FLUORESCENCE SPECTROSCOPY BY DETECTION OF GLUCOSE CONCENTRATIONS IN DMEM-SOLUTIONS AND ITS PERSPECTIVES FOR NON-INVASIVE MEASUREMENT

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Abstract: An easy accessible and low-cost method for glucose concentration monitoring and diabetes management will be a great help for more than 250 millions of diabetic patients worldwide to avoid the risks and the complications caused by hyper- or hypoglycemia. This paper shows the results obtained using fluorescence spectroscopy for detecting the glucose concentrations in DMEM solutions. By irradiating DMEM solutions that have different glucose concentrations with light, a few wavelengths in UV- and visible-range for the calculations of glucose concentrations using fluorescence spectroscopy are applied and the detected signals were analyzed. For the detection of glucose concentration noninvasively using various optical methods, the interaction between light and definite glucose solutions was studied. The developed compact system will enable the application of different LASER diodes (LD`s) or light emitting diodes (LED`s) in the range of UV and NIR in an easy manner. Variable intensity, frequency and duty cycle can be adjusted for fluorescence and other optical measurements. A multi-sensor taking all perturbations into account will be a good choice for glucose monitoring. Fluorescence measurements at wavelengths below 800 nm and especially the measurements at the wavelength 485 nm give reproducible glucose concentrations results from DMEM glucose solutions at a constant temperature.

1 INTRODUCTION

Continuous non-invasive monitoring of blood glucose can achieve a great enhancement by glucose management to attain a better normal life for diabetics. Detection of blood contents like Glucose non-invasively in an easy manner can reduce morbidity and mortality by diabetics. Design of a system for the detection of glucose in solutions and non-invasively can be a great help for diabetics.

Diabetes risk lies in its complications like heart diseases and infarcts, stroke, blindness, kidney disease, nerve disease, diabetic foot and amputation. The current applied invasive methods are intermittent, inconvenient and painful, having infection risk, blood loss and time delay, need consumables materials, needles and strips. The invasive method cannot be applied continuously, and hence hypo- or hyperglycemia may be not detected. A continuous blood glucose monitoring is essential for glucose management. Noninvasive blood glucose concentration monitoring will reduce mortality and morbidity and improve life quality of more than 250 Million diabetic patients worldwide.

1.1 Fluorescence Spectroscopy

Glucose in water shows no fluorescence. In DMEM solution a glucose dependent autofluoresence can be observed. The fluorescence differs from the process of Raman effect in that the incident light is completely absorbed and the system is transferred to an excited state from which it can go to various lower states only after a certain resonance lifetime.

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Fluorescence spectroscopy and time resolved fluorescence are now dominant methodologies and used extensively not only in biochemistry and biophysics, but also in biotechnology, medical dioagnostics and genetic analysis (Moschou, 2004, Pickup, 2005, Lakovics, 2006). The technique is extremely sensitive. There are increasing examples of even single-molecule detection using fluorescence methods. Many studies indicate that fluorescent technology has real sensitivity especially in low glucose ranges. In addition, since near-infrared light passes through several centimeters of tissue, with the appropriate choice of fluorophore, molecules can in theory be excited and the emission interrogated from outside the body providing the potential for completely non-invasive sensing. A convenient way of classifying fluorescence-based glucose sensors that involve measurements of fluorescence is either according to the type of molecular receptor for glucose, or whether cells or tissues are used to signal glucose concentrations and/or glucose metabolism. A review of the principles of operation and current status of the various approaches to fluorescence-based glucose sensing are described in D'Auria, 1999.

2 APPARATUS AND METHOD

The results shown below are obtained using a fluorescence spectrometer having the stimulation/emission wavelengths of 360 nm /465 nm, 430 nm / 535 nm and 485 nm / 535 nm.

The method discussed here will be applied for invasive and non-invasive measurement. Photodiodes or phototransistors for fluorescence detection in the visible and NIR spectrum and light emitting diodes LED or LASER diodes as light sources will be applied. Variable frequency and duty cycle can be adjusted for time resolved fluorescence signal detection.

The block diagram of the principle of a flexible developed measuring system is shown in Figure 1. The developed system is flexible and can be used for the development purposes, where different parameters have to be adjusted. Then light intensities, duty cycle, different LASER types and variable amplifications can be achieved by using this system.

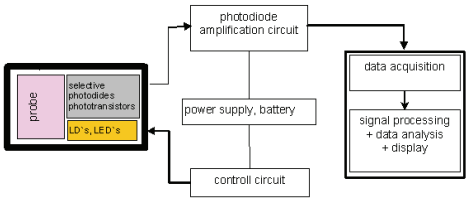

Figure 1: Block diagram of an optical sensitive measuring system.

3 RESULTS AND DISCUSSION

The results obtained below by using a fluorescence spectrometer show the emitted light by stimulation of a DMEM solution with different glucose concentrations. The detected signal with 465 nm by the stimulation in the UV light at the wavelength of 360 nm is not highly correlated with the glucose concentration (Figure 2). But an increasing tendency of the emitted light with the increasing glucose concentration is shown.

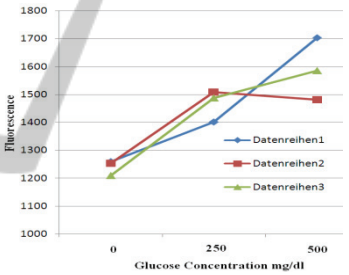

Figure 2: Fluorescence by stimulation/emission wavelengths of 360 nm /465 nm.

The detected signal at 535 nm shows a high correlation with glucose concentration when stimulated with 485 nm (Figure 3). In the contrary to the detected signals at 465 nm mentioned above, a decreasing tendency of the emitted light with the increasing glucose concentration is shown.

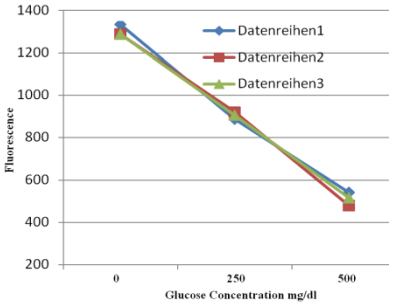

Figure 3: Fluorescence by stimulation/emission wavelengths of 485nm /535 nm.

Figure 4 shows also a high correlation of the detected signal at 535 nm with known glucose concentration when stimulated with 485 nm at different glucose concentrations. We try here to obtain a high resolution and accuracy at the lowest normal level (about 50 g/dl) of glucose concentrations in order to detect the hypoglycemia. Also high accuracy has to be achieved around the glucose concentrations of 180 g/dl in order to detect hyperglycemia with an acceptable clinical accuracy.

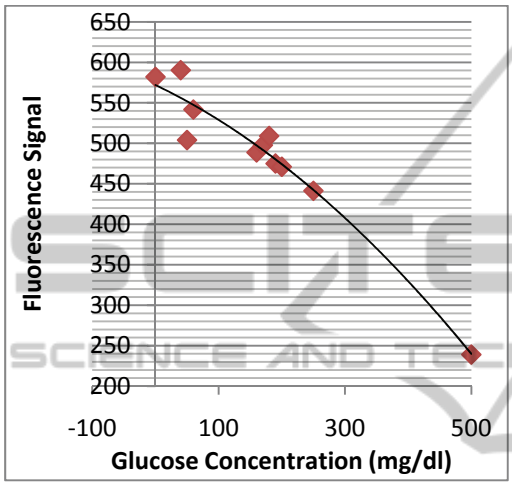

Figure 4: Fluorescence by stimulation/emission wavelengths of 485nm /535 nm.

Similar relationship between the detected signals at the wavelength of 535 nm and the glucose concentrations is shown in Figure 5 for stimulation at the wavelength 430 nm. A decreasing tendency of the emitted light with the increasing glucose concentration is shown in Figure 4 and Figure 5.

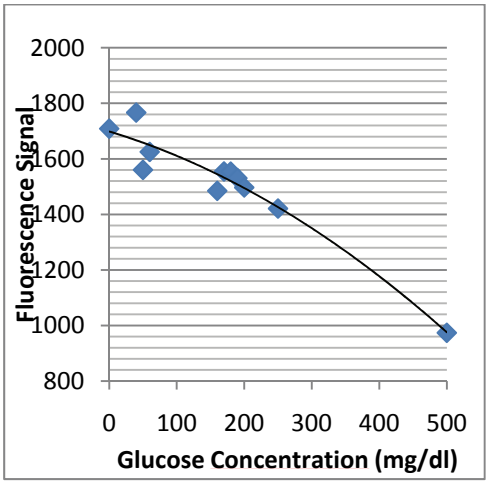

Figure 5: Fluorescence by stimulation/emission wavelengths of 430nm /535 nm.

The detected signal at 535 nm shows in accordance to other measurements a higher correlation with glucose concentration and a better reproducibility when stimulated with 485 nm instead of 430 nm.

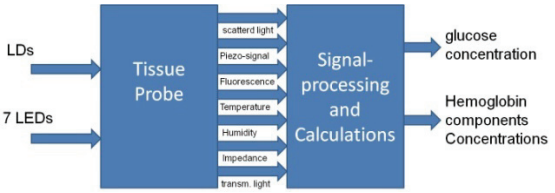

Figure 6: Schematic of a multisensor for non-invasive detection of blood glucose, hemoglobin concentration, and fractional oxygen saturation.

The detected glucose signals are too small and should be processed carefully. Also the *in vivo* measurements are subjected to more noise and motion artifacts. An adaptive filtering will be needed for eliminating these perturbations. A Noise Reference Signal is generated by means of a Synthesizer or piezoelectric element and will be adjusted as much as possible to the real noise contained in the corresponding measurement by the adaptive filter based on the least mean square optimization algorithm. This algorithm has delivered very good results by testing it for the non-invasive calculations of oxygen saturation by artificial vibrations of the hand, where a pulse oximeter sensor is applied at the finger subjected to these artifacts.

4 CONCLUSIONS AND FUTURE WORK

A non-invasive continuously blood glucose concentration monitoring will improve the management of diabetes mellitus. It will be important for diabetics to avoid the complications caused by high glucose level in blood. IR-spectroscopy has the potential for the development of a simple cost effective sensor for glucose monitoring that can be used for home care.

Problems with existing methods have encouraged alternative approaches to glucose sensing, and those based on fluorescence intensity and lifetime have special advantages, including sensitivity and the potential for non-invasive measurement when UV, visible or NIR light is used (Yamakoshi, 2006; Evans, 2005; Evans, 2003; Pickupa, 2005). The fluorescence signals using UV

light as stimulus and detection of fluorescence at violet or blue have shown a very good correlation with the glucose concentrations in DMEM solution. Light stimulation with blue light and the detection of fluorescence by green region shows also a high correlation with the glucose concentrations. The detected glucose signals will be then subjected to perturbations from the surroundings and from the background of the measured locations due to tissue alteration and physiological parameter variations. All perturbations such as temperature, humidity and applied pressure variations have to be included by the calculations, as illustrated by Figure 6.

The drift of the characteristics of the electronic and optical components may cause high disturbances to the measurements. The integration of further parameters may enhance the reproducibility but decrease the accuracy due to the measurement errors. The system complexity and the number of the measured parameters have to be minimized.

There are few fluorescence-based glucose detection methods that have reached the stage of testing in vivo, but none have entered clinical practice for diabetes management. This will be an area of active investigation in a future work. We will need to explore different interferences and the stability as well as accuracy under normal life conditions.

There is no doubt that fluorescence technologies have considerable promise for glucose sensing.

As a future work, all developed sensors will be integrated in one system that enables the simultenous processing of the detected signals (Caduff, 2009). Other blood components like total hemoglobin concentrations and fractional oxygen saturation measured non-invasively have to be taken as parameters by the glucose calculations. The suitable locations for measurements may be earlobe for transmission measurements or forehead as well as abdomen for reflection measurements has to be chosen. Applying the Twersky theory or diffusion theory by the calculations are our next perspectives. After that a clinical study for non-invasive measurements and applying the neural fuzzy techniques the results and the system will be optimized.

Using a daily, disposable contact lens embedded with newly developed boronic acid containing fluorophores may also be suitable for the continuous monitoring of tear glucose levels.

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