

2.2 Absorption spectroscopy
NIR spectroscopy of biological tissue is based on the different absorption wavelengths of different tissues. Glucose absorbance is the most significant at wavelengths of 1420–1480 and 1630–1730 nm. Water has one major absorption band near 1450 nm in the region of 1280–1849 nm. The absorption band near 1450 nm is due to the combination of the OH symmetric and antisymmetric stretching modes of water. In the NIR spectroscopy range of 1420–1480 nm, the absorption coefficient decreases with increasing glucose concentration. The NIR absorption coefficient increases with a rise in the glucose concentration at wavelengths of 1630–1730 nm. The absorption coefficient of a glucose molecule is higher than that of water due to the C–H stretch vibrations in the first overtone (1550–1850 nm). Based on the aforementioned properties of NIR spectroscopy, LEDs with wavelengths of 1450 and 1650 nm were selected as the light sources in the present study.

3. Experimental methods

3.1 Sample preparation
The glucose aqueous solution was a blend of high-purity glucose [d(+)–glucose monohydrate, Panreac] dissolved in two different solutions: PBS (pH 7.4, 0.2 g KCl, 1.44 g NaHPO4, 8 g NaCl, and 0.24 g KH2PO4 in 1 : 1 deionized water, Sigma-Aldrich) and fresh human blood (from a 26-year-old male) diluted in PBS. The blood sample was provided by the blood bank of Dong Hai medical laboratory in Tainan City, Taiwan. The authorization of the study was granted by the National Science Council of Taiwan. In order to simulate the light-absorbing interference, 4 ml of fresh human blood was diluted with 200 ml of PBS solution (1 : 50 dilution, in order to reduce the glucose concentration adjustment error). Then, d(+)–glucose was added to the only PBS/blood solution to a concentration of 0–20 wt % (PBS is saturated at a glucose concentration of 20 wt %).

3.2 Fabrication of the dual-wavelength optical fluidic glucose sensor
Glucose absorption occurs at wavelengths of 1000–2600 nm in the NIR spectral region. In this paper, two GaInAs PDs (Hamamatsu L10899-005k) were used as light detectors. Figure 1 shows the sensitivity of the PDs and the relative radiant output range of the NIR LEDs. The PD detectors converted the received light into an electrical signal (voltage or current), which was then quantified by electronic processing. The employed PDs have a wavelength range of 500–1700 nm, an active area of 0.5 mm, and a dark current of 0.5–2.5 nA at 1 V. Two NIR LEDs (Hamamatsu L10660, 1450 nm, and Hamamatsu L10823, 1650 nm) were used as the optical sources for the DOWFGS. The two LEDs and two PDs were placed in a poly(methyl methacrylate) (PMMA) microfluidic structure. Using the two LEDs for measurement allowed the results to be double checked. The signals of the different LEDs were staggered at different phases or positions. Light at two different wavelengths was transmitted through a blood glucose solution, which flowed through the solution inlet and outlet, to two PDs receiving light at wavelengths of 1450 and 1650 nm. The intensity of the light received by the PDs decreased as glucose concentration increased because of the increased absorption rate. As shown in Fig. 2(a), the DOWFGS is fabricated with four optical paths for glucose measurement. The direct light received by the PDs from the LEDs travels an optical path length of 2 mm (A and C), and the non-corresponding lights have an optical path length B and D of 5 mm. Each photodiode not only receives the light signal from its corresponding LED but also detects the light from the non-corresponding LED. Figure 2(a) shows the DOWFGS for the four optical paths (zones A and C: 2 mm; zones B and D: 5 mm). As shown in Fig. 2(b), the function generator produces a square waveform. The light transmitted by the LED (I0) passes through the sample under test (SUT) and the light (I0) received by the PD. In the schematic, R1 is the current-limiting resistor, which adjusts the LED light intensity.
intensity, $I_{ct}$ is the current that passes through the LED and $R_1$, $V_{ct}$ is the voltage of $R_1$, $R_2$ is the resistance below the PD, $I_{cr}$ is the current that passes through the PD and $R_2$, and $V_{cr}$ is the voltage of $R_2$ that detects the signal of the $R_2$ voltage and amplifies this signal. The PD has an internal resistance $R_{PD}$. According to the Beer–Lambert law, the absorption of light by the test sample written in the exponential form is

$$I_{ao} = I_{ao}e^{-2.34Cf}.$$ \hspace{1cm} (4)

In our experiment, the voltage response and the $R_{PD}$ both change when the PD receives light. In Eq. (5), the relationship between the glucose concentration ($C$) and PD output ($V_o$) equivalent circuit is written as

$$V_o = I_{ct} \times \left( \frac{R_{PD}R_2}{R_{PD} + R_2} \right) \times e^{-2.34Cf}.$$ \hspace{1cm} (5)

Figure 2(c) shows a block diagram of the glucose concentration measurement with DOWFGS. The experimental setup consisted of a DOWFGS and included a signal generator (Tektronix AFG 3102), a power amplifier (AD620), and an oscilloscope. First, the glucose in the PBS/blood solution was injected into the tube through the inlet. A black cover was used to prevent interference from ambient light. The square wave generator was applied to light from the two LEDs. The LEDs were activated with a square wave (3 Vpp 15% duty cycle, with 90 kHz). Between measurements, the DOWFGS flow channel was washed twice with alcohol and deionized (DI) water to prevent any interference from solution residues. Two different signals from the two LEDs passed through the test sample simultaneously. The two PDs detected the optical signal from the test sample. The optical signal was transformed into an electrical signal by the PDs.

Figure 2. (Color online) (a) DOWFGS fabricated with four optical path lengths. (b) Schematic diagram of the DOWFGS electronic circuit. (c) Block diagram of glucose concentration measurement with DOWFGS.
The response waveforms from the PDs were recorded using a digital oscilloscope. The waveform data were saved on a personal computer and used to analyze the PD voltage response of the waveform.

3.3 Timer-series analysis process

The TSA procedure has eight steps: (1) First, the brightness of the NIR LEDs was adjusted to a light intensity suitable to the glucose absorption rate in an electronic circuit. (2) Then, two LEDs transmitted light through the sample solution, and the PDs received the transmitted light and converted it to a voltage response waveform. (3) The measurement data were then recorded using a digital oscilloscope. (4) The PD voltage response waveforms were calibrated to a reference waveform for a glucose measurement of 0%. (5) The time scale of the PD response waveform is selected as 10 to 90% \((t_{V10\%} - t_{V90\%})\) of maximum voltage. (6) The ARMA model was used to determine the variation in the rising edge of the signal waveform. (7) The blood glucose concentration was determined using the sum of the PD response amplitudes. (8) Finally, the curve-fitting method was employed to model the relationship and fit a linear equation to the observed data. Figure 3 shows the block diagram of the measurement of glucose concentration using the rising-edge time-series analysis methods by the DOWFGS. The LED light was transmitted through the sample liquid to the PD, and the PD voltage response waveform showed slight variation in the rising edge of the time-scale curve. In the original TSA method, background noise effects have to be removed. Spectral interference subtraction can be applied to the TSA-corrected data when light scatter is not a serious issue.33) The reference waveform \(W_{ref \%} (0\%)\) curve and the test sample waveform \(W_{sample \%} (5 \text{ to } 20\%)\) curve are the light absorption changes in the DOWFGS PD response to blood glucose concentration. The calibration process is shown in Eq. (6).

After the calibration process, the recorded curve of the rising-time-scale data was selected from the time series of 10 to 90% \((t_{V10\%} - t_{V90\%})\) maximum PD voltage. Then an integration formula was used to analyze the rising edge of the waveform of the PD response voltage from \(t_{V10\%}\) to \(t_{V90\%}\). In this study, an ARMA model with a fixed structure is used in the TSA of the DOWFGS. Using Eq. (1), the measured waveform in the AR model can be described as

\[
X_t = \sum_{i=1}^{n} \Psi_i(\text{ICr}_{t-i}) + \varepsilon_t + \mu.
\]

where \(\Psi_0, \Psi_1, \Psi_2, \ldots, \Psi_p\) are parameters, \(\mu\) is a constant, and the random variable \(\varepsilon_t\) is white noise. Substituting Eq. (6) with Eq. (7) yields

\[
X_t = \sum_{i=1}^{n} \Psi_i(W_{ref \%}_{t-i} - W_{sample \%}_{t-i}) + \varepsilon_t + \mu.
\]

Then, using the MA model to filter the white-noise error terms yields Eq. (9)

\[
X_t = \sum_{i=1}^{n} \Psi_i(W_{ref \%}_{t-i} - W_{sample \%}_{t-i}) + \sum_{j=1}^{n} \theta_j \varepsilon_{t-j-1} + \varepsilon_t + \mu.
\]

Equation (10) describes the analysis process using the rising-time response voltage waveform edge to integrate the curve amplitude, which results in the determination of the variation in the rising edge.
The sum of the rising-time response amplitude was used to calculate the variation of light absorption in the comparative analysis of glucose concentration. The glucose concentration was identified by curve-fitting to the analyzed AR model. To judge the quality of the curve fitting, the coefficient of determination was used

$$R^2 = \frac{\sum_{i=1}^{n} (y_i - \frac{1}{n} \sum_{i=1}^{n} y_i)^2}{\sum_{i=1}^{n} (y_i - \frac{1}{n} \sum_{i=1}^{n} y_i)^2},$$

where $R^2$ is the coefficient of determination, $y_i$ is the data point of the trend line, $y'_i$ is the data point from the DOWFD measurements of the glucose concentration, and $n$ is the number of data points. Finally, the glucose concentration can be obtained from the sum of the PD voltage response rising-time waveforms.

### 4. Results and discussion

#### 4.1 Measurement of glucose concentrations

Glucose concentrations were measured using the DWOFGS described above in the experimental setup section. Any glucose solution remaining in the fluidic channel influenced PD response voltage results. Thus, prior to each measurement, the fluidic channel was washed twice with DI water. The measurement was repeated twice at each of the five glucose concentrations (i.e., glucose concentrations of 0–20% in increments of 5%). Each measurement cycle required 3 min to complete. Figure 4(a) shows the glucose measurement waveform obtained using the DWOFGS for four optical path lengths. Table I shows the DWOFGS glucose measurement waveform of the PD voltage response peak value.

![Diagram](Fig. 4. (Color online) (a) Glucose measurement waveform obtained using the DWOFGS for four optical path lengths (zones A and C: 2 mm; zones B and D: 5 mm). (b, c) Rising-edge time ($t_{V90%}$–$t_{V90%}$) waveform of the DOWFGS glucose measurement voltage response to zones A–D.)

| Glucose concentration (%) | PDs response (V) | Standard deviation |
|---------------------------|------------------|--------------------|
|                           | Zone A | Zone B | Zone C | Zone D | Zone A | Zone B | Zone C | Zone D |
| 0                         | 1.938  | 0.657  | 1.918  | 0.678  | 0.0067 | 0      | 0      | 0      |
| 5                         | 1.940  | 0.619  | 1.900  | 0.640  | 0.0067 | 0.0063 | 0      | 0      |
| 10                        | 1.938  | 0.596  | 1.880  | 0.619  | 0      | 0.0097 | 0      | 0      |
| 15                        | 1.932  | 0.683  | 1.840  | 0.581  | 0.00719| 0      | 0      | 0.0103 |
| 20                        | 1.938  | 0.539  | 1.812  | 0.559  | 0      | 0      | 0      | 0      |
have higher identification rates (28–62 mV). The slight variation of the PD response peak value can cause errors in the measurement results when the solution cannot absorb more light in the absorption steady state. The DWOFGS voltage response waveform of the $V_{10\%} - V_{90\%}$ rising-time scale was extracted from Eq. (6) for the calibrated waveforms shown in Figs. 4(b) and 4(c).

The variation in the curve of glucose absorption after the calibration process and after filtering the noise using Eq. (9) is shown in Figs. 5(a), 5(b), 5(c), and 5(d) for zones A, B, C, and D, respectively. In this paper, the rising-edge time-series curve varied with light absorption by glucose.

Figure 6 presents the curve-fitting of the AR model to the sum of the variation in the rising-edge time responses for the four optical paths. The PD response shows higher absorbance sensitivity (17.202 V/glucose %) for zone A, but the response is not linear ($R^2 = 0.8359$) because zone A (1450 nm) also contains the major water absorption band near 1450 nm. The glucose molecule begins its bending motion at equilibrium with a nonzero dipole moment, so the molecule bending mode is infrared active. However, water is a nonlinear triatomic molecule, and its equilibrium dipole moment is nonzero. The second higher sensitivity of absorbance, $V_n = \alpha C_n - F$, is

$$V_A = 17.202C_A - 4.4479, \quad \text{(13)}$$
$$V_B = 4.108C_B - 0.7039, \quad \text{(14)}$$
$$V_C = 8.9981C_C - 3.9084, \quad \text{(15)}$$
$$V_D = 5.8565C_D - 0.6366. \quad \text{(16)}$$

Table II shows the DWOFGS absorbance sensitivity and the linearity of the sum of the rising-edge time responses for the four optical paths. The PD response shows higher absorbance sensitivity (17.202 V/glucose %) for zone A, but the response is not linear ($R^2 = 0.8359$) because zone A (1450 nm) also contains the major water absorption band near 1450 nm. The glucose molecule begins its bending motion at equilibrium with a nonzero dipole moment, so the molecule bending mode is infrared active. However, water is a nonlinear triatomic molecule, and its equilibrium dipole moment is nonzero. The second higher sensitivity of absorbance, $V_n = \alpha C_n - F$, is

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Table II. The DWOFGS sensitivity of absorbance and linearity of the sum of rising-time edge voltage responses in four optical path lengths.

| Path type | Optical path length (mm) | Sensitivity (mV/glucose %) | Linearity ($R^2$) |
|-----------|-------------------------|---------------------------|-------------------|
| A1450nm  | 2                       | 17.2                      | 0.8359            |
| B1650nm  | 5                       | 4.11                      | 0.9859            |
| C1650nm  | 2                       | 8.99                      | 0.9921            |
| D1450nm  | 5                       | 5.86                      | 0.9973            |
sorbance (8.9981 V/glucose %, $R^2 = 0.9921$) were obtained when $n = C = 1650$ nm, with linearity better than that obtained of $n = A$ at 1450 nm. The greater linearity of absorbance (5.8565 V/glucose %, $R^2 = 0.9973$) obtained of $n = D$ at 1450 nm.

CGM data are often inaccurate due to sensor calibration problems, which can cause uncertainty in the measurements (i.e., noisiness). CGM data combined with time-series data should be more detailed and accurate. This study presented a TSA method for obtaining blood glucose measurements by DWOFGS. However, the TSA method using the sum of the rising edge has a better PD voltage response noise margin. The results of the glucose concentration are obvious in slightly vary from the rising-edge time-series scale by the TSA methods.

5. Conclusions

This paper described a method for determining blood glucose concentration based the difference in the absorption of aqueous glucose at dual wavelengths as measured using a DWOFGS. TSA is used to analyze the changes in the rise time and waveform with varying glucose concentration. The glucose concentration affected the NIR absorption. The intensity of the light transmitted through the solution decreased as glucose concentration increased. Time-series analysis of the pulse-edge and the rising-edge responses of the waveform can be used to calculate the concentration of glucose in the solutions based on the variation in the rising time. DWOFGS four light absorbance paths enable detection of D(+)glucose concentrations. The TSA method can be calibrated photodiodes voltage response background changes during measurements to make the glucose measurement more precise and dependable. The results illustrate the promise of the rising-edge TSA method in the analysis of biological signals. In the future, DWOFGS can be applied to determine the amount of sugar in fruits or for self-measurement of blood glucose levels.

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