CW-PHOTOACOUSTIC-BASED PROTOCOL FOR THE NON-INVASIVE DETECTION OF AQUEOUS GLUCOSE AT LOW MG/DL CONCENTRATION LEVELS

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ABSTRACT
This paper reports an improved protocol for measurements of aqueous glucose solution at low concentration levels. This contribution results from our effort to develop a non-invasive and continuous blood glucose sensor based on the continuous-wave-photoacoustic (CW-PA) technique. We recently proposed a method called Frequency Shift (FS) and demonstrated a linear response for glucose concentrations of up to 2 g/dL. However, the final target application requires a detection limit of about 70 mg/dL. We then investigated our technique's performance at several times lower concentration levels and achieved a detection limit in the 10 mg/dL range.

KEYWORDS: Blood Glucose Sensor, CW-Photoacoustic, High Sensitivity, Frequency-Shift Protocol

INTRODUCTION
Over the last few decades, the patient population suffering from diabetic mellitus disorder has been increasing worldwide. Due to the potential high impact of a non-invasive blood glucose sensor, this research area has been extensively studied and several techniques investigated [1]. Among those techniques, the photoacoustic one (PA) is a promising candidate with two standard setups: the pulse configuration and the continuous wave (CW) one. However, despite potential higher sensitivity, CW-PA has received almost no interest for this kind of application because of cavity dimension dependence of resonance. Nevertheless, we recently proposed a method called Frequency Shift (FS) [2] that not only resolves this issue of cavity dimension dependence but also takes advantage of this characteristic: the FS method is equivalent to an acoustic velocity measurement and therefore independent of the optical wavelength and cavity dimensions. We have already demonstrated a linear response for glucose concentrations of up to 2 g/dL. However, the final target application requires a detection limit of about 70 mg/dL, and anticipating a decrease of performance when moving from the in vitro to in vivo environment, we then investigated our technique's performance at several times lower concentration levels, typically in the 10 mg/dL range.

EXPERIMENTAL
The concept of the PA method is schematically shown in the left part of Fig. 1. An absorbing medium, typically water, is illuminated by an amplitude-modulated light beam at a fixed wavelength. Acoustic waves are then generated inside the medium through the photothermal effect and propagate until the transducer, which converts the acoustic pressure into electrical signal. This electrical signal is then post-processed in order to specifically extract the information about glucose concentration.

We designed a custom-made measurement cell and integrated it within a closed-loop system (right part of Fig. 1). The measurement cell is cylindrical in shape whose inner diameter is fixed (10 mm) while its length can be adjusted continuously from few millimeters to two centimeters. Two apertures designed to fit the optical fiber and acoustic transducer face each other, and two microfluidic ports (inlet/outlet) enable one to transfer the liquid sample inside the measurement area.

Figure 1: Schematic view of PA concept (left) and the experimental setups we developed for performing a CW-PA measurement (right).
The closed-loop system includes a several elements whose functions may be briefly explained as follows. The function generator creates a square wave voltage signal that triggers the lock-in amplifier and drives the distributed feedback laser diode (DFB-LD) through the LD driver as well. The optical signal modulated in intensity is then sent to the measurement cell through a single-mode optical fiber. The acoustic signal sensed by the transducer is first pre-amplified before it is fed into the lock-in amplifier. A computer pilots all the different elements via a lab-view interface. This experimental system enables amplitude and phase measurements at any frequencies within the frequency generator's capabilities.

As pointed out earlier, we used the FS protocol to measure glucose concentration at low milligram per deciliter levels. This protocol uses the frequency shift induced by changing the glucose concentration of the sample solution through the acoustic velocity change. A measurement based on the frequency then ensures high sensitivity, but this method also exhibits sensitivity to temperature, which can bias the measurements, especially at low concentration levels. We then first designed an experimental setup to ensure better control of the sample temperature all along the measurement sequence.

**RESULTS AND DISCUSSION**

Figure 2 (left) shows a schematic view of our setup with a heat source providing hot water flow, a small container inside a bigger one to prevent the hot water flow from directly striking the detection cell, several temperature sensors (S1, S2, and S3) to monitor the temperature distribution, and plastic beads (PBs). The PBs are used to reduce the evaporation ratio and thereby decrease the energy loss for better feedback control. On the right of Fig. 2, the temperature stability at S1 after reaching the stable regime (plateau) with a 36-degreeC assignment exhibits a consequent improvement by using the PBs. With the PBs, we could improve the temperature control and reached an accuracy below the temperature sensors' one (better than +/- 0.05 deg.C).

![Figure 2: Experimental set-up for examining the performance of low-concentration-level glucose measurement in thermo-regulated conditions (left) and the effect of PBs on the feedback control efficiency: the use of PBs improves the temperature control efficiency, with an accuracy better than the temperature sensor precision (+/- 0.05 deg.C) (right).](image)

We then carried out measurements of glucose solution at low concentration levels. The left part of Fig. 3 shows a typical experimental result at one glucose concentration; the right part summarizes all the experimental data from two setups. With the so-called "system_1", we first performed measurements over a wide range of concentrations. In this particular case, the sample solution was kept outside the container filled with temperature-controlled water, at room temperature. The first results over large concentration span appeared linear. However, when zooming below the 50 mg/dL level, we found huge error bars (equivalent to +/- 26 mg/dL) that limit the sensor performance at low concentration range. We then devised a new protocol where the sample load is immersed inside the thermo-regulated bath long enough to decrease the thermal shock when switching the sample solution. With the further optimized "system_2", despite longer measurement time, we could drastically decrease the error bars down to a +/- 3.5 mg/dL (left of Fig. 3). These error bars are attributed to the noise levels at the two plateaux (before and after the transfer of the new solution) that are inherent to the PA protocol and the devices used here. Nevertheless, the data points in the right part of Fig. 3 seem almost randomly distributed around the average curve. Since the FS method is equally sensitive to glucose and temperature, the optimization towards high sensitivity leads to a protocol highly sensitive to both. From experimental data, we estimated these temperature differences between samples to be about +/- 0.015 and +/- 0.035 deg.C for system_2 and system_1, respectively. The two values reported here are far below the temperature sensor detection limit and rely on independent estimation of the sensor sensitivity to temperature.

Further optimization of the *in vitro* experimental setup may lead to a decrease of the temperature fluctuations down to a level of few milligrams per deciliter equivalent. However, when thinking about the final application with the temperature fluctuation inherent to the human body, further improvement of the temperature controlling system will not bring any valuable supplementary information about our sensor characteristics.
Figure 3: Experimental result when measuring the phase shift induced by switching (T) the sample solution from 0 mg/dL (STEP 1) to 11.6 mg/dL glucose concentration (STEP 2) (left), and the sensor response in the low glucose concentration range (inset: below 1000 mg/dL): comparison between the two setups providing different temperature control accuracy at levels below 50 mg/dL (right).

CONCLUSION
We recently proposed the FS measurement protocol to measure non-invasively the glucose concentration based on the CW-PA technique. To estimate its detection limit and resolution, we designed a thermoregulated bath system and performed measurements at concentration levels below 50 mg/dL. These experimental results were sufficient to estimate the resolution of our sensor in an in vitro and well-controlled environment, with a limit of about +/-3.5 mg/dL considerably below the required detection limit of 70 mg/dL. However, these results also point out the selectivity to glucose as being a major issue for the FS-based measurement protocol, especially when dealing with real patients and environments undergoing simultaneous changes of different parameters, including temperature. As a consequence, the selectivity issue should be addressed as a first priority by combining this method with a compound-specific protocol.

REFERENCES

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