

OPTICAL SPECTROSCOPY AND OBSTACLES BY NON-INVASIVE DETECTION OF GLUCOSE CONCENTRATION BY HOME MONITORING

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Abstract: Tight glycemic monitoring and control is the main goal in successful diabetes management to avoid its complications. Frequent blood glucose measurements with a combination of regimented diet, exercise and insulin administration can accomplish this task. Different methods are applied for non-invasive measurement of blood glucose concentration. Despite the great interest and the intensive research in this field since 1980s, there is no convenient device at the market that can measure the glucose concentration non-invasively in an easy manner. This paper discusses the different methods for detecting the glucose concentration. Elastic and inelastic (Raman) scattering as well as fluorescence and IR Spectroscopy measurements will be shown and discussed for the development of a compact non-invasive device for home monitoring. In conclusions, an optical multi-sensor measuring the fluorescence and light scattering in the tissue optical window in and around visible range (360 nm – 1200 nm) taking the perturbation factors into account is promising and under development.

1 INTRODUCTION

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. An easy accessible and low-cost method for continuous 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 hypoglycaemia. Standard treatment includes lifestyle changes, medication and frequent monitoring of blood glucose levels. Insulin and other

diabetes medications are designed to lower the blood sugar level when diet and exercise alone aren't sufficient for managing diabetes. By the development of a compact system, different LASER diodes (LD's) or light emitting diodes (LED's) in the range of UV and NIR will be used. Light scattering and fluorescence spectroscopy can be applied for non-invasive measurement of blood components like glucose concentration or the early detection of pathological variations like melanoma.

The complications of diabetes are largely avoidable and may be reversed by strict control of blood sugars through medication and diet. Patients with type 2 diabetes mellitus are at increased risk for macrovascular disease complications (Gaster, 1998; Mezzetti, 2000; Pambianco, 2006). Detection of blood contents like Glucose non-invasively in an easy manner can reduce morbidity and mortality by diabetics.

1.1 Non-invasive Methods for Detecting Glucose Concentration

In-vitro Glucose concentration measurements depend on chemical principles. The non invasive monitoring methods are much more difficult to apply with the required accuracy. Glucose concentrations in blood are very low compared for example with that of hemoglobin. The effect of glucose on the measured signals is too low and hence a high amplification is needed, which means that other background and surrounding noises will also be amplified and hence the measurement will be very sensible for tiny perturbations.

The different methods for non-invasive glucose concentration monitoring are discussed in diverse literature (Khalil, 2004; Yamakoshi, 2006; Maruo, 2003; Tura, 2010). Raman and fluorescence spectroscopy as examples of promising optical methods will be briefly discussed in the next section.

1.2 Optical Spectroscopy

The optical methods rely on the interaction between light and tissue. They are widely used by different research groups and companies.

1.2.1 Raman Spectroscopy

Raman spectroscopy measures scattered light that has been influenced by the oscillation and rotation of the scattered molecules. Various Raman techniques have been attempted in blood, water, serum, plasma solutions and the eye, but multiple problems remain before human studies can be accomplished. Analytical problems include instability in the laser wavelength and intensity, errors due to other chemicals in the tissue sample and long spectral acquisition times. The applying of special types of Raman spectroscopy can greatly enhance the signal noise ratio, the resolution and sensitivity. Resonance Raman (RR) scattering, surface enhanced Raman spectroscopy (SERS)¹², coherent anti-Stokes Raman scattering spectroscopy (CARS), and Stimulated Raman scattering (SRS) are examples of the Raman enhancement methods.

1.2.2 Fluorescence Spectroscopy

Fluorescence spectroscopy and time resolved fluorescence are dominant methodologies and used extensively not only in biochemistry and biophysics, but also in biotechnology, medical diagnostics 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.

In DMEM solution a glucose dependent autofluorescence 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.

2 APPARATUS AND METHOD

Our measurements were obtained using a micro Raman spectrometer, based on Olympus IX71 microscope by Fraunhofer Institute in Stuttgart. The separation of spectrums is achieved using holographic grill in spectrographic HoloSpec f/1.8 (Kaiser Optical Systems). Spectrum detection attained using a CCD for NIR (DU420A-BR-DD, 1024x256 Pixel von Andor). The measurements are done using a glass bottom dish Willco Welles.

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

The developed system is flexible and can be used for the development purposes, where different parameters have to be adjusted. Light intensities, duty cycle, different LASER types and variable amplification can be acquired using this system.

In the meantime we are developing a time resolved spectroscopy system in order to take more parameters into account by the calculation of glucose with a multisensor. By applying a multisensor for the detection of glucose concentration a great attention has to be given for the calibration method.

As we discussed in other papers (Abdallah, 2010) and as we can see in diverse literature the signal to noise ratio has to be kept as high as possible, but the glucose signal is too low, so that in addition to diminish the noise and considering all parameters affecting the measurements an adequate calibration is necessary for accurate glucose measurement non-invasively. The scattered signals may come mainly from deep tissue and blood glucose may be taken as reference. Moreover hemoglobin concentration and oxygen saturation have to be taken in to account by these measurements. Direct invasive measured values can be taken as reference. But fluorescence signals may come mainly from intestinal skin fluid ISF. Transfer of glucose from the blood to the ISF compartment occurs by passive diffusion through an established concentration gradient. The mass transfer rate is affected by several variables, such as the blood flow rate to the site, rate of glucose uptake by the surrounding tissue, and capillary permeability. Nevertheless, as discussed in the literature [Barman 2010], a simple mass transfer model can be written for the ISF volume (VISF).

The review by Ziegler (Zierler, 1999) describes major factors that, singly or together, influence the concentration and distribution of D-glucose in humans, with emphasis on rest, physical activity, and alimentation. It identifies areas of uncertainty: distribution and concentrations of glucose in interstitial fluid, kinetics and mechanism of transcapillary glucose transport, kinetics and mechanism of glucose transport via its transporters into cells, detailed mechanisms by which hormones, exercise, and hypoxia affect glucose movement across cell membranes, whether translocation of glucose transporters to the cell membrane accounts completely, or even mainly, for insulin-stimulated glucose uptake, whether exercise stimulates release of a circulating insulinomimetic factor, and the relation between muscle glucose uptake and muscle blood flow. It was pointed out that there is no compartment of glucose in the body at which all glucose has the same concentration, and that models of glucose metabolism, including effects of insulin on glucose metabolism based on assumptions of concentration homogeneity, cannot be entirely correct.

3 RESULTS AND DISCUSSION

The results obtained by applying a simple system with costs effective components and using DMEM solutions show that the measurements are reproducible under the same conditions. Using different light sources (LASER, LED, IR-emitter) having wavelengths in the visible and IR and using photodiodes and thermopile as detectors have shown the same tendency by measurements. A few wavelengths have demonstrated more dependency on the glucose concentrations. As an example of the measurements, figure 1 shows the high dependency of the detected light from glucose concentrations in glucose DMEM solutions by IR around the wave number 3200 cm^{-1} and between 1000 and 1700 cm^{-1} .

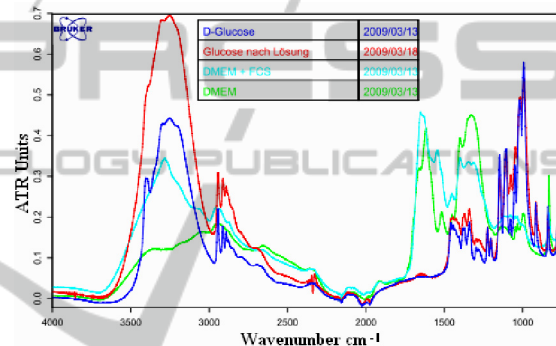


Figure 1: IR-Spectroscopy by DMEM glucose solution.

The tissue light absorption in the IR-range is too high due to the high water absorption, so that the light penetration in tissue is too small. Light emitting diodes and IR-detectors are also too expensive in this range. The non-invasive glucose measurement in this range is then too difficult and very expensive for home monitoring. Using an IR-emitter as an example of the results by measurements in DMEM solution and a thermopile as detector of scattered IR radiation between 9000 nm and 10000 nm have shown good results. Instead of IR-Emitter and thermopile, Laser sources and pyroelectric infrared sensors can be utilized.

By Raman scattering without enhancement the resolution of the measurements was not enough for the glucose concentrations monitoring. Increasing the measuring time will increase the resolution and the detection threshold. Figure 2 shows that the increase of the detection time increases the signal quality and the resolution; where as the resulting error will be reduced. The measuring time in Figure 2 by measurements an DMEM-solutions having different glucose concentrations is five minutes. As shown in the non-processed signal, the detection

threshold is ca. 30 mg/dl and the resolution seems to be around 10 mg/dl. Increasing the measuring time may be possible by in-vivo measurements. The motion artifact will cause large perturbations to the detected signals, which can be minimized by applying an adaptive filter. The enhancement of the Raman scattering by applying the previous mentioned methods can produce results having a high resolution and the detection time may be reduced.

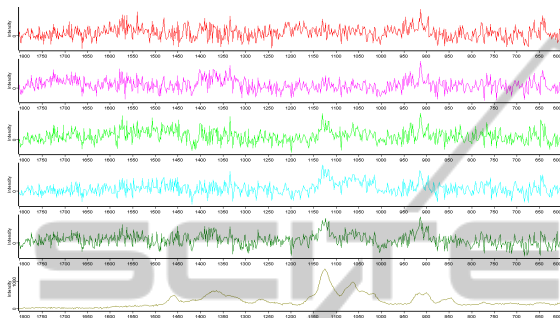


Figure 2: Raman scattering measured using a LASER diode with wavelength 785 nm and 80 mW power. From top: 300 s measuring time; a. 10mg/dl, b. 20 mg/dl, c. 30 mg/dl, d. 40 mg/dl, e. 50 mg/dl, f. reference solution with a very high glucose concentration.

The realization of a compact simple device for home monitoring using Raman spectroscopy seems to be very difficult, but it may be possible using miniaturized components and intelligent methods.

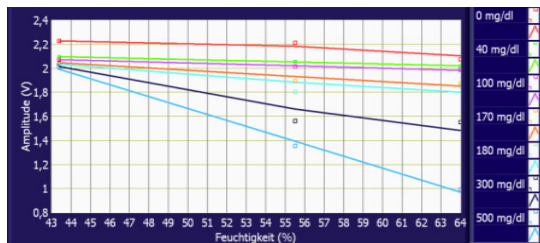


Figure 3: Scattered measured signal humidity for different glucose concentrations at parameters.

The relation between the scattered measured signal and humidity for different glucose concentrations are shown in Figure 3. The results obtained 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. But an increasing tendency of the emitted light with the increasing glucose concentration is registered.

The detected signal at 535 nm shows a high

correlation with glucose concentration when stimulated with a light by the wavelength 485 nm. 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. Despite of the variations of the detected signals around the wavelengths 465 nm when stimulated with the UV-light at the wavelength 360 nm, non-invasive measurement may deliver very good results due to other fluorophores in the skin.

A high correlation of the detected signal at 535 nm with known glucose concentration when stimulated with 485 nm or 430 nm at different glucose concentrations was obtained in DMEM-solutions.

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. We are applying a multisensor technology that overcomes the obstacles others have faced trying to measure blood glucose optically through the skin.

4 CONCLUSIONS AND FUTURE WORK

Optical methods are valuable and promising for the non-invasive detection of blood glucose. Raman scattering can have a very high sensitivity and resolution, but the development of an in-vivo simple device till now, seems to be very difficult. IR-spectroscopy is promising for the development of a cost effective sensor for home monitoring. Because of the fact that the detected glucose signals are too small and subjected to a lot of disturbances from the surroundings and from the background of the measured locations due to tissue alteration and physiological parameter variations, a high signal to noise ratio measuring system is essential for this difficult task. Tiny perturbations such as

temperature, humidity and applied pressure variations can adulterate the measurements. Also small drift of the characteristics of the electronic and optical components can cause great disturbances to the measurements reducing the accuracy or even yielding invalid measurements. In addition to use a robust hardware and apply advanced signal processing methods by the glucose detection, all these factors have to be taken into account.

The results obtained using a fluorescence spectrometer having the stimulation/emission wavelengths of 360 nm /465 nm, 430 nm / 535 nm and 485 nm / 535 nm (Abdallah, 2011) as well as our further fluorescence measurements at these and further wavelengths have shown that fluorescence spectroscopy is a very promising method. We have already developed a compact sensible system and sensor for that aim.

The integration of further parameters can enhance the accuracy, but the system complexity, its size and costs have to be minimized to enable the applying of the device for home monitoring. Also the number of the measured parameters has to be minimized in order to reduce the resulting error caused by the measurements variations..

Also by applying light sources with wavelengths in IR over 1400 nm the penetration depth of light in the tissue will be very small because water has a high absorption of IR-light. This will be important by detecting glucose or cholesterol using IR-spectroscopy. The reflective sensors can be applied proximal in order to avoid the perfusion problem by applying sensors (in case of transmission) distal to body extremity like fingers or earlobe. Light reflected from the tissues and detected by photodetectors and then the findings can be interpreted by the software in the sensor. The reflection sensor can be applied on forehead, back, breast etc., and hence diagnose the central parts of the body.

For the detection of glucose concentration noninvasively using various optical methods, the interaction between light and definite glucose solutions was studied. 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 multiparameter like scattering, 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 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 considered by the calculations, as illustrated by Figure 4. The drift of the characteristics of the system components may cause high disturbances to the measurements.

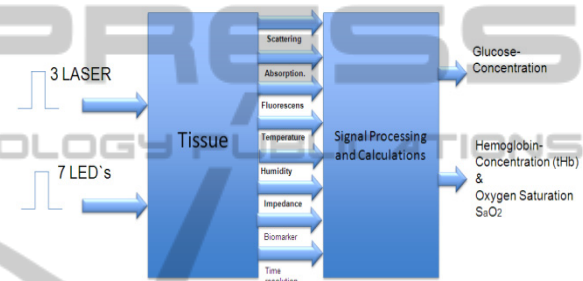


Figure 4: Schematic of a multisensor for non-invasive detection of blood glucose, hemoglobin concentration, and fractional oxygen saturation.

There is no doubt that the multiparametric measurement depending on scattering, absorption and fluorescence technologies have considerable promise for glucose sensing.

As a future work, all developed sensors will be integrated in one system that enables the simultaneous 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. For reflection measurements forehead as well as abdomen or arm can 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 will be conducted. Applying the neural fuzzy techniques, the results and the system will be optimized to obtain the required resolution and accuracy.

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