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Near Infrared Spectrometric Investigations on the behaviour of Lactate

N Baishya, K Budidha, M Mamouei, M Qassem, P Vadgama and P A Kyriacou

Abstract— In patients with life-threatening illnesses, the metabolic production and disposal of lactate are impaired, which leads to a build-up of blood lactate. In critical care units, the changes in lactate levels are measured through intermittent, invasive, blood sampling and *in vitro* assay. Continuous monitoring is lacking, yet such monitoring could allow early assessment of severity and prognosis to guide therapy. Currently, there is no routine means to measure lactate levels continuously, particularly non-invasively. The motivation of this study was to understand the interaction of lactate with light in the Near Infra Red (NIR) region of the electromagnetic spectrum. This was to create an opportunity to explore the possibility of a non-invasive sensing technology to monitor lactate continuously.

In vitro studies were performed using solution samples with varying concentration levels of sodium lactate in isotonic Phosphate Buffer Solution (PBS) at constant pH (7.4). These samples were prepared using stoichiometric solution compositions and spectra for each sample were taken using a state-of-the-art spectrometer in the NIR region. The spectra were then analysed qualitatively by 2D correlation analysis, which identified the regions of interest. Further analysis of these regions using linear regression at four randomly selected wavelengths showed bathochromic shifts, which, moreover, showed systematic variation correlating with lactate concentration.

I. INTRODUCTION

Glycolysis is a metabolic pathway, which takes place in the cytosol of any living cell, wherein, glucose is converted to pyruvate along with the production of Adenosine Triphosphate (ATP) and reduced Nicotinamide Adenine Dinucleotide (NADH). The pyruvate then enters the Kerbs cycle under ideal aerobic respiration conditions. However, in a deficit of oxygen, the pyruvate converts to lactate through the action of Lactate Dehydrogenase (LDH). LDH is a ubiquitous enzyme that catalyzes the interconversion of lactate and pyruvate with respective use of NAD+ and NADH cofactor. Lactate is produced in the human body, even at rest, mostly by the skeletal muscle (40-50%), the brain (13%) and adipose tissue (variable). The renal medulla, gastrointestinal (GI) tract, skin, red & white blood cells and platelets are also lactate-producing sites [1]. In blood, lactate production by the red blood cells, leukocytes (predominantly neutrophils) and platelets is 80%, 13%, and 7%, respectively [2]. The major lactate consuming tissues include the liver (20-30%), the renal cortex (20%) and the myocardium (5-15%). Lactate produced by muscle is converted to glucose by the liver and following its return to muscle is recycled as lactate by muscle glycolysis: the Cori Cycle [1]. The kidneys clear excess lactate in the blood, the renal threshold being 6-10 mmol/L. However, in patients with renal failure, notably in critical care, hyperlactataemia can result from impaired lactate clearance rather than overproduction, with levels overshooting the clearance threshold value [3]. In addition, for patients with sepsis in critical care, an increase in pyruvate production is seen because of an increase in glycolytic flux to such an extent that it exceeds the oxidative capacity of the mitochondria, which leads to excess lactate production [4]. In an experimentally induced sepsis, lactate production was seen to have increased by 6-fold in the leukocyte due oxidative burst of neutrophils [2].

According to the Surviving Sepsis Campaign (SSC) guidelines, sepsis is a medical emergency. SSC has suggested lactate level measurement to be an important hemodynamic marker for hypoperfusion and the onset of sepsis. It also states that if the initial lactate level during admission is ≥ 2 mmol/L, the patient needs urgent resuscitation with continued re-measurement every 2-4 hours until the lactate levels are stabilized [5].

Lactate measurements in critical care are often undertaken using arterial blood gas (ABG) samples, which are collected in heparinized syringes or *via* an arterial catheter line. The samples are then analyzed in the laboratory using analyzers based on enzymatic colorimetry or amperometry, which are reaction based assays and may be subjected to imprecision [6]. There are other factors, which can affect the concentration of lactate in a sample, for example: (i) the blood sample could be cross-contaminated by intravenous fluid; (ii) patients who have a tremor or vascular occlusion may have locally elevated lactate values, which can be misleading [7]; (iii) site & type of the sample: arterial blood samples are considered gold standard, since venous samples exhibit slightly elevated levels as compared to the arterial ones, while capillary samples may show much higher levels of lactate [1, 7, 8]; and (iv) handling of the sample: the samples are advised to be measured within 15 minutes from collection time because the red cells utilize the glucose and generate lactate [1], however this time is extended through using sample preservative. This identifies the need for a new and disruptive rapid measurement technology to monitor lactate levels non-invasively and continuously.

As a way forward towards an alternative technology, we propose the use of Near Infrared spectroscopy (NIR), to determine the concentration of lactate non-invasively on the hypothesis that light in the NIR region is sensitive to changes in lactate concentration. NIR spectroscopy has previously shown significant potential in monitoring tissue-oxygenation [8] and blood metabolites, including glucose [9, 11] and lactate [10]. Although such studies contributed to a predictive analysis of lactate concentration, the results were inconclusive and the interaction of light with the lactate molecule needs further investigation with more basic study and improved instrumentation.

This study focuses on qualitatively analyzing the changes in the concentration of lactate in buffer solution in a controlled experimental laboratory set-up. As such, the basic investigation would enable understanding of the changes in the absorption profile of lactate in the NIR region. This will be particularly useful whilst developing a technology, which requires no sample preparation and can utilize the whole NIR absorption spectrum for the continuous measurement.

II. MATERIALS AND METHODS

A. Preparation of Reagents and Solutions

Na Lactate and isotonic Phosphate Buffer Solution (PBS) salts, analytical grade, were obtained from Thermo Fisher Scientific (*Massachusetts, United States*). A stock solution of 600 mmol/L was prepared using 67.236 g of Na lactate powder in 1L of deionized water (*Deionised Water Company, UK*) and aqueous PBS made at X1 concentration. The lactate stock solution was then diluted to make 37 samples of 30mL each; 21 samples of 0-5 mmol/L lactate were prepared in concentration steps of 0.25 mmol/L and 16 samples of 5-20 mmol/L concentration, in concentration steps of 1 mmol/L. All test solutions were at pH 7.4 (±0.2) and tested at 24^0C; pH was checked using an Orion Star A211 Advanced pH Benchtop Meter Kit, from Thermo Fisher Scientific, (*Massachusetts, United States*) just before experiments. The lactate concentrations of the test samples was verified using an LM5 lactate analyzer from Analox Instruments Limited, (*Stourbridge, UK*).

B. NIR spectrometry

Three repeat continuous NIR absorption spectra from 800-2600 nm, with increments of 1 nm, were collected using a Lambda 1050 dual beam spectrophotometer, Perkin Elmer Corp (*Massachusetts, USA*). The three spectra were then averaged and the resulting spectrum from each sample was used for further analysis. The test samples were chosen at random during spectral collections to prevent bias. The following setup was maintained for the acquisition of spectra in the Lambda 1050: (1) a halogen tungsten lamp used as light source, (2) a Indium Gallium Arsenide, InGaAs photodiode used as detector for 800-1800 nm and (3) a Lead Sulfide, PbS detector used for the rest of the wavelength range. The gain & response time for the InGaAs and PbS detector were kept at 5 & 0.2s and 1 & 0.2s, respectively.

The attenuator settings were set to 1% for the reference beam and 100% for the sample compartment. This was done in order to reduce noise for high absorption values. Furthermore, an initial baseline correction of 100% Transmittance/ 0% Absorption was added. The sample was then placed in the sample compartment in Quartz cuvettes of 1 nm path length obtained from Hellma (*GmbH & Co., Germany*). The reference cuvette, of the same specification, was kept empty.

C. Spectral analysis

Once all 37 spectra of varying concentration were obtained, a spectral difference analysis was first performed on the spectra. In this operation, the spectrum of the blank (0 mmol/L concentration of lactate) was subtracted from all the other spectra. Following this, Extended Multiplicative Scatter Correction (EMSC) was applied on the resulting spectra to minimize any multiplicative effects and to enhance spectral features. To further enhance and highlight the spectral absorption features and to reduce noise, a Savitzky-Golay derivation (SG) was performed. The polynomial order, derivative and window length of the SG filter were 2, 2 and 71 respectively. These values were decided on as a trade-off between noise suppression and feature enhancement in the plots.

2D correlation analysis was then applied to the pre-processed spectra to visualize the systematic variations in spectral intensities induced by changes in the concentration of lactate. The constructed 2D correlation synchronous plot was then used as a guide to understand the chromic changes occurring in the spectra. All the aforementioned spectral analysis tasks were executed on MATLAB R2018a, MathWorks (*Massachusetts, USA*).

III. RESULTS AND DISCUSSION

Fig. 1 shows the raw NIR absorption profile of the thirty-seven different lactate concentrations varying from 0-5 mmol/L (at 0.25 mmol/L intervals) and 5-20 mmol/L (at 0.5 mmol intervals). Good quality raw spectra with clear spectral features were acquired from the Lambda 1050 spectrometer.
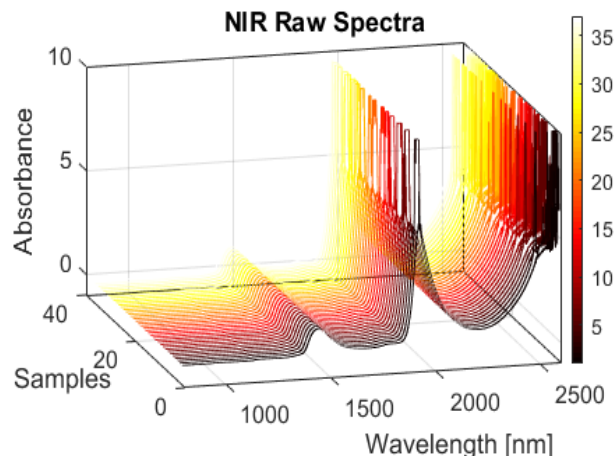

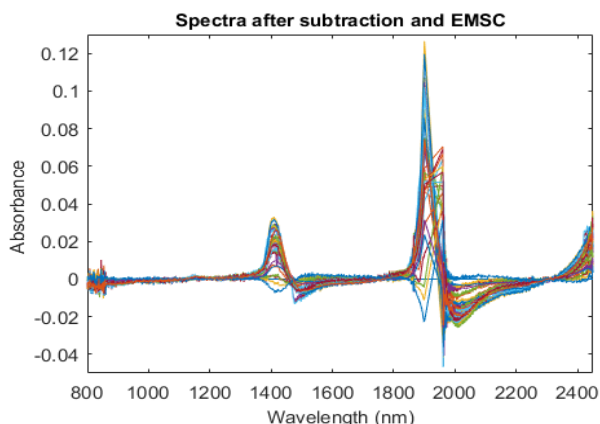Fig. 1: Raw Near Infra-Red (NIR) Absorbance Spectra of 37 samples.

Fig. 2: 37 spectra of varying lactate concentration after subtraction of base sample from all the samples and applying Extended

The raw spectra acquired are comparable to previous attempts made in understanding the absorption of glucose in water [12]. The absorption profile (Fig 1) shows two evident peaks at around 1470 nm ($av_1+ bv_3$, $a+b=2$) and 1900 nm ($av_1+ v_2+ bv_3$, $a+b=1$), which can be associated with overtone bands of water, where a and b are integers, ≥ 0, and v_1, v_2, v_3 are symmetric stretch, bending and asymmetric stretch of the covalent bonds, respectively.

From Fig. 1, it can also be observed that there are high frequency noise peaks in the region from 1900-1960 nm and 2350-2600 nm. This is due to detector saturation resulting from the high absorption of water. These regions were removed manually from the spectra to reduce unwanted correlations in the 2D correlation analysis. The base concentration of test solutions (0 mmol/L concentration of lactate) was subtracted from all the other spectra, followed by EMSC, as shown in Fig. 2. As can be seen from Fig. 2, the acquired 37 spectra of varying lactate concentration, after base subtraction and EMSC, contained significant noise, which was removed using SG derivation.

Fig. 3 depicts the 37 spectra of varying lactate concentration after the SG derivation. The flat lines in the region from 1900-1960 are due to the deletion of high

contour plot of synchronous 2D correlations

contour plot of synchronous 2D correlations
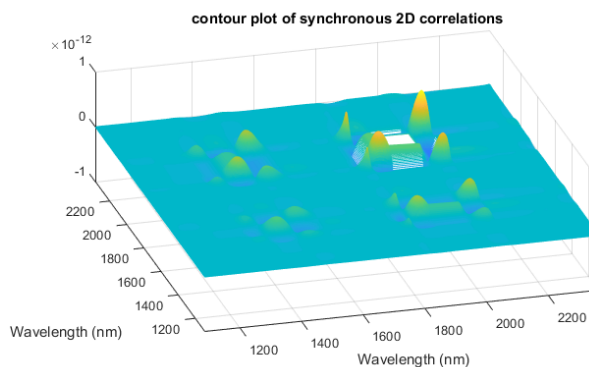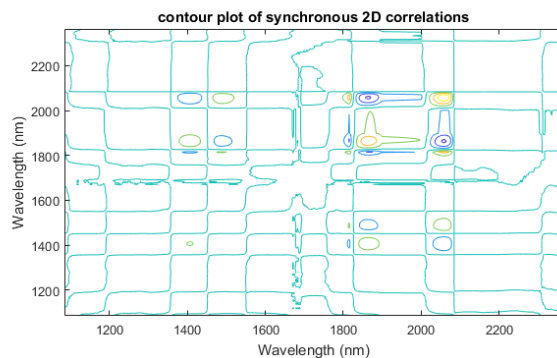
Fig. 4: Two dimensional synchronous correlation plot depicting the correlation changes in the 37 spectra.

frequency noise. Since lactate absorption in the solution is very weak compared with water absorption at these wavelengths, we cannot see large differences in the SG derivative spectra. However, what can be clearly seen is that the water absorption peaks experience a bathochromic shift. These changes are a likely consequence of varying lactate levels causing minor changes in the spectra, as the H^+ concentration remains unchanged in the buffered solution. These spectral changes might have been due to minor difference in lactate ionization correlated with the concentration of lactate. As the lactate level increases, the Degree of Ionization intensifies, since lactate is a weak acid, with a pK_a of 3.8. To assess these systemic changes further, 2D correlation analysis was performed on the 37 spectra of varying lactate concentration. From Fig. 4, systematic variations were found in two regions of the NIR spectra. The regions are 1380-1540 nm and 1860-2370 nm, which as stated earlier, correlate with changes in water absorption.

Four wavelengths from each of these regions were chosen at random to evaluate the bathochromic shifts further. Two wavelengths on each side of the maximum water absorption peaks (1470 nm & 1900 nm), were chosen. A linear regression was performed on the 37 spectra, at each of these wavelengths, separately. Table 1 shows the wavelengths, coefficients and the p-values of the regression.

As seen from Table 1, the p-values of the wavelengths, 1388 nm, 1435 nm, 1888 nm and 1899 nm are significant, with $p \leq 0.05$. The peaks at 1388 nm, 1435 nm, 1888 nm, and 1899 nm lie on the left side of the water absorption peaks, i.e. 1470 nm and 1900 nm. Hence, at these wavelengths, the
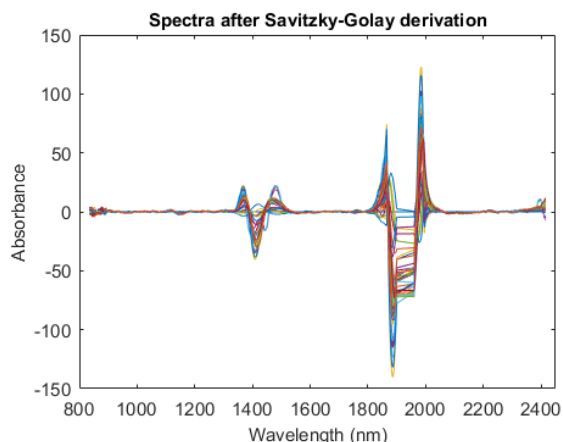

Fig. 3: 37 spectra of varying lactate concentration after pre-processing; subtraction of base sample, Extended Multiplicative Scatter Correction (EMSC) and Savitzky- Golay (SG) derivation.

TABLE 1: Values of randomly selected wavelengths, with the coefficients and p-values of the linear regression

Wavelength (nm)	Coefficient	p-values
1388	-484.97	0.0184
1435	-887.53	0.0239
1499	202.32	0.255
1532	291.48	0.239
1888	-162.19	0.0118
1899	-98.693	0.0028
2028	51.276	0.5774
2093	246.14	0.2181

coefficients are expected to be negative, indicating a negative correlation. However, the peaks at 1499 nm, 1523 nm, 2028 nm and 2093 nm have relatively high p-values, which indicates that the right side of the peaks at 1470 nm and 1900 nm are of no significance.

CONCLUSION

The results of this study has clearly demonstrated that lactate changes even at small concentrations can be detected using NIR spectroscopy. These qualitative results have indicated changes in lactate concentrations, which are eclipsed by the overtones of water in NIR region. However, this study provides the necessary confidence to further quantitatively analyze the spectra using advanced mathematical tools, which may allow accurate prediction of lactate levels.

ACKNOWLEDGMENT

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