In vitro quantification of lactate in Phosphate Buffer Saline (PBS) samples

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***Abstract*— Continuous measurement of lactate levels in the blood is a prerequisite in intensive care patients who are susceptible to sepsis due to their suppressed immune system and increased metabolic demand. Currently, there exists no non-invasive tool for continuous measurement of lactate in clinical practice. The current mode of measurement is based on arterial blood gas analyzers which require sampling of arterial blood. In this work, we propose the use of Near Infra-Red (NIR) spectroscopy together with multivariate models as a means to non-invasively predict the concentration of lactate in the blood. As the first step towards this objective, we examined the possibility of accurately predicting concentrations of sodium lactate (NaLac) from the NIR spectra of 37 isotonic phosphate buffer saline (PBS) samples containing NaLac ranging from 0 to 20 mmol/L. NIR spectra of PBS samples were collected using the Lambda 1050 dual beam spectrometer over a spectral range of 800 - 2600 nm with a quartz cell of 1 mm optical path. Estimates and calibration of the lactate concentration with the NIR spectra were made using Partial Least-Squares (PLS) regression analysis and leave-one-out cross-validation on filtered spectra. The regression analysis showed a correlation coefficient of 0.977 and a standard error of 0.89 mmol/L between the predicted and prepared samples. The results suggest that NIR spectroscopy together with multivariate models can be a valuable tool for non-invasive assessment of blood lactate concentrations.**

I. INTRODUCTION

Sepsis is the leading cause of acute hospital mortality and commonly results in multi-organ dysfunction secondary to culture-positive or negative infection [1]. It is defined as unrecoverable hypotension despite adequate fluid replacement according to the Surviving Sepsis Campaign (SSC) Guidelines. The incidence of severe sepsis within the first 24 hours of admission to an intensive care units (ICU) in the UK is as high as 27.1%, and the mortality associated with these patients is predicted to be as high as 50% (40,000 deaths/year) [2]. When extrapolated worldwide, sepsis affects 26 million people and claims 6 million lives every year, making it a bigger killer than stroke, heart attack, or Chronic Obstructive Pulmonary Disease (COPD) [2], [3]. The fiscal and economic impact of sepsis on the UK national health services (NHS) is also significant, with more than £15.6 billion spent every year [4].

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Reducing mortality in patients with sepsis warrants early diagnosis followed by aggressive therapy. Patients with a late diagnosis are usually left with long-lasting effects after treatment such as missing limbs, organ dysfunction like kidney failure or cognitive impairment. Currently, the diagnosis and recognition of sepsis is based on screening tools such as the quick-Sepsis-Associated Organ Failure (qSOFA), National Early Warning Score (NEWS) guidelines, and The National Institute for Health and Care Excellence (NICE) guidelines (NG51), which check for hypothermia ($< 36^o$ C), hypotension (systolic BP < 90mmHg), tachycardia (> 130 BPM), tachypnoea (> 25 breaths per min), hyperlactatemia (> 2 mmol/L) and physical appearance of rashes [5], [5]. Nonetheless, with overwhelming clinical evidence available in recent years, the clinical decision making and patient management are predominantly based on persistent hypotension after fluid resuscitation and elevated serum lactate levels.

The relationship between elevated serum lactate levels and sepsis is marked by hypoperfusion. Inadequate perfusion with low oxygen delivery to the tissues results in increased anaerobic glycolysis. Which in turn causes a build-up of its by-products (i.e., pyruvate, NADH and H^+) and ultimately lactate [6]. Although lactate clearance typically happens in the liver and kidneys, these organs cannot overcome lactate overproduction, and this condition usually worsens in septic shock status. Hence, the measurement of lactate is a critical criterion for the timely resuscitation of septic shock patients. Currently, the changes in lactate levels are measured using blood gas analyzers, where arterial blood is sampled with a syringe or an arterial catheter usually from the radial artery. However, these instruments are costly, complex to operate, have long analysis times, require large volumes of blood (100–200 μl), and are not portable for bedside application. Most importantly, they are invasive and provide intermittent measurements [7].

Although not widely used in clinical practice, amperometry and photometry (which sample blood, saliva or tears) based devices are also available in the market for measuring lactate. These devices require a smaller sample volume and offer quick response time and portability. However, they suffer from single-point measurements and do not provide data on lactate level fluctuations during the period of interest. Other attempts to monitor blood lactate continuously such as the subcutaneous needle-based sensors and wearable bioelectronic devices have been confined to research as their reproducibility and accuracy are poor and are still being investigated [7]. Hence, there exists an unmet clinical need for a reliable, simple-to-use, reproducible, and cost-effective

technique that can measure blood lactate levels continuously and non-invasively.

We propose the use of optical spectroscopy combined with multivariate spectroscopic analysis as a means to non-invasively predict the concentration of lactate. The hypothesis behind this method is that the absorbance of light in the visible, near and mid-infrared regions is indirectly sensitive to variations in blood lactate levels and would hence show a variation in the absorption spectrum. However, these absorption peaks may be masked by the absorption of other absorbents such as tissue, hemoglobin, water etc. Hence multivariate models such as Principal component analysis (PCA), Partial Least Squares regression (PLS), and Pattern recognition are proposed to effectively analyze and quantify the (linear) systematic changes in the electromagnetic spectrum that are caused by changes in lactate levels. A similar approach, previously undertaken by Lafrance *et al* has resulted in promising yet inconclusive results, and the light interaction with lactate molecules remains unexplored [8], [9].

Hence to achieve the above objective, the first step of the approach proposed is to investigate the optical properties of lactate in a simple blood analogs with fewer absorbents and in a large physiological range (0 to 20mmol/L). The investigation involved acquiring absorption spectra in visible/UV, NIR and mid-IR regions of the electromagnetic spectra. However, this current paper focuses on the acquisition and quantification of changes in the lactate concentration in the NIR region.

II. MATERIALS AND METHODS

A. Sample preparation

Thirty seven equivolume (30 mL) samples of isotonic Phosphate Buffer Saline (PBS) with varying concentration of sodium lactate (NaLac) ranging between 0 and 20 mmol/L were prepared for this investigation. Isotonic PBS was used as the osmolarity and ion concentrations of PBS correspond to that of the human body. Analytical grade Sodium L-lactate ($C_3H_5NaO_3$ – $98 + \%$) *(L14500, Alfa Aesar, Lancashire, UK)* and isotonic PBS *(Thermo Fisher Scientific, Massachusetts, USA)* were first acquired in powder form and stock solutions were prepared. Stock NaLac solution of 600 mmol/L was prepared by dissolving 67.236 g of NaLac powder in a liter of deionized water *(Deionised Water Company, UK)*. A liter of aqueous PBS (1X) was made by dissolving 9.89 g of PBS 10x powder in a liter of deionized water. The NaLac stock solution was then serially diluted with PBS to produce thirty seven 30mL samples. The NaLac concentration in the first 21 samples was varied from 0 - 5 mmol/L with 0.25 mmol/L interval, and in the rest of samples the concentration was varied from 5 - 20 mmol/L with steps of 1 mmol/L. The concentration of each sample solution was verified before obtaining optical spectra using the LM5 lactate analyzer *(Analox Instruments Limited, Stourbridge, UK)*. All the test solutions were maintained at a pH of 7.4 ($\pm$ 0.2) and 24^{o}C, measured by Orion Star A211 Advanced pH Benchtop Meter *(Thermo Fisher Scientific, Massachusetts, USA)*.

B. Acquisition of Spectra

NIR spectral acquisition of NaLac samples was performed using the Lambda 1050 dual beam UV/Vis/NIR spectrophotometer *(Perkin Elmer Corp, Massachusetts, USA)*. The spectrophotometer was configured as follows:

- The light source used for NIR light transmission was a halogen tungsten lamp
- Two photodetectors, namely indium gallium arsenide detector (InGaAs) and lead sulfide detector (PbS) were used to detect the transmitted light photons in the regions between 800 – 1800 nm and 1800 – 2600 nm respectively
- The spectral resolution of the acquired spectra was set to 1 nm
- The slit setting for the InGaAs and PbS detectors were set on "servo mode", whereby the system monitors the reference beam energy and adjusts the slits accordingly to avoid over saturation of the detectors
- The gain of the InGaAs and PbS detector were set to 5 and 1 respectively, while the response time of both the detectors was set to 0.2 seconds
- The attenuation in the sample and reference beam were set to 100% and 1% respectively. This was to ensure stable spectral acquisition with high signal-to-noise (SNR) of high absorbance samples
- Prior to the acquisition of NaLac spectra, baseline correction was performed on the spectrophotometer at 100% Transmission / 0% absorbance to remove background noise.

Once configured, 300 μl of each NaLac sample was transferred into a macro quartz cuvette (λ : 200 nm – 3500 nm) *(Hellma GmbH & Co.KG, Jena, Germany)* with a light path length of 1 mm, and placed in the sample compartment of the spectrophotometer. An identical blank cuvette was inserted into the reference compartment. Three spectra of each sample was acquired in the desired NIR range. The three spectra were then averaged and the resulting spectrum from each sample was considered for further analysis. The test samples were chosen at random during spectral collection, to prevent bias.

C. Analysis of spectra

Once all the raw spectra was acquired using the UVWinlab software (Perkin Elmer Corp, Massachusetts, USA) that accompanies the Lambda 1050 spectrophotometer, it was pre-processed and analyzed in MATLAB R2018a *(The Math Works Inc., Massachusetts, USA)*. The first step in pre-processing was to perform a baseline spectral subtraction. Whereby, the spectra of the sample with base NaLac concentration (0 mmol/L) was subtracted from all the other spectra. This allows for the suppression of spectral features that are not pertinent to NaLac samples and highlights the change in the magnitude of spectral peaks among the spectra. Following this, the spectra was filtered and smoothed to

remove instrumental high frequency noise and enhance signal properties using Savitzky-Golay (SG) filter. The polynomial order and the window length of the filter were 2 and 51 respectively.

Partial Least Squares (PLS) regression analysis was then used to selectively extract lactate information from NIR spectra in the presence of other chemical interferents such as water, sodium and potassium chloride. PLS regression is a multivariate method used to determine correlations between concentrations of analytes and spectral responses, assuming a linear relationship between the two. The method extracts orthogonal factors or latent variables (LV) to model the spectral features that correlate with the analyte concentrations [10]. Each LV describes the proportion of co-variance in a descending order between the calibration spectrum and the concentrations of the analyte. The LVs reduce the dimensionality of the independent variables (wavelengths with no relevance to lactate absorption) space by projecting them onto lower dimensional spaces. The optimal number of LVs required for accurate estimation of lactate concentrations was determined using the Prediction Error Sum of Squares (PRESS) . The PLS model developed was validated by leave-one-out cross-validation method, where a spectrum of unknown concentration is set as test subject and the remaining spectra are used to develop a model. The model was then used to predict the lactate concentration of the unknown test spectrum. This process was repeated 37 times, until every spectra has been used as a test subject and its concentration was estimated. The prediction accuracy of the calibration model was then described by coefficient of determination (R^2) between the estimated and actual lactate concentration, and by the estimated measurement error, which was calculated as the root mean squared error of prediction (RMSEP).

III. RESULTS AND DISCUSSION

The raw NIR absorption spectra of thirty-seven PBS samples with varying lactate concentrations between 0 – 20 mmol/L is presented in Fig. 1. Good quality raw spectra with clear spectral features were acquired from the Lambda 1050 spectrometer. As with most NIR spectra, the absorption in the region was dominated by the hydrogen atoms. Two prominent peaks in the spectra can be observed at the 1450 nm and 1920 nm, which can be associated with the O-H stretching fundamental (1^{st} overtone of water) and the combination band of O–H stretch with O–H bend respectively [11]. However, it is also important to realize that these broad absorptions are caused by multiple narrow, overlapping absorptions. Hence to visibly see changes in other regions more relevant to lactate molecules, the baseline water absorption (NaLac = 0 mmol/L) was subtracted from the rest of the spectra. Before the base spectral subtraction, the high frequency noise peaks in the regions between 1900 – 1960 nm and 2350 – 2600 nm (Fig. 1) were removed manually from the spectra to reduce the estimated errors in further analysis. This noise is due to detector saturation resulting from high absorption of water.

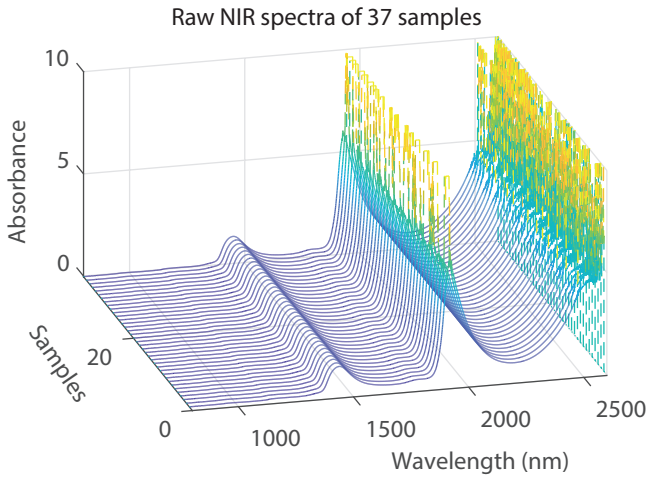

Fig. 1. Raw Near Infra-red (NIR) spectra of thirty-seven Phosphate Buffer Saline (PBS) samples with varying concentration of sodium-lactate ranging between 0–20 mmol/L

Fig. 2 shows the filtered spectra (SG filter) of five randomly selected samples of NaLac after the base spectra subtraction. In Fig. 2, an absorption with a magnitude greater than zero should theoretically reflect NaLac absorption, while absorption with magnitude below zero represents water (hydrogen atoms) absorption. Although, this is not entirely true in this case due the small concentration of NaLac ($<$ 20 mmol/L). Nonetheless, distinguishable peaks can now be observed in regions other than the water absorption peaks. These absorption peaks are in between 2200 – 2400 nm (centered at 2187, 2266 and 2299), 1660 – 1780 nm (centering at 1640 and 1743), and 1050 – 1220 nm (centering at 1147). Absorption in these NIR regions is linked to the combination of C–H stretch with C–H bend (2200 – 2400 nm) and C-H stretching (1660 – 1780 nm is the 1st C-H overtone and

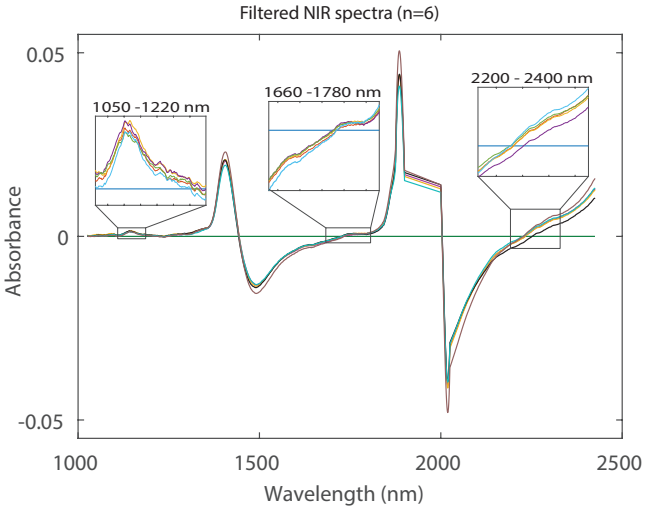

Fig. 2. NIR spectra of six randomly selected samples after base lactate correction and Savitzky-Golay (SG) filtering. Absorption peaks pertinent to NaLac can be seen in the regions between 2200 – 2400 nm, 1660 – 1780 nm, and 1100 – 1220 nm.

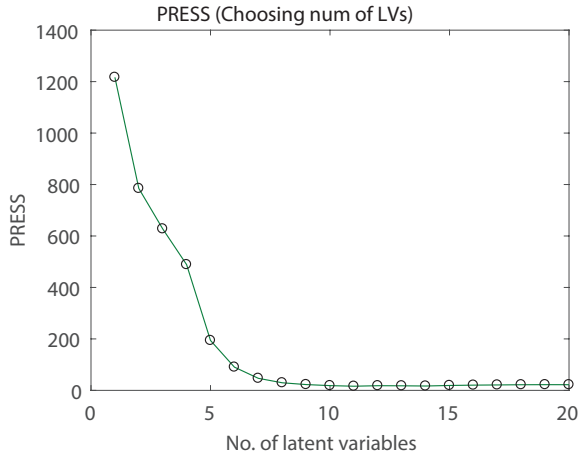

Fig. 3. PRESS versus number of latent variables (LV). Optimal number of LVs used for building the PLS model was eight.

1100 – 1220 nm is the 2nd C-H overtone) respectively [11], [12]. These results are in agreement with the previous work conducted by Lafrance *et al*, where they reported NaLac absorption peaks between 2100 – 2400 nm [9].

To examine the underlying relationship between the detected absorption peaks and NaLac concentrations and to explore the possibility of accurately predicting NaLac concentrations, a PLS calibration model was created using all the spectra. As mentioned earlier, PRESS was used to determine the simplest model, i.e. the model with the minimum number of LVs. This criterion, helps avoid over-fitting. Fig. 3 shows the graph of predicted residual sum of squares (PRESS) vs the number of latent variables. From Fig. 3, it can be observed that the first few factors contribute the most to the PLS model. After the 8^{th} LV, it is unlikely that addition

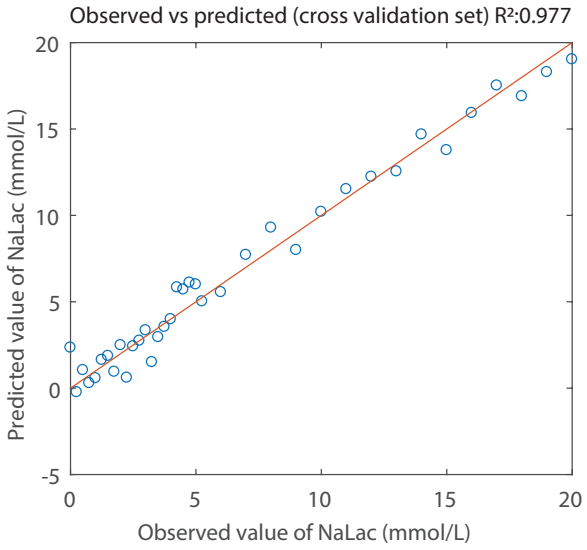

Fig. 4. Predicted NaLac concentration vs the NaLac concentration of thirty-seven PBS samples prepared in the laboratory. The correlation coefficient (R^2) and the root mean square error of prediction (RMSEP) was 0.984 and 0.89 mmol/L respectively.

of more LVs will significantly improve the PLS prediction. Hence, eight LVs were used to build the PLS model.

The dot product of the calibration coefficient vector and each spectrum of the data set was then used to estimate NaLac levels in PBS samples using PLS. Fig. 4 shows the plot of reference NaLac concentrations and predicted NaLac concentrations in the leave-one-out routine, using eight PLS factors. The diagonal line in the figure represents a great match between the measured and predicted NaLac values. The high correlation between the predicted values and the reference values resulted in a coefficient of determination, R^2, of 0.976. The root mean square error of prediction (RMSEP) on the linear regression was 0.89 mmol/L. This indicates that although the tight correlation of the data at low lactate concentrations is not apparent, it is easy to distinguish the large change in NaLac concentrations in PBS solution.

IV. CONCLUSIONS

In summary, we have demonstrated that NaLac concentrations in buffered solutions can be successfully estimated from NIR spectra using the PLS method. The results from the study provide the necessary confidence to carry out further investigations in complex solutions with more absorbents such as serum and whole blood. Future work will focus on improving the prediction algorithms used in this study to quantitatively predict the NaLac concentrations from NIR spectra of blood.

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