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Multispectral photoacoustic sensing for accurate glucose monitoring using a supercontinuum laser

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Accurate monitoring of glucose levels constitutes the most important parameter for diabetes management and treatment planning. In this work, we report on an *in vitro* glucose monitoring system based on multispectral photoacoustic sensing (MSPAS) using a cost-effective supercontinuum (SC) laser. We demonstrate for the first time, to the best of our knowledge, how the use of a broadband SC source allows the identification of distinct absorption characteristics of two major analytes (glucose and cholesterol) present in the human body in the extended near-infrared 1540–1840 nm spectral range. Employing the reported SC-based MSPAS system with a ratiometric analysis, we were able to accurately (coefficient of determination ≥0.938) measure a wide range of glucose concentration levels *in vitro*. We further demonstrate clinically accurate prediction of glucose concentrations over commonly encountered physiological levels inside the human body (0–400 mg/dL) with reference to a Clarke error grid analysis. These findings pave the way for devising potentially noninvasive and label-free continuous glucose monitoring systems.

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1. INTRODUCTION

Diabetes mellitus (DM) is a fatal metabolic disease with 424.8 million people affected worldwide, and this number is predicted to rise to 629 million by 2045 [1]. Improper diagnosis and monitoring of DM can lead to the onset of serious complications affecting microvascular and macrovascular vessels inside the human body [2]. To prevent such chronic complications, DM patients have to monitor their glucose levels frequently. The most widely used and established approaches for monitoring glucose levels inside the body rely on amperometric detection and enzyme reactions [3–5]. However, these techniques are mostly invasive (based on finger-pricking). Moreover, compared to other label-free techniques, the enzyme-based approaches suffer from reduced sensitivity due to degradation of the enzyme with time [6]; therefore, several attempts are being made for devising reliable noninvasive and label-free techniques for the monitoring of glucose levels inside the human body [7–10].

Photoacoustic (PA)-based sensing is a powerful noninvasive technique, which has attracted significant attention recently for determination/analysis of glucose levels [8,11]. The PA-based sensing techniques were widely employed for glucose detection in the mid-infrared region due to the presence of fundamental absorption bands of glucose (9–11 μm) [5,12]. However, the strong water absorption within this spectral region imposes significant limitations for noninvasive glucose measurements. On the other hand, the extended near-infrared (NIR) region optical window in the biological tissue (1500–1850 nm) is propitious for devising a noninvasive glucose monitoring technique due to the higher penetration depths compared to the techniques based on a shorter wavelength [13]. Figure 1 shows the measured spectral characteristics of two different analytes (distilled water and glucose) indicating the well-differentiated absorption peaks between the two. The spectral difference between the two analytes can be attributed to the overtone and combination bands of C—H and O—H bonds [14]. *In vitro* PA studies on aqueous glucose within this spectral region have been performed using commercially available monochromatic laser sources mainly at 1550 nm [13]. However, because the absorption spectrum of glucose has identical characteristics with other analytes inside the human body, such as water and lipids, these systems are prone to limited sensitivity [15]. MSPAS [16]-based glucose sensing is a promising technique, which employs a tunable excitation source for establishing the absorption characteristics of glucose with respect to the other analytes, thereby enabling the spectral region where glucose has higher absorption contrast compared to other analytes inside the human body. Such *in vitro* MSPAS studies have been recently performed in the NIR and extended NIR region (850–1900 nm) with tunable optical parametric oscillators (OPOs) [17–19]. However, while allowing wide wavelength tunability,

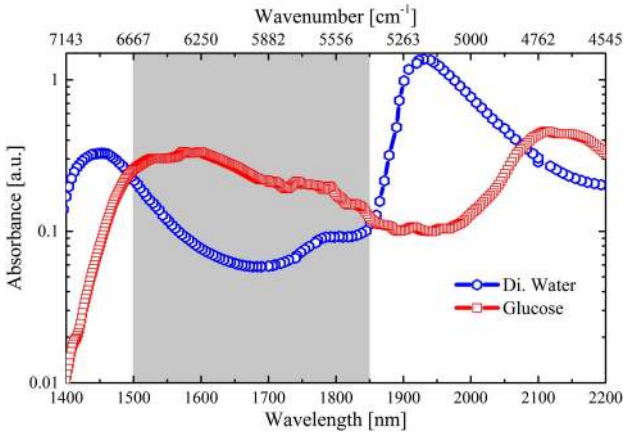

Fig. 1. Absorption spectra of distilled water (measured in transmission mode) and D-glucose (measured in diffuse reflection mode) measured using a commercial FTIR spectrometer (ABB Bomem FTLA2000). The inset (highlighted area) showing dominant absorption region of the glucose due to overtone and combination bands of C—H and O—H bonds in glucose.

OPOs suffer from high cost and a large footprint, making them unsuitable candidates for portable MSPAS glucose monitoring systems.

In this study, we developed, for the first time to our knowledge, a supercontinuum (SC) laser-based MSPAS system for glucose monitoring in the first overtone band at 1540–1840 nm. We demonstrate how the proposed system can be used to identify the absorption characteristics of various analytes and then select a suitable wavelength region for further investigations. Based on a simple ratiometric analysis, we demonstrate the feasibility of the system for accurate monitoring of glucose over a wide range of concentrations. The concentrations used in the experiments varied from 0–8 g/dL, covering commonly encountered physiological glucose levels (0–400 mg/dL) inside the human body. We further employ a linear regression analysis to predict various glucose concentrations with clinically acceptable accuracies with respect to a Clarke error grid (CEG) analysis standard, thereby revealing its true potential toward noninvasive and label-free continuous glucose monitoring applications.

2. MATERIALS AND METHODS

A. Sample Preparations

The glucose samples were prepared by the process of dissolution, i.e., different proportions of glucose (D-Glucose, VWR) (1–8 g in steps of 1 g and 0–400 mg in steps of 50 mg) were measured using a balance (Entris 224 – 1×, Sartorius) with a precision of 0.2 mg. The weighed samples were subsequently dissolved in distilled water [pure distilled water (D-Water, VWR) served as reference]. The glucose solutions were then transferred to the sample holder and replaced using a syringe after every measurement. The cholesterol used in the experiment was commercial grade cholesterol (C8667, ≥99%, Sigma-Aldrich). During the experiments pure cholesterol was filled in a polymer capillary and placed inside the sample holder.

B. Experimental Setup

The MSPAS system developed for the characterization of the different analytes and the glucose concentration experiments is shown in Fig. 2. A home-built high-energy SC laser source based on a telecom range diode laser-based amplifier and a few meters of standard single-mode fiber was used as an optical excitation source. The pulse energy of the SC laser at 30 kHz pulse repetition rate is 13.3 μJ over a bandwidth of 400 nm (1500–1900 nm). The detailed configuration of the SC laser source is described in our previous study [14]. The output from the SC laser source is collimated using an achromatic lens (L1) (RC02FC-P01, Thorlabs). The excitation band used for the PA generation was filtered using a linear variable filter (LVF) (1.25–2.5 μm, Vortex Optical Coatings), steered using a mirror (M1) and then focused into the transparent sample holder (about 5 mm above the surface of the sample holder) using

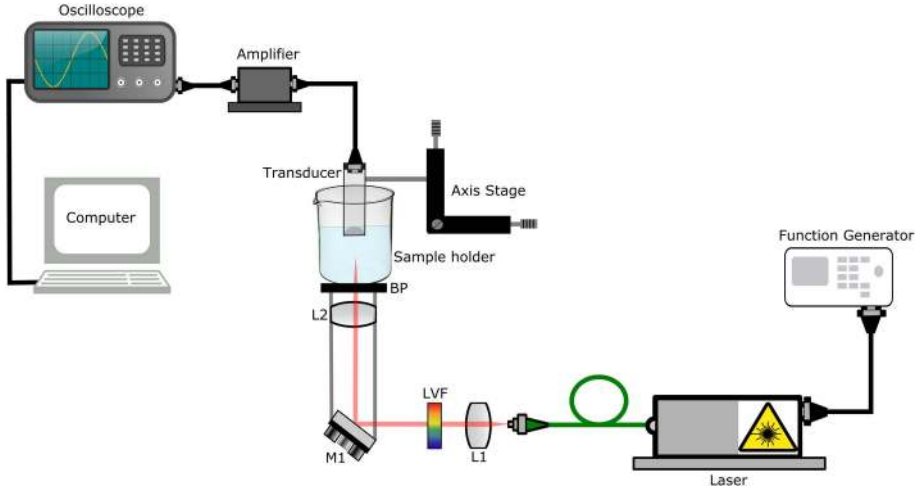

Fig. 2. Schematic of the MSPAS system. A home-built high-energy SC laser is used as optical excitation source; the output from the laser is collimated using a lens (L1), filtered using an LVF, and then focused inside the solution placed on top of a base plate (BP) using an objective lens (L2). The generated PA signals are detected using a focused transducer, amplified using a low-noise amplifier, sent to an oscilloscope, and saved to the computer.

a 5× objective lens (L2). The sample holder is specially designed to have direct access to the glucose solutions without traversing any cover slips.

The generated PA signals were detected using a focused ultrasonic transducer (V320, Panametrics) with a central frequency and 6 dB bandwidth of 7.5 MHz and 5.8 MHz, respectively. The detection sensitivity of the setup can be maximized by confocal aligning of both the foci [excitation (spot size, 19 μm) and detection (spot size, 0.6 mm)]. Therefore, a high precision stage was used to align the transducer to a confocal excitation and detection. The detected signals were then amplified using two cascaded wide-band low-noise amplifiers (ZFL-500 LN, Mini-Circuits) and further digitized using a high-resolution oscilloscope (HDO 9404, Teledyne Lecroy). The digitized signals from the oscilloscope were then transferred to a computer for further postprocessing.

C. Methodology and Data Analysis

1. Prediction of Glucose Concentration

The amplitude of the pressure wave generated due to the laser pulse excitation at the transducer is given by [20,21]

$$P = \mathrm{K}\Gamma\alpha E_0, \tag{1}$$

where K is a constant incorporating the geometrical parameters, Γ is the Grüeneisen parameter, which depends on the physical parameters of the sample, α is the absorption coefficient, and E_0 is the excitation pulse energy.

The amplitude of the photoacoustic signal ($\mathrm{PA_{sig.}}$) detected by the piezoelectric transducer due to the pressure can be written as

$$\mathrm{PA_{sig.}} = \mathrm{const.}P, \tag{2}$$

$$\mathrm{PA_{sig.}} = K'\Gamma\alpha E_0, \tag{3}$$

where K' is a constant that includes the geometrical parameters as well as the response properties of the piezoelectric transducer.

In the concentration monitoring applications, the change in the concentration of the sample affects the physical properties of the sample (Γ) in addition to the optical properties (α) of the sample, thereby resulting in the stronger PA amplitudes. Previous studies [8,19,22] have already confirmed that the variation of the PA signal is linear with the glucose concentration (for the concentration range used in the experiment). Therefore, a linear regression was used to predict the PA signal of unknown glucose concentrations ($\mathrm{PA_{sig.unk.}}$) using known glucose concentrations.

2. Data Analysis

The acquisition and analysis of the raw data were accomplished using a MATLAB routine. The routine takes acquired PA signals from pure distilled water (reference) and the test glucose solution as two inputs. (To have high accuracy and a signal-to-noise ratio, 500 PA signals are acquired at every test solution.) The PA amplitude of the recorded PA signal is the computed area under the curve of its envelope; therefore, the Hilbert transformation was used to calculate the envelope of the recorded PA signal, and the area under the envelope is extracted. The PA amplitude at each concentration was then estimated using the ratiometric analysis of the PA amplitude of the test glucose solution at respective concentrations and the reference. The total measurement duration at each concentration was about 1.6 ms.

3. RESULTS AND DISCUSSION

To assess feasibility of the MSPAS system for the characterization of two analytes (glucose and cholesterol), a tunable excitation source (high-energy SC with LVF) was used to first establish the absorption characteristics of both the analytes.

Figure 3(a) shows the output power spectral density (PSD) of the SC laser. The inset shows one of the excitation bands at 1620 nm filtered using the LVF.

The PA spectra of both the aqueous glucose solution (1 g/dL) and the cholesterol were scanned in the wavelength

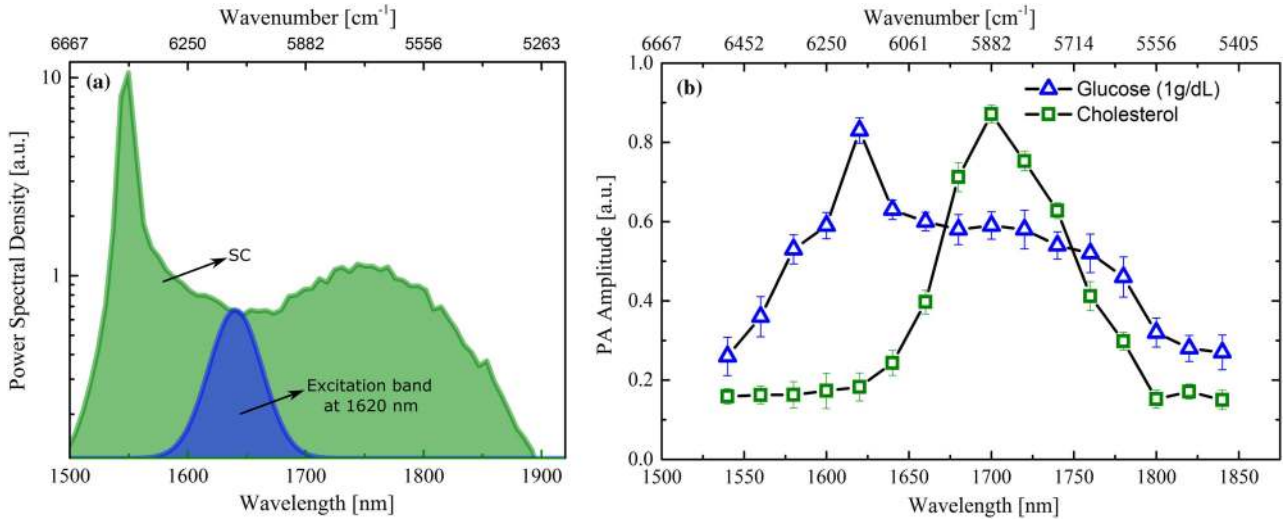

Fig. 3. (a) Output power spectral density of the SC source. The blue inset shows one of the excitation bands (center wavelength 1620 nm) filtered using the LVF. (b) The PA spectra of the glucose (1 g/dL) and cholesterol, recorded in the wavelength region 1540 to 1840 nm, with wavelength steps of 20 nm.

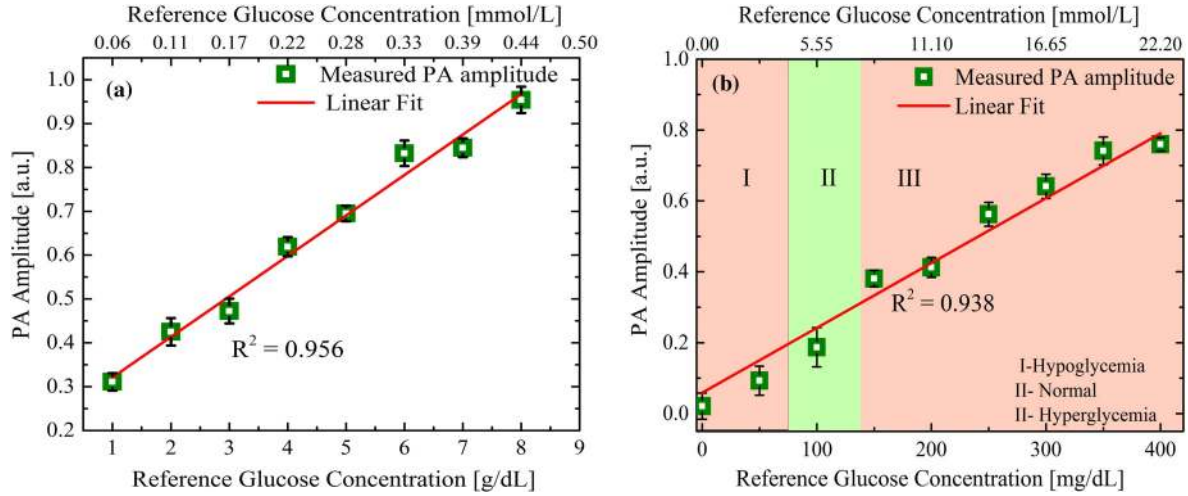

Fig. 4. Variation of the PA amplitude as a function of the reference glucose concentration. (a) Higher glucose concentrations levels. (b) Physiological glucose concentration levels; highlighted areas indicate the regions of hypoglycemia, both normal and hyperglycemia levels.

region from 1540–1840 nm in steps of 20 nm. Figure 3(b) presents the PA spectra of both analytes. The analytes have distinct absorption peaks separated by approximately 80 nm in the wavelength region of interest. The peaks in the PA amplitude can be attributed to the increased absorption due to the first overtone and combination region of C—H and O—H bonds of the analytes. The most pronounced PA amplitude was recorded at 1620 nm for the glucose and 1700 nm for the cholesterol. To have high sensitivity and a signal-to-noise ratio, the 1620 nm excitation band was therefore selected for the *in vitro* glucose concentration experiments.

To further explore the potential of the current system for accurate glucose monitoring, the PA amplitudes of different glucose concentrations varying from 1–8 g/dL in steps of 1 g/dL were recorded. Figure 4(a) presents the variation of the PA amplitude with respect to the concentration of glucose in the test solution. It can be clearly observed that the PA amplitude variation shows a close to linear correlation, as the increase in the concentration of glucose in the test solution increases the overall absorption, thereby increasing the detected PA amplitude. A linear regression applied to the data set yields a coefficient of determination (R^2) of 0.956. The experiments were repeated for physiological glucose concentrations present inside the human body (0–400 mg/dL) (i.e., concentrations ranging from hypoglycemia to hyperglycemia). Figure 4(b) shows the variation of PA amplitude with respect to the concentration of the glucose in the test solution. A close to linear correlation was still valid with a coefficient of determination (R^2) of 0.938.

Furthermore, to show the feasibility of the method for clinical applications, CEG analysis was employed. CEG is a widely used standard to determine the accuracy of glucose monitoring techniques [23]. CEG divides the correlation plot of glucose measurements into five different regions—namely, regions A, B, C, D, and E. It defines a region of sufficient accuracy (within 20% of the reference sensor, zone A) and a region of low but clinically acceptable accuracy without inappropriate treatment of the patient (zone B). The results in zones C, D, and E are

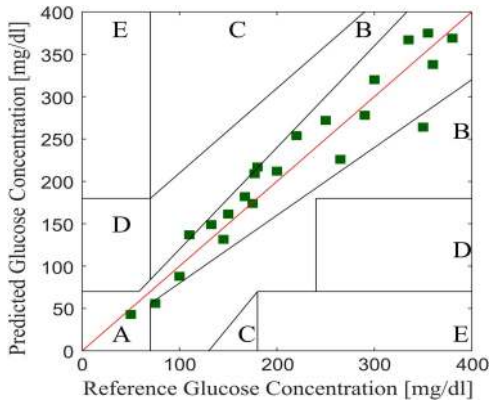

Fig. 5. Predicted glucose concentrations as a function of the reference glucose concentrations plotted on CEG.

potentially dangerous and are therefore clinically significant errors. The PA signals from 22 different samples with random glucose concentrations are recorded, and the concentrations of the glucose inside the samples are predicted (as detailed in Section 2.C.1). The correlation between the predicted and the reference glucose concentrations (measured using high-precision balance) overlaid on the CEG is shown in Fig. 5. As can be seen in Fig. 5, all the predicted concentrations of glucose fall in the acceptable accuracy region of the CEG with a coefficient of determination (R^2) of 0.901, thereby showcasing the potential of the measurement technique for further noninvasive *in vivo* applications.

4. CONCLUSION

In summary, we have demonstrated the development of an SC laser-based MSPAS sensing system for *in vitro* glucose monitoring. The system was used to identify the absorption characteristics of two major analytes (glucose and cholesterol) over a wavelength region of 1540–1840 nm. Glucose and cholesterol

show distinct absorption peaks at 1620 nm and 1700 nm, respectively, and the absorption peaks can be attributed to the first overtone and combination region of C—H and O—H bonds. We demonstrated how the proposed system can be used to measure glucose concentration using ratiometric analysis over a broad range of concentrations, from physiological concentrations commonly occurring inside the human body to concentrations as high as 8 g/dL. Using CEG analysis we further demonstrated that glucose concentrations can be determined for clinical applications with sufficient accuracy over the entire range of commonly encountered physiological levels inside the human body.

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