In-vivo quantification of lactate using Near Infrared reflectance spectroscopy

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***Abstract*— Elevated lactate levels in blood (hyperlactatemia) are indications of hypoperfusion or sepsis in critical care conditions. Quantification and monitoring of this important marker is performed using intermittent blood sampling, which fails to provide a complete scenario to aid clinicians in di- agnosis. The feasibility of Near Infrared (NIR) Spectroscopy as an alternative to state-of-the-art techniques in critical care environments for non-invasive and continuous monitoring of lactate has previously been established. Nevertheless, the challenge lies in translating this research from bench to bedside monitoring. For this reason, a pilot investigation was carried out with a portable NIR spectrometer, where spectra in the range of 900-1300 nm were collected from 8 healthy human volunteers undertaking a high intensity incremental exercise protocol for lactate monitoring. This paper reports on the measurement set-up, spectra acquisition and analysis of diffuse NIR reflectance spectra of varying concentrations of lactate. The results obtained by 2D correlation analysis and linear regression are promising and show that the wavelengths 923 nm, 1047 nm, 1142 nm, 1233 nm, 1280 nm and 1330 nm are significant for lactate concentration determination in the NIR region. This provides the necessary confidence for using NIR sensor technology for lactate detection in critical care.**

***Keywords*- Lactate, Lactic Acid, Critical Care, Biosensors, Spectroscopy, Near Infrared**

I. INTRODUCTION

Lactate is one of the key metabolites in blood which serves as an important marker for the detection and diagnosis of several critical conditions such as stroke and sepsis [1]. For this reason, continuous, bedside, non-invasive sensing of lactate would be very desirable in critical care settings. The feasibility of Near Infrared (NIR) Absorption/Reflectance Spectroscopy, as a rapid and non- invasive tool for lactate detection has been established in the recent decades.

In 1996, Burger et al. identified lactate in an aqueous medium with the help of NIR Raman Spectroscopy [2]. In the same year, Chung et al., identified lactate using NIR Spectroscopy in the wavelength range 2000-2500 nm, also in an aqueous medium [3]. Again, in 1998, McShane etal., reported a prediction error of 8 % for lactate in a cell culture medium [4]. These reported investigations used multivariate analysis, such as, Partial Least Squares (PLS), for the identification of lactate in a pool of analytes with similar chemically structures. However, it was McShane et al., who also reported that these algorithms would require further development for better predictive analysis in NIR spectroscopy [5].

Furthermore, in 2002, Yano et al. identified lactate in peritoneal dialysis solutions using NIR Absorption Spectroscopy in the wavelength range of 400- 2500 nm and predicted lactate with $R^2 = 0.99$, using multivariate analysis algorithms [6].

During the same period, Riley et al., successfully isolated lactate from 5 different analytes in an aqueous medium in the wavelength range of 2083.33 to 2380.95 nm. They further resolved lactate from 19 different analytes in an animal cell culture medium using Fourier Transform NIR (FT-NIR) Spectroscopy [7]–[9].

The most significant contribution in NIR Absorption/ Reflectance Spectroscopy for lactate detection has been carried out by Lafrance et al. In 2000, Lafrance et al., predicted lactate in human plasma using FT-NIR in the wavelength range of 2050 to 2400 nm. The study reported a lactate prediction coefficient, $R^2 = 0.99$ with an error value of 0.51 mM, when compared against a commercial enzymatic electrochemical device [10], using multivariate analysis. Subsequently, in 2003, they predicted lactate in whole human blood with an $R^2 = 0.98$ and a standard error of 0.65 mM in the same range of wavelengths [11]. Ultimately, in 2004, an *in-vivo* study was conducted, where NIR diffuse reflectance spectra were collected in the wavelength range of 1500 to 1750 nm using a bench-top FT-NIR spectrometer. These spectra were recorded from the fingernail bed of healthy human volunteers, undergoing an incremental exercise protocol. Prediction of lactate was reported with an $R^2 = 0.97$ and a standard error of 0.76 mM [12]. This study confirmed the feasibility of using NIR Absorption/Reflectance Spectroscopy for lactate concentration prediction using multivariate analysis algorithms.

However, all the investigations that have been carried out previously used bench top spectrometers for lactate detection. Therefore, there is a need to investigate the feasibility of lactate detection using portable spectrometers for translating the research from bench-top spectrometers in a laboratory to bedside continuous monitoring. As a preliminary, the wavelengths pertinent to lactate in the NIR region needs investigation. Through this study, a fundamental understanding of the linear behaviour of the wavelengths pertinent to the lactate molecule would be investigated using a portable spectrometer, with the overarching aim of bedside lactate detection and monitoring.

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II. MEASUREMENT SET-UP AND PROTOCOLS

The investigation was carried out at the Research Center for Biomedical Engineering (RCBE) at City, University of London. During which, eight healthy volunteers (4 males and 4 females) were recruited for the investigation, with a mean age ± SD of 28 ± 6.7. The participant exclusion criteria included the following: prescribed medication, cardiovascular disease, lack of good balance and upper body strength, use of recreational drugs in the last month, alcohol consumption within 48 hours, smokers, and intake of stimulating supplements, both natural or synthetic in the last 48 hours. Ethical approval to conduct this study was obtained from the Senate Research Ethics Committee (SREC) at City, University London (SREC 17-18 02 13.1 10 10 2017).

Prior to the investigation, participants were were asked to fast for at least three hours to avoid any discomfort during the exercise protocol. The investigation commenced once a written consent was obtained, followed by an initial blood lactate measurement using a hand-held finger-prick meter Lactate Pro 2 from Arkay Inc, (*Flat Rock, MI, USA*). The Lactate Pro 2 analyser was chosen for this study, as it has been demonstrated to give stable, accurate and reliable results in independent comparative studies [13]–[15]. Spectra from the upper part of the right thumb of each volunteer were also collected using a portable NIR spectrometer and a fibre optic cable NANOQ-PROBE-600-VIS-NIR, from Ocean Optics (*Largo, FL, USA*). These measurements were recorded by allowing the volunteer to place the right thumb in the opening of the reflectance probe holder, as shown in Figure 1. The set-up was established to minimise volunteer movement which affects the quality of the acquired spectra.

Following the initial measurements, participants were then asked to sit on a standard exercise indoor Pro/Trainer bike from Wattbike *(Nottingham, UK)*. They were asked to cycle for 1 minute on an unloaded bike at a constant cadence of 60 rotations per minute (rpm). This was followed by a 1 minute rest period, where lactate concentrations were measured using the Lactate Pro 2 and NIR spectra were also recorded. The incremental exercise protocol was repeated, where after every rest period, the air resistance was increased by 2 units magnetic resistance constantly at 6 units, and constant cadence of 60 ± 2 rpm. Subsequent two minute exercise-rest sequences were performed until exhaustion of the volunteer or upon reaching 90 % of predicted maximal heart rate (derived using the equation max heartrate = 220 – age in years) for each volunteer.

The lactate concentration measurement and spectra collection were continued for three or more cycles (every minute) even after the exercise protocol ceased. This was conducted to ensure that the lactate concentration of the volunteer had receded to a normal range. Moreover, this also provided a range of lactate concentrations and spectra from each volunteer. The spectra collection of each volunteer is discussed in detail in the next section.

III. NIR REFLECTANCE SPECTROMETRY

A. Spectrometry

A commercial portable spectrometer, NIRQuest, from Ocean Optics (*Largo, FL, USA*) was used to collect *in- vivo* NIR reflectance spectra from 900 to 1700 nm. This spectrometer was equipped with 512 pixels, Hamamatsu G9204, InGaAs linear array detector. The light source used was a halogen-tungsten lamp, LS-1-LL, also from Ocean Optics. A slit width of 25 μm was attached to the light source to obtain an optical resolution of 3.1 nm.

The reference spectrum of the lamp was collected using the Spectralon Diffuse Reflectance Standard from Lapsphere *(North Sutton, NH, USA)*, as a white background. The dark spectrum was collected by covering the end of the fibreoptic cable by a black sheet with the lamp turned off. The integration times of all the spectra was kept as automatic.

Ten spectra were collected during each measurement, which were then averaged for further pre-processing and analysis.

B. Spectral Analysis

A total of fifty nine diffused reflectance spectra were collected from 8 volunteers. The concentration of lactatein these spectra was found to vary from 1.6-19 mM, onthe basis of the hand held meter measurements (Lactate Pro 2). However, due to the noise in the recorded spectra, only twenty nine of these spectra could be used for further analysis. The noise was mostly from a few volunteers which might have been due to the skin properties of the volunteers.

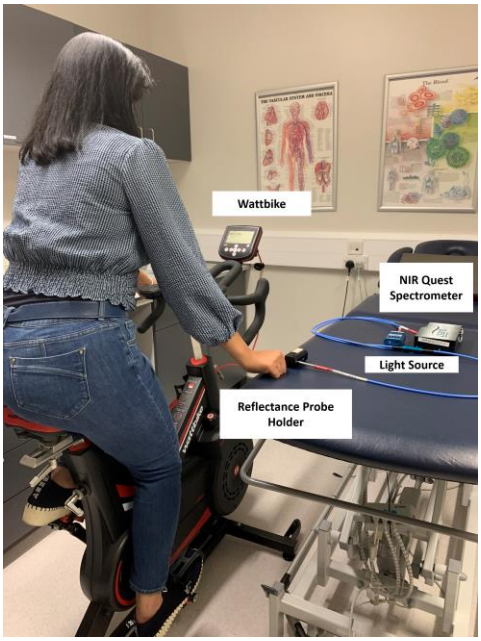

Fig. 1. The measurement set-up of an in-vivo volunteer investigation.The set-up demonstrates an incremental exercise bike study, where lactate concentrations are measured using a commercial portable NIR spectrometer, a light source and a reflectance probe holder, which connects the fiber optic cable to the spectrometer.

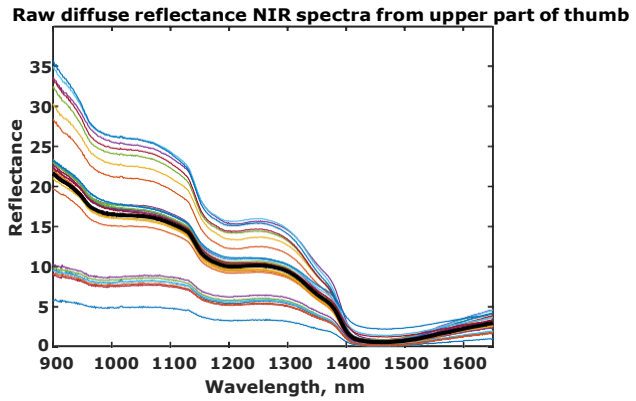

Fig. 2. Raw diffuse NIR reflectance Spectra from the upper part of the human thumb collected from 8 different volunteers.

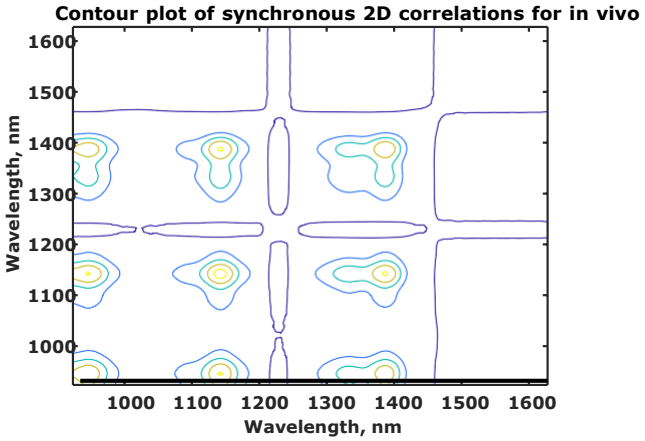

Fig. 3. 2D correlation of the in vivo NIR reflectance spectra collected from 8 different volunteers.

Outlier detection was performed solely by visual inspection, and the remaining spectra were pre-processed using the following techniques in sequence:

- Spectral Subtraction: where the spectra of 1.6 mM concentration of lactate was subtracted out from the rest of the spectra,
- Savitzky-Golay Derivation (SG): 2^{nd} order polynomial fit and 31 points on a second derivative was applied on the remaining 28 spectra
- Multiplicative Scatter Correction (MSC): was also performed in order to reduce noise and enhance the spectral features.

After the pre-processing of the remaining spectra, they were analyzed using 2D correlation analysis. The highlighted wavelengths were then linearly analyzed. Visualization, pre-processing and analysis of the spectra was carried out using the MATLAB 2020b software (*MathWorks*$^{T\ M}$, *Massachusetts, United States*).

IV. RESULTS AND DISCUSSION

The objective of this study was to make headway towards clinical translation, bring the proven feasibility of measuring lactate using NIR spectroscopy to a point of care device instead of using large bench top spectrometers. Diffuse reflectance with NIR detectors has been proven to potentially acquire data from subcutaneous tissues in the human body [16], [17]. Hence, in this study, elevated lactate concentrations in healthy volunteers were following a high intensity cycling experiment. During the rest cycles, concentrations of lactate were measured using a finger-prick blood sampling and diffuse NIR reflectance spectra was collected from the right thumb, in the range of 900-1700 nm, as shown in Figure 2. The wavelength range chosen was based on that previously reported as the "optical window" for lactate; 600-1300 nm for non- invasive chromophore detection [18]. In figure 2, variations in the spectra can be seen as indicative of lactate concentration changes and were not affected by individual volunteer skin characteristics at the sample collection site. This shows that the thumb is an excellent location for sampling NIR diffuse reflectance spectra, which had notbeen probed in the previous studies. The reflectance of these recorded spectra might have resulted from the Red Blood Cells (RBCs) in the capillaries, in addition to the connective tissues in the thumb. Variations in the spectral features could possibly be the result of minor de-protonation/ionization of lactic acid to lactate ion ($CH_3CH(OH)COO^-$), since lactic acid is a weak acid [19], [20]. However, only a 900-1300 nm wavelength range was chosen for this study to avoid unwanted ionization effects; the OH first overtone lies in the wavelength range of 1300-1600 nm [21].

The concentrations of lactate the twenty nine spectra from each subject were assumed to track with the 1.6-19mM found in the blood samples, but it is also the casethat lactate in tissue will have some absolute and temporal differences, specially under dynamic conditions. However, these differences would be very minute and should not interfere during the processing of the spectra. Pre-processing of the spectra was performed in order to remove any additive effects on the spectra by removing the background and noise.

Figure 3, plots the 2D correlated plot of the wavelengths in the NIR region and the highlighted wavelengths are: 923 nm, 1047 nm, 1142 nm, 1233 nm, 1280 nm and 1330 nm. These wavelengths are in line with the in vitro data previously reported, considering the pH of a healthy volunteer is close to 7.4 [22].

The data in Table I shows that the p-values are all $\leq$ 0.05 which makes them all statistically significant, proving that these wavelengths reflects lactate concentration changes linearly, obeying the Beer-Lambert law. Hence, there is a possibility of developing the model for in vivo monitoring of lactate. Estimated lactate concentrations show equivalent linearity in the wavelengths of interest. Also, when p-values were compared with regards to in-vitro NIR and buffer values, they were in line [22]. There is also no evidence of inter individual characteristic differences in the correlations, which proves that NIR Reflectance Spectroscopy could be an alternative to the state-of-the-art for non-invasive lactate detection and monitoring.

TABLE I

P-VALUES FOR SIGNIFICANT LACTATE WAVELENGTHS FOR *in vivo* DIFFUSE REFLECTANCE NIR SPECTRA.

Wavelengths (nm)	923	1047	1142	1233	1280	1330
p-values	7.18E-05	2.47E-04	2.08E-05	8.08E-08	5.80E-05	1.20E-05

V. CONCLUSIONS

The results of this feasibility study have indicated forthe first time that a portable NIR spectrometer could beused for monitoring reliably changes in lactate concentrationsin vivo. However, due to the limited number of spectra available for building multivariate predictive models, thewavelengths of interest for lactate were analysed using linear regression. Nonetheless, this study provides the necessary confidence for further investigations using more extensively collected spectra from a wider subject pool and use advanced computational tools for data extraction to more accurately predict concentrations of lactate in critical care environments.

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