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, secretion and excretion of lactate is impaired which leads to a build-up of lactate levels in the body. In critical care units, the changes in lactate levels are measured invasively using intermittent blood gas analysers. Continuous monitoring of these changes can, however, be used for early prognosis and to guide therapy. Currently, there is no means to continuously measure lactate levels, particularly non-invasively. The motivation of this paper is to understand the interaction of lactate with light in the Near Infra-Red (NIR) region of the electromagnetic spectrum. This will enable an opportunity to explore the possibility of finding a non-invasive sensing technology to continuously monitor lactate.**

In-vitro studies were performed in solution samples with varying concentration levels of sodium lactate in an isotonic Phosphate Buffer Solution (PBS) of a constant pH (7.4). These samples were prepared using solution stoichiometry and spectra of each sample were taken using a state-of-the-art spectrometer in the NIR region. The spectra was then analysed qualitatively by 2D correlation analysis which prompted the regions of interest. Further analysis in these regions by linear regression in four randomly selected wavelengths shows bathochromic shifts, which indicates systematic variations in the changes of the concentrations of lactate.

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 Adenosine Triphosphate (ATP) and reduced Nicotinamide Adenine Dinucleotide (NADH). The pyruvate, then enters the Kerb's cycle in ideal aerobic respiration. However, in a deficit of oxygen, the pyruvate metabolizes into lactate, with the help of Lactate Dehydrogenase (LDH). LDH is an enzyme that catalyzes the reaction of lactate to pyruvic acid, by converting NAD+ to NADH, in a reversible process and is found in almost all body tissues. L-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, the lactate production by the red blood cells, leukocytes (predominantly neutrophils) and platelets are 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%), where it is converted to glucose and returns to the muscles to be broken down again to lactate by Cori's Cycle [1]. The kidneys usually clear up the excess lactate build up in the body with the threshold value being 6-10 mmol/L. However, in patients with renal failure, especially in critical care, hyperlactemia can result from impaired lactate clearance instead of overproduction, when it overshoots the clearing threshold value [3]. In addition, for septic patients in critical care, an increase in the pyruvate production is seen because of an increase in the glycolytic flux, to such an extent that it exceeds the oxidative capacity of mitochondria, which leads to enhanced lactate production [4]. In an experimentally induced sepsis, the lactate production was increased to 6-fold in the leukocyte due to oxidative burst of neutrophils [2].

According to the Surviving Sepsis Campaign (SSC) guidelines, sepsis is considered a medical emergency. SSC has suggested lactate level measurements as a very important hemodynamic marker for hypoperfusion and onset of sepsis. It is also mentioned 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 usually done using arterial blood gas (ABG) samples, which are collected in heparinized syringes or arterial catheter. The samples are then analyzed in the laboratory using instruments based on enzymatic colorimetry and amperometry, which are indirect measures of L-lactate [6] and are hence, less precise. There are other factors, which directly affects the concentration of lactate in the samples, for example: (i) *blood collection technique*: arterial catheterization should be done by trained personnel, as there is often the possibility of contamination of samples by the intravenous fluid. Patients who are trembling or having a vascular occlusion can give elevated lactate values, which can be misleading [7]; (ii) *site & type of the sample*: arterial blood samples are considered gold standard, venous samples might exhibit slightly elevated levels as compared to the arterial ones, while capillary samples, show much higher levels of lactate [1, 8, 9]; and (iii) *handling of the sample*: the samples are advised to be measured within 15 minutes from their collection time because the cells utilize the glucose present in blood and converts it into lactate, which can be misleading, as well [1]. This identifies the need for a new and distuptive rapid sensor technology to continuously monitor lactate levels non-invasively.

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N Baishya, K Budidha, M Mamouei, M Qassem, and P A Kyriacou are with the Research Centre for Biomedical Engineering at City, University of London, Northampton Square, London, EC1V 0HB (phone: 020-7040-3878; e-mail: nystha.baishya@city.ac.uk).

P Vadgama is with Queen Mary University of London, Mile End Road, London, E1 4NS.

As a way forward towards such sensor technology we propose the use of Near Infrared spectroscopy (NIR), to non-invasively predict the concentration of lactate on the hypothesis that light in the NIR region is sensitive to changes in lactate concentrations. NIR spectroscopy has previously shown a lot of potential in monitoring tissue-oxygenation [10] and blood metabolites, including glucose [11] and lactate [12]. Although studies were previously carried out to predict lactate concentrations, the results from those studies were inconclusive and the interaction of light with the lactate molecule needs further investigation, with more basic studies and improved instruments [12].

This study focuses on qualitatively analyzing the changes in the concentration of lactate in a buffer solution in a controlled experimental laboratory set-up. As such a basic investigation would enable to understand the changes in the absorption profile of lactate in the NIR region. This will be particularly useful while developing a sensor technology, which will require no sample preparation and can take into consideration the whole NIR absorption spectra for continuous measurements.

II. Materials and Methods

A. Preparation of Reagents and Solutions

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

B. NIR spectrometry

Three continuous NIR absorption spectra from 800-2600 nm, with increments of 1 nm, were collected using the Lambda 1050 dual beam, Perkin Elmer Corp (*Massachusetts, USA*). 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. The following settings were maintained for the acquisition of spectra in the Lambda 1050: (1) halogen tungsten lamp was used as a light source, (2) InGaAs photodiode was used as a detector for 800-1800 nm and (3) PbS detector was 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 also 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 the 37 spectra were obtained, spectral difference analysis was first performed on the spectra. Whereby, the spectra of the base concentration (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 enhance spectral features. To further enhance and highlight the spectral absorption features and reduce noise, 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 as a trade-off between noise suppression and feature enhancement in the plot.

2D correlation analysis was then applied on the preprocessed spectra to visualize the systematic variations in spectral intensities induced by changes in 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.

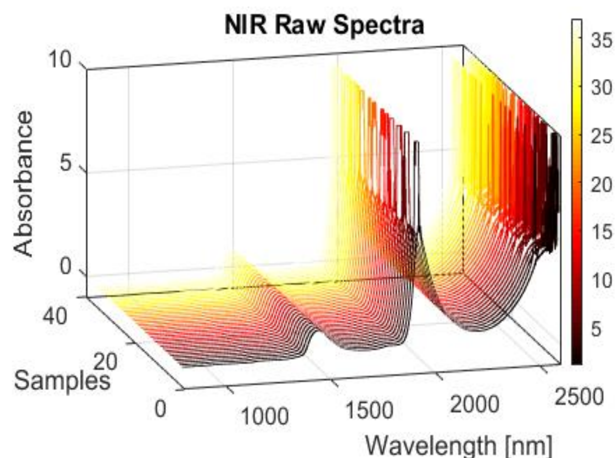


Fig. 1: Raw Near Infra-Red (NIR) Absorbance Spectra of 37 samples.

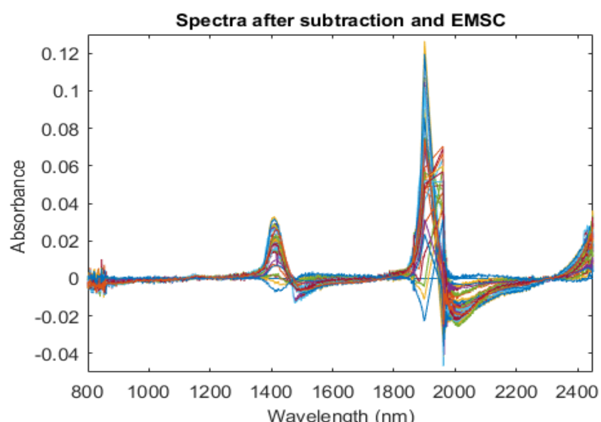


Fig. 2: Spectra after subtraction of base sample (0 mmol/L of lactate concentration) from all the samples and applying Extended Mean Signal Correction (EMSC).

The raw spectra acquired was comparable with previous attempts made in understanding the absorption of glucose in water [13]. The absorption profile (Fig 1) shows two evident peaks at around 1470 nm ($a\nu_1 + b\nu_3$, a+b=2) and 1900 nm ($a\nu_1 + \nu_2 + b\nu_3$, a+b=1), which can be associated with overtone bands of water, where a and b are integers, ≥0 and ν_1, ν_2, ν_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 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 spectra after base subtraction and EMSC, contained noise. Which was then removed using SG derivation.

Fig. 3 depicts the 37 spectra after the SG derivation. The flat lines in the region from 1900-1960 are due to the deletion of high 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 consequence of varying lactate levels causing a minor change in the pH of the solution. The pH of the solution varies minutely, since lactate is a weak acid by itself, with a pK_a of 3.8. To assess these systemic changes further, 2D correlation analysis was performed. 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 mentioned earlier, are correlated to changes in water absorption.

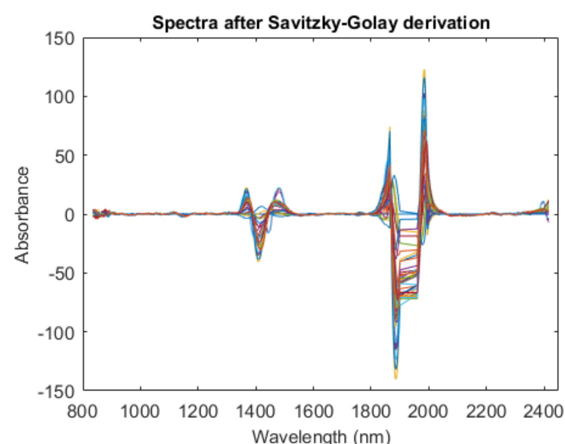
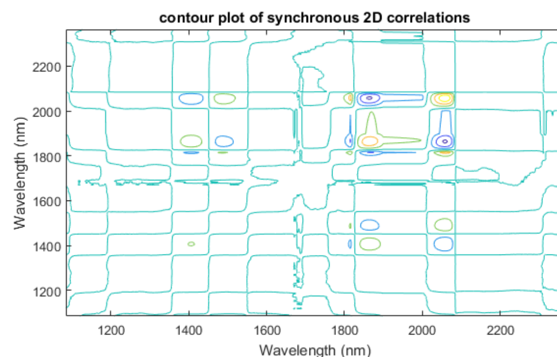


Fig. 3: Spectra of all the samples after pre-processing; subtraction of base sample (0 mmol/L of lactate concentration), Extended Mean Signal Correction (EMSC) and Savitzk- Golay (SG) derivation.



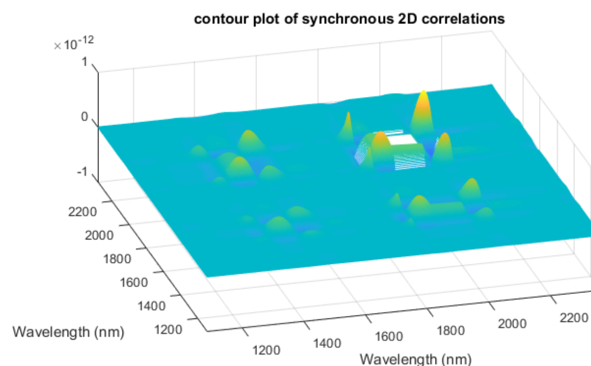


Fig. 4: Two dimensional synchronous correlation plot depicting the correlation changes in the 37 spectra.

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. Table 1 shows the wavelengths, coefficients and the p-values of the regression.

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

As seen from Table 1, the p-values of the wavelengths, 1388 nm, 1435 nm, 1888 nm and 1899 nm are significant, with values ≤ 0.05. The peaks 1388 nm, 1435 nm, 1888 nm, and 1899 nm lie on the left side of water absorption peaks, i.e. 1470 nm and 1900 nm. Hence, at these wavelengths, the coefficients are expected to be negative, indicating a negative correlation. Whereas, the peaks 1499 nm, 1523 nm, 2028 nm and 2093 nm has relatively high p-values, which indicate that the right side of the peaks 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 predictions of lactate levels.

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