International Journal of Electrical and Electronics Engineers
Vol. No.7 Issue 02, July-December 2015
www.arresearchpublication.com
IJEEE
ISSN 2321 - 2055

A REVIEW ON NON INVASIVE CONTINUOUS BLOOD GLUCOSE MEASUREMENT TECHNIQUES

Gaurav Kumar Sangal[1], Pranjal Kaushal[2], Ruchir Sharma[3], Nandit Kaushik[4]

[1]Asst. Prof., ECE Deptt., [2,3,4]Student ECE Deptt., Hindu College of Engg., Sonepat

ABSTRACT

This study focused on the investigation of the possibility of monitoring blood glucose levels in humans by non-invasive techniques. The techniques used here is reverse iontophoresis and one of spectroscopy method called raman spectroscopy. The first technique is used to measure glucose level by extracting glucose and lactate from blood through the skin using reverse iontophoresis. In vitro reverse iontophoresis studies have indicated that the optimum switching mode for reverse iontophoresis of lactate and glucose are continuous direct current and direct current with electrode polarity reversal every 15 minutes, respectively. The application of a current combined with electrode polarity reversal every 15 minutes has been suggested for use in humans. The reverse iontophoresis technique was applied to 10 healthy volunteers. Glucose and lactate were successfully extracted through the subjects' skin into the methylcellulose gel of the electrodes. A fair-good correlation (r^2 = 0.62) between the subject's blood glucose level and the ratio of glucose to lactate levels in the collection gels was observed after two outliers were removed from the regression equation. The result suggests that it may be possible to non-invasively monitor the blood glucose levels using this new approach free of the need for calibration with a blood sample.

It is the successful study of the use of Raman spectroscopy for quantitative, non invasive (``transcutaneous'') measurement of blood analytes, using glucose as an example. As an initial evaluation of the ability of Raman spectroscopy to measure glucose transcutaneously, we studied 17 healthy human subjects whose blood glucose levels were elevated over a period of 2±3 h using a standard glucose tolerance test protocol. During the test, 461 Raman spectra were collected transcutaneously along with glucose reference values provided by standard capillary blood analysis. A partial least squares calibration was created from the data from each subject and validated using leave-one-out cross validation. The mean absolute errors for each subject were 7.8%, 61.8% (mean 6 std) with R^2 values of 0.83 60.10. We provide spectral evidence that the glucose spectrum is an important part of the calibrations by analysis of the calibration regression vectors.

Keywords: Reverse Iontophoresis, Iontophoresis,Raman Spectroscopy, Glucose, Monitoring, Non-Invasive.

I. INTRODUCTION

Iontophoretic drug delivery is now an accepted method of drug therapy which is gaining wide popularity especially in the area of pain relief. This technique provides a means for regulated non-invasive systemic

administration of minute amounts of drug transdermally which is especially useful in patients who require long - term medication as in chronic pain, diabetics, hypertensive, rheumatoid etc. It negates the need for needle sticks, the pain and anxiety involved and minimises the trauma and risks of infection associated with it. This mode of drug delivery is simple, versatile, effective, and reliable and can be tailored for individual needs.

1.1 Iontophoresis

Different investigators have given different definitions because one simple definition cannot explain all the mechanisms involved. But for the sake of simplicity, "Iontophoresis is a process of transportation of ionic molecules into the tissues by passage of electric current through the electrolyte solution containing the ionic molecules using a suitable electrode polarity." This means it would involve an electromotive force. In the body, ions with a positive charge(+) are driven into the skin at the anode and those with negative charge (-) at the cathode. Iontophoresis is sometimes confused with electrophoresis and electro-osmosis, the former involving movement of the colloid (dispersed phase) and the latter involving the liquid (dispersion medium), which are quite different. Iontophoresis may however cause an increased transport of method of penetration of non electrolytes through tissues.

1.2 Reverse-Iontophoresis

The same technique is reversed here to continuous measure the blood glucose level and it is called reverse iontophoresis Raman spectroscopy as a method to measure the concentrations of blood analytes non invasively.

It is estimated that the number of people affected with diabetes mellitus will increase from 150 million to 220 mil-lion worldwide from 2000 to 2010.[1] There are many serious long-term complications, the most significant being cardio-vascular, retinal, renal and neuropathic. The Diabetes Control and Complications Trial report makes it clear that tight control of blood glucose levels, which entails frequent blood sampling, significantly delays occurrence of these complications, resulting in improved quality of life and reduced burden on the health care system.[2] Conventional blood sampling methods are painful and have other undesirable features. Non invasive “tanscutaneous"! Blood sampling methods are an attractive alternative for monitoring glucose, as well as other blood analytes. Several transcutaneous techniques are under development; for a review see Ref. 3. Methods employing near-infrared ~NIR! spectroscopy combined with multivariate regression analysis are among the most promising.[4 ± 6] Of the non invasive techniques for measuring glucose reported in the scientific literature, none has demonstrated sufficient accuracy for non adjunctive clinical use.[7] In addition, there has been no substantial proof that the measured signals result from the actual glucose concentrations.[3] Instead, it has been shown that the calibration models derived easily become over deter-mined, and that chance correlations can be interpreted as variations in glucose concentrations.[8,9] This indicates the need for a non invasive method providing greater speciality.

In this paper we demonstrate the use of another optical technique, Raman spectroscopy, for transcutaneous monitoring of glucose concentrations. Raman spectra exhibit distinct narrow features characteristic of the molecules present in the blood-tissue matrix, including glucose. Despite its weak sig-nals, Raman spectroscopy has been shown to provide detailed

quantitative information about the chemical composition of skin ~proteins and lipids!,[10,11] and corresponding changes associated with the development of cancer[12,13] and atherosclerosis.[14] Because spectra from blood or

tissue are composed of contributions from many constituents, extraction of quantitative information requires use of a reliable multi-variate calibration method, such as partial least-squares ~PLS! regression analysis.[15] PLS analysis of Raman spectra has been successfully applied to quantitative measurements of glucose and other analytes in serum[16] and whole blood samples.[17] The present study employs Raman spectroscopy for quantitative transcutaneous measurements. We show that glucose concen-tration variations in human volunteers can be quantitatively measured. We also present clear spectral evidence that the spectrum of the glucose molecule is an important part of the calibration, the first such demonstration using a noninvasive optical technique.

Skin provides a unique gateway for non-invasive transdermal monitoring. Recently, the reverse iontophoresis technique has been used for patient monitoring and non-invasive diagnosis. Reverse iontophoresis refers to the passage of a low level of current through the skin to promote the transport of both charged and neutral molecules. The major mechanisms are either the electromigration of charged species to the electrode of opposite polarity, electro-osmosis of neutral molecules to the cathode or anode, or a combination of these two processes. Topically, the most successful reverse iontophoresis application for patient monitoring has been non-invasive and continuous blood glucose detection. GlucoWatch® biographer is the best example of a commercial product utilising the techniques of reverse iontophoresis and biosensor to non-invasively and continuously monitor blood glucose. However, GlucoWatch® still has some problems, such as it does not reliably detect hypoglycaemia (The Diabetes Research in Children Network (DirecNet) Study Group, 2004). Also, before each use, GlucoWatch® must be calibrated with a blood sample assayed in the conventional way. This provides considerable impetus for the improvement of the existing system or the development of a new system, which is free of the need for calibration with a blood sample, to non-invasively and continuously monitor blood glucose level.

Lactate is known to be a major regulatory substrate in carbohydrate (e.g. glucose) metabolism. Therefore, lactate may be also a good parameter to monitor blood glucose level. Until now, no research has been done on investigating reverse iontophoresis of lactate in humans. Therefore, in this study, glucose and lactate were used as parameters to establish a new approach free of the need for calibration with a blood sample to measure blood glucose level in humans by reverse iontophoresis.

Hydrogen and hydroxyl ions are the two ionic species inherently present in the skin. Use of constant direct current (DC) may localise hydroxyl ions at the anodal skin region and hydrogen ions at the cathodal skin region. This process can cause a polarization of the skin which leads to stinging and erythrema (Howard, Drake & Kellogg, 1995). The use of pulsed DC, a constant DC delivered periodically, has been suggested to prevent skin polarization (Chien, Lelawongs, Siddiqui, Sun & Shi, 1990). During the “off time,” the skin naturally depolarizes itself and returns to its near initial electric condition. The use of a bipolar current, a constant DC which changes its current flow direction periodically, has also been suggested to overcome skin polarization (Tomohira, Machida, Onishi & Nagai, 1997; Howard et al., 1995). Tomohira et al. (1997) used an *in vivo* rat abdominal skin model to study the effect of electrode polarity switching (i.e. bipolar DC current profile) in iontophoresis of insulin and calcitonin. They found that bipolar DC current profile could enhance the absorption of the insulin and calcitonin. Hirvonen, Hueber and Guy (1995) used a diffusion cell with mouse skin to study the impact of different applied current profiles in regulating the permeation of two charged amino acids, lysine

and glutamic acid. They found that bipolar current profiles and constant DC current profile resulted in comparable transport rates, which are higher than that of pulsed DC current profile. Thus, in order to achieve a high transport rate, it is better to use bipolar current profile or constant DC current profile rather than pulsed DC current profile. However, constant DC is thought to cause a polarization of the skin which leads to stinging and erythrema. Therefore, a bipolar current profile is used in this study as it can provide a high transport rate and reduce skin irritation.

The aims of this current work were to establish the optimum switching mode for reverse iontophoresis of both glucose and lactate and to study the reverse iontophoretic extraction of glucose and lactate across human skin barriers. The effects of switching mode for reverse iontophoresis of glucose and lactate were investigated using *in vitro* diffusion cells. The best switching mode was applied to healthy human volunteers, and glucose and lactate was extracted through their skin using a specially-designed transdermal reverse iontophoresis collection device. The ratio of glucose to lactate levels in collection gels and the glucose level in healthy volunteers' blood were compared.

II. MATERIALS AND METHODS

2.1 Constant Current Source

The constant current source, designed and developed by the authors (Ching, Camilleri & Connolly, 2005), was used to deliver iontophoretic current for both *in vitro* and humans reverse iontophoresis experiments.

2.2 In Vitro Reverse Iontophoresis Experiments

N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES), sodium chloride (NaCl), sodium hydroxide (NaOH), hydrochloric acid (HCl) and lactate were purchased from Sigma Chemical Company (St. Louis, MO). Glucose was purchased from BDH Limited (Poole, England). Lactate reagents (LC 2389) and glucose reagents (GL 26233) were purchased from Randox Laboratories Limited(Antrim, UK). De-ionized water (resistivity $\geq 18x10^6$ Ωcm) that had been purified by a Millipore System (Milli-Q UFplus; Bedford, MA) was used to prepare all solutions. Nanoporous membrane with a net negative charge at pH 7 was used as the artificial skin model (Spectra/Por® CE (cellulose ester) Dialysis Membranes MWCO: 500, Spectrum Laboratories, Inc., Canada).Silver-silver chloride (Ag/AgCl) electrodes were prepared by chloridizing silver wire (1mm diameter, 25mm length, 99.99% pure; Aldrich Chemical Company Inc., Milwaukee, WI) immersed in 0.1M HCl solution (Pt-cathode) for 90 minutes at an applied current of 314μA.

All experiments were performed using diffusion cells (Connolly, Cotton & Morin, 2002), in which both electrode chambers were located on the same surface side of a nanoporous membrane. These chambers were filled with 350μl of 25mM, pH 7.4, HEPES buffer containing 133mM NaCl. The lower chamber of the diffusion cell contained an electrolyte solution comprising 133mM NaCl, buffered to pH 7.4 with 25mM HEPES, and either 5mM glucose or 10mM lactate. Each electrode chamber contained a Ag/AgCl electrode. The surface area of the nanoporous membrane exposed to the electrode in each chamber was $0.2cm^2$ and the electrode chambers were 11mm apart. An iontophoretic current of $0.3mAcm^{-2}$ at 4 different switching manners (the polarity of electrodes reversing at intervals of 5, 10 and 15 minutes, or without reversing) was passed for 60 minutes via the Ag/AgCl electrodes. The entire content of the electrode chambers were removed at the end of the experiment to

quantify the amount of glucose or lactate extracted. For the control experiments, all the experimental arrangements and procedures were the same as that described in reverse iontophoresis experiments except no current was applied in the control experiments.

Spectrometric assay, using a spectrometer (Multiskan Ascent®, Labsystems Oy, Finland), was used to quantify the amount of glucose (determined by Glucose reagents) or lactate (determined by Lactate reagents) extracted through the nanoporous membrane. Excellent linear relationships ($r^2 > 0.99$ for both cases) between lactate/glucose concentrations and their relative absorbance were found, allowing the lactate/glucose concentrations to be calculated simply by linear regressions.

2.3 Humans Reverse Iontophoresis Experiments

Sodium phosphate monobasic (USP grade) and NaOH were purchased from Sigma Chemical Company (St. Louis, MO). Methylcellulose (MC) was purchased from The DOW Chemical Company (USA). 0.1M phosphate buffer solution (PBS) was prepared by dissolving 1.1998g of sodium phosphate monobasic in 100ml de-ionized water and adjusting the pH to 7.4 using a 40% w/v solution of NaOH. 4% MC gel was prepared by mixing 4g of MC with 100ml of 0.1M PBS. De-ionized water (resistivity $\geq 18x10^6$ Ωcm) that had been purified by a Millipore System (Milli-Q UFplus; Bedford, MA) was used to prepare all solutions.

Circular-shaped (11.3mm diameter) screen-printed Ag/AgCl electrodes (SPE) were fabricated and used in this study. Each SPE contains 90µl MC (4%) gel which acts as a conductive media for the transmission of iontophoretic current from the

SPE to the human skin and also acts as a collector for the storage of the extracted lactate and glucose.

Ten healthy human volunteers (8 men and 2 women), aged between 22 and 35 years (mean = 26.8, SD = 3.7), with no history of dermatological disease participated in this study. The subjects were required to maintain the sites under investigation free from application of any cosmetic topical formulations for at least 5 days before the study. The study was approved by the Strathclyde University Ethics Committee and informed consent was obtained from each volunteer.

The areas of skin of the subject's inner forearm where SPEs to be located were prepared by briskly rubbing the areas for 6-8 seconds with alcohol prep pads to remove dry skin, oils and other contaminants. The areas were then allowed to dry thoroughly. Then, the four SPEs were positioned on the subject's inner forearms and fixed in position with surgical tapes. Each pair of SPEs was about 23mm apart between the electrode centres and the two pairs of SPEs were about 50mm apart. An iontophoretic current of 0.3mA, with polarity of SPE reversing at intervals of 15 minutes, was passed between one pair of SPEs in conditions of room temperature for a period of 60 minutes. The second pair of SPEs was used as control with no current passing through it. At the end of the experiment, 20µl MC gel was carefully pipetted from each SPE and stored separately in microcentrifuge tubes at 4°C for later quantification of lactate and glucose. The subject's blood glucose level was also measured before experiments by a portable glucose meter (FreeStyle Blood Glucose Monitoring System, TheraSense Ltd., UK).

180µl of 0.1M PBS was pipetted to each microcentrifuge tube containing the extracted 20µl MC gel. The mixtures were then mixed well. From those mixtures, the amount of lactate (determined by Lactate reagents) and glucose (determined by Glucose reagents) extracted through the human skin was determined by spectrometric assay using spectrometer (Multiskan Ascent®, Labsystems Oy, Finland). Excellent linear

relationships (r^2 > 0.98 for both cases) between lactate/glucose concentrations and their relative absorbance were found, allowing the lactate/glucose concentrations to be calculated simply by linear regressions.

2.4 Raman Instrumentation

Raman spectra were collected by means of a specially de-signed instrument, optimized to collect Raman light emitted from a scattering medium ~tissue! with high ef®ciency. The setup ~Fig. 1! used an 830 nm diode laser ~PI-ECL-830-500, Process Instruments, Salt Lake City, UT! as the Raman exci-tation source. The beam was passed through a bandpass ®lter ~Kaiser Optical Systems, Ann Arbor, MI!, directed toward a paraboloidal mirror ~Perkin-Elmer, Azusa, CA! by means of a small prism, and focused onto the forearm of a human volun-teer with an average power of 300 mW and a spot area of ;1/mm^2. Backscattered Raman light was collected by the mirror and passed through a notch ®lter ~Super Notch Plus, Kaiser Optical! to reject the backscattered Rayleigh peak and the specular re¯ection at 830 nm. The ®ltered light was trans-ferred to a spectrometer ~Holospec f/1.8 *i* , Kaiser Optical! by means of an optical ®ber bundle ~Romack Fiber Optics, Wil-liamsburg, VA!, which converted the circular shape of the collected light to a single row of ®bers, in order to match the shape of the spectrometer entrance slit. The spectra were col-lected by a cooled charge coupled device array detector ~1340 31300 pixels, Roper Scienti®c, Trenton, NJ! corrected for the image curvature in the vertical direction caused by the spec-trometer optics and grating and then binned in the vertical direction, resulting in a spectrum with intensities at 1340 fre-quency intervals.

The intensity level of excitation light used in this experi-ment was based upon a thorough study in which tissue samples were irradiated with various ¯uences ~J/cm^2! of 830 nm light. The samples were then examined by a pathologist for changes in histology. The selected 300 mW level was substantially lower than the levels that caused histological changes. Mechanisms for cooling present *in vivo*, such as blood ¯ow, were not included in this study.[18]

With this result as an input, our protocol was approved by MIT's Committee on the Use of Humans as Experimental Subjects. A derma-tologist examined the skin of the ®rst volunteer before and after the measurements and observed no change. Except for one volunteer who developed a small blister, none of the vol-unteers experienced any discomfort during the test or exhib-ited any skin damage afterwards.

At this power level, our signal to noise ratio ~SNR!, cal-culated as the ratio of the collected signal to the noise at each wave number value for a 3 min measurement averaged across the spectral measurement range, 355±1545 cm^{21}, was 6500:1.

2.5 In Vivo Data Collection

Raman spectra were collected from the forearms of 20 healthy Caucasian and Asian human volunteers following the intake of 220 mL of a beverage ~SUN-DEX! containing 75 g of glucose. For each volunteer, all spectra were measured from the same area. The data from three of the volunteers were not included in the study because of problems such as excessive movement during the test with two of the volunteers and a small blister developed by the third. Using the data from the remaining 17 volunteers, each spectrum was formed by aver-aging 90 consecutive 2 s acquisitions ~3 min collection times.

Spectra were acquired every 5 min over a period of 2±3 h ~2.3 h, on average!, forming a ``measurement series" for each vol-unteer ~27 spectra per series, on average!. During this period, the blood glucose concentration typically doubled and then returned to its initial value. The glucose concentrations for all volunteers ranged from 68 to 223 mg/dL. During the measure-ments, reference capillary blood samples were collected from ®nger sticks every 10 min

~277 total! and analyzed by means of a Hemocue glucose analyzer, with a one std precision speci®ed by the manufacturer as <6 mg/dL. Reference mea-surements with this amount of imprecision could have added approximately 10% to our reported error in glucose measure-ment. Spline interpolation was used to provide reference val-ues at the 5 min intervals.

2.6 Raman Spectral Pre-Processing

Raman spectra in the range 355±1545 cm^{21} were selected for processing. Spectra collected *in vivo* consisted of large, broad backgrounds superposed with small, sharp Raman features. We utilized two methods of processing the collected spectra. In the ®rst method, the background was removed by least-squares ®tting each spectrum to a ®fth order polynomial and subtracting this polynomial from the spectrum, leaving the sharp Raman features. In the second method, the spectra were analyzed without removal of the background. Removing the background offers the advantage of more clearly showing the Raman spectra. All of the Raman spectra illustrated in the ®gures were pre-processed in this way. However, we found that somewhat more accurate calibrations were obtained using data without the background removed ~mean absolute error of 7.8% versus 9.2%!. Intensity decreases and spectral shape changes in the background signal were observed during the course of measurements on each individual. The effect of the polynomial subtraction method on Raman spectra extracted from background signals with these changes may be the rea-son that the errors are higher when the background is re-moved. Therefore, the performance results discussed below are based upon measured spectra without background re-moval.

2.7 Chemical Composition

The features of the observed *in vivo* Raman spectra were seen to be dominated by spectral components of human skin. These contributions were evaluated by least-squares ®tting the ob-served Raman spectra to Raman spectra of the key constitu-ents: human callus skin ~thickened stratum corneum with high keratin content!, collagen I and III to model dermal and epi-dermal structural protein, and triolein ~a triglyceride! to model subcutaneous fat. A Raman spectrum of human hemoglobin was also included to account for the blood volume probed. The spectra of other possible components, such as water, cho-lesterol, elastin, phosphatidylcholine and actin, were also in-cluded. The spectrum for each component was normalized by its total Raman signal strength.

2.8 Spectral Data Processing

The combined background/Raman spectra from each volun-teer were analyzed by means of partial least-squares regression.[15] The spectra were smoothed with a 13 point Savitsky±Golay algorithm to increase the effective SNR and then mean centered.

A PLS calibration was created, using Pir-ouette software ~Infometrix, Bothell, WA! and validated using leave-one-out cross validation.[19] A PLS calibration regression vector was formed from between 3 and 10 loading vectors from each calibration set. In most cases, the method utilized to determine the optimal number of factors was to ®rst deter-mine the number of factors that produced a minimum Stan-dard Error of Validation ~SEV!. Then, to reduce the chance of over®tting, the model chosen was the one with the lowest number of factors such that there was not a signi®cant differ-ence in its error compared to the model with the lowest SEV.[15]

International Journal of Electrical and Electronics Engineers
Vol. No.7 Issue 02, July-December 2015
www.arresearchpublication.com
IJEEE
ISSN 2321 - 2055

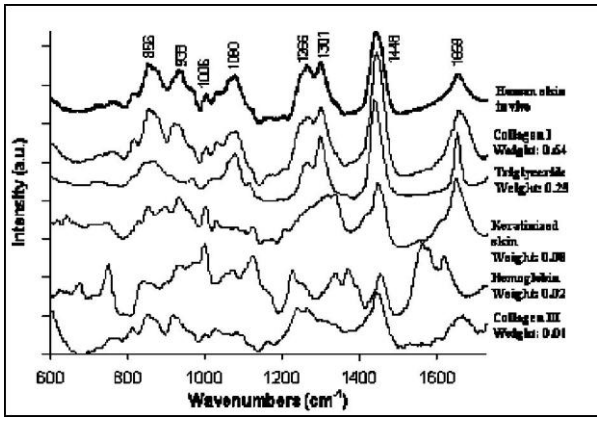

Fig. 2 Raman Spectra of Human Skin and its Primary Chemical Com-Ponents.

With four sets of data, we utilized more than the number of factors determined optimal by the above method to obtain calibrations that are more strongly in¯uenced by glucose. This is explained further in the Analysis and Discussion section. The predicted glucose concentrations were then obtained as the scalar product of the measured Raman spectra and the calibration regression vector plus the mean value of reference glucose concentrations. A mean absolute error was calculated for the predicted glucose concentrations of the *n* samples in each data set as

$$MAE5\ \frac{1}{n}\left(\sum_{i\ 51}^{n} Abs\sim\sim glu_{meas}2glu_{ref}!/glu_{ref}\right).$$

III. CONCLUSIONS

This study demonstrates the feasibility of noninvasive blood glucose measurements using Raman spectroscopy. This result combined with our earlier report on whole blood measure-ment of a number of analytes[17] suggests the feasibility of noninvasive measurement of other blood analytes as well. It also projects the promise that technology based upon Raman spectroscopy can be developed to meet clinical accuracy re-quirements. To our knowledge, this is the firrst report of optical noninvasive glucose measurements to clearly demonstrate that the spectral features of the glucose molecule are an important part of the calibrations.on the other hand the another technique reverse iontophresis by application of a current can facilitate movement of both glucose and lactate across the nanoporous membrane and the optimum switching mode for reverse iontophoresis of lactate and glucose are DC and DC with electrode polarity reversal every 15 minutes, respectively. However, the application of current combined with electrode polarity reversal every 15 minutes is recommended to be used in clinical situations as lactate extraction under these conditions was still good and skin irritation may be minimised. On the other hand, long duration bipolar direct current with 15 minutes electrode polarity reversal can also facilitate both glucose and lactate extraction across the human skin *in vivo*. A fair-good degree of relationship was found between the subject's blood glucose level and the ratio of glucose to lactate levels in collection gels after two outliers were removed from the regression equation. This possibly permits a non-invasive glucose sampling methodology free of the need for calibration with a blood sample.

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