Near Infrared and Aquaphotomic analysis of water absorption in lactate containing media

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***Abstract*— Increased concentrations of lactate levels in blood are often seen in patients with life-threatening cellular hypoperfusion or infections. State-of-the-art techniques used in clinical practice for measuring serum lactate concentrations rely on intermittent blood sampling and do not permit continuous monitoring of this all important parameter in critical care environments.**

In recent years, Near Infrared (NIR) Spectroscopy has been established as a possible alternative to existing methods that can mitigate these constraints and be used for non-invasive continuous monitoring of lactate. Nevertheless, the dominant absorption of -OH overtone bands of water in the NIR presents a challenge and complicates the accurate detection of other absorbers such as lactate. For this reason, comprehensive analysis of the -OH overtone bands with systematic lactate concentration changes is essential. This paper reports on the analysis of NIR spectra of two aqueous systems of varying concentrations of lactate in saline and whole blood using the principles of Aquaphotomics.

The results show distinctive conformational and structural differences in lactate-water binding, which arise due to the molecular interactions of bonds present in respective solvents.

***Keywords*- Lactate, Lactic Acid, Critical Care, Biosensors, Spectroscopy, Aquaphotomics**

I. INTRODUCTION

Serum lactate levels are often measured in emergency and trauma departments to assess the severity of disease states, support diagnosis and prognosis and suggest viable treatments [1]. In recent studies, it has been found that lactate concentration levels can predict mortality rates in critically ill patients and reflect the transitions in health conditions even after months of the initial readings made during admission [2]. These measurements, however, cannot be made frequently due to the invasive nature of measurements, and in recent years, this limitation has prompted rigorous investigations into the development of non-invasive techniques for continuous monitoring of blood lactate levels.

Near Infrared Spectroscopy (NIR) has been demonstrated as a potential rapid and non-destructive tool for determining lactate levels in various media [3]–[8]. However, a major challenge in NIR spectroscopic measurement of biological analytes with Absorbance in the NIR region, is presented by the dominant absorption of water-related overtone and combination bands. These bands, specifically around 1,450 nm & 1,940 nm, eclipse all other weaker absorbance bands, and makes direct and accurate quantification of these absorbers highly difficult. For this reason, previous attempts of lactate determination or quantification using NIR spectroscopy have generally disregarded these regions in their analysis. However, the development of a novel approach for indirect estimation of the solutes present in an aqueous solution called Aquaphotomics, has placed significant emphasis on these regions of the NIR spectrum [9]. This approach extends the possibility of uncoupling the latent information in the NIR region through the *"water mirror approach"*; where spectral analysis for aqueous systems are executed by mapping the external perturbations in the water absorbance bands of NIR spectra.

The process requires the acquisition of the NIR spectra of perturbed samples in the NIR region, especially identifying the first water overtone region 1300-1600 nm known as Water Absorbance Bands (WABS). Thereafter, the 12 Water Matrix Absorbance Coordinates (WAMACS), are identified which highlights the activated water absorbance bands. These activated water absorption bands produce patterns known as Water Absorbance Spectral Pattern (WAPS) that are pertinent to the aqueous system chosen in this study. The mapping of these WAMACS is usually carried out with the aid of Aquagrams [10]. To date, this technique has been used to understand several biological systems [9], and in addition to applications such as water and food quality, Aquaphotomis has been utilized in various fundamental biochemical systems applicable to animal and human medicine [11].

Another major challenge in NIR Absorption/Reflectance Spectroscopy is the depth of penetration of NIR light [12]. Besides blood, lactate can be produced by skin, adipose tissues and skeletal muscles, all of which contain considerable amounts of water [13]. These soft tissue aqueous systems appear on the surface of blood vessel network and further complicate the identification of light penetration depth in *in-vivo* settings. This is necessary to understand because distinct lactate producing sites in the body produce dissimilar concentrations of lactate, which if not fully understood, can provide erroneous output readings and mislead interpretations of lactate values by clinicians [14].

The motivation of this study is to observe the perturbations in the form of lactate concentration changes in two aqueous media; namely, Phosphate Buffer Saline (PBS) and whole blood, in accordance to the principles of Aquaphotomics. This study focuses on the identification of clusters in WAMACS for WAPS in the two media by constructing Aquagrams that will provide better understanding of water-lactate

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molecular conformations. Such knowledge could further aid in the development of a non-invasive lactate sensor for critical care applications using NIR spectroscopy.

II. Reagents and Materials

Na-L-Lactate and Phosphate Buffer Saline (PBS) powder (analytical grade) were purchased from Thermo Fisher Scientific (*Massachusetts, United States*) and a 600 $mmols/L$ (stock solution) of lactate was prepared. The PBS was diluted to X1 concentration in de-ionized water (*Deionized Water Company, UK*), with a pH of 7.4. Forty one sample solutions of varying lactate concentrations (0-20 $mmols/L$, at intervals of 0.5 $mmols/L$), of 30 mL each were prepared. All the solutions were maintained at room temperature of 24°C. Sheep Blood in Alsever's was acquired from TCS Biosciences Ltd (*Buckingham, United Kingdom*). Sheep Blood was chosen for this study because its lactate concentration was the closest resemblance to normal lactate levels in human blood. The study was approved by the Senate Research Ethics Committee (SREC), City, University of London as it involved biological fluids. 1 mL of lactate and PBS samples were then mixed with 19 mL of sheep blood to obtain 41 samples (for each set) of varying lactate concentrations of 20 mL each. The initial lactate concentration of the commercially acquired sheep blood was around 3.8 - 4 $mmols/L$. The pH of the prepared samples varied between 7.1-7.4, after adding the lactate solutions of known concentrations to the whole blood (with initial values of 7.4). The lactate and pH concentrations of all the prepared samples were measured using a Blood-Gas Analyser (BGA), ABL 825 from Radiometer UK Limited (*England, United Kingdom*).

III. NIR Spectrometry

A. Lactate in PBS samples

The spectra of the lactate in PBS samples were collected in the same way as described in our previous work [15].

B. Lactate in Blood samples

NIR spectra of whole blood samples were also collected using the Lambda 1050 dual beam spectrophotometer from Perkin Elmer Corp. (*Massachusetts, USA*), using a 100 mm InGaAs integrating sphere detector set at 0 $\deg$ transmission mode. Spectra were acquired in the wavelength range 870-2600 nm at 2 nm data interval. The integrating sphere detector was used in order to obtain a homogenised scattering of the transmitted light through the sample solutions as whole blood is a biological fluid with complex scattering properties. The Gain and Response Times for the InGaAs detector was maintained at 0 and 0.2 s, while the slit size was kept at 2 nm. These settings were maintained to prevent the detectors from oversaturation. Reference beam attenuation was set at 1%, and the same quartz cuvettes were used.

C. Spectral Analysis

In order to understand the perturbations in the system due to the varying concentration of lactate in both media, acquired spectra were pre-treated prior to performing further analysis. Since the whole (sheep) blood set of samples contained an initial level of lactate, only higher concentrations of lactate solutions were considered during pre-treatment and analysis in order to maintain consistency amongst both sample sets. Also, outliers were removed from both sets of sample. The following steps were performed sequentially:

- Spectral Subtraction: where the spectra of the base lactate from each set was subtracted from the rest of the spectra,
- Savitzky-Golay Derivation (SG): 2^{nd} order polynomial fit and 31 points on a second derivative
- Multiplicative Scatter Correction (MSC): in order to reduce noise and amplify the spectral features.

The pre-treated spectra were then examined by constructing aquagrams for the two sets. Visualization and processing of spectra was carried out using the MATLAB 2019b software ($MathWorks^{TM}$, *Massachusetts, United States*).

IV. Results

The raw spectra (1300-1600 mm) for both sets (lactate concentration changes in PBS and PBS & whole blood) can be seen in Figure 1 (a) and (b). From visual inspection of both sets it is quite evident that a marked distinction could not be made in the spectra as a result of changes in lactate concentration, as all the spectra seem to overlap. Figure 2 (a) and (b) presents the pre-treated spectra that were produced following the subsequent processes mentioned in the previous section. Here, clear and distinctive spectral patterns (WAPS) could be seen, and finally, Aquagrams (Figure 3(a) and (b)) were constructed, showing the 12 identified Water Matrix Absorbance Coordinates (WAMACS), in the region from 1300-1600 mm, which reflected the changes of the lactate concentrations in both sets.

V. Discussion

An extensive understanding of water-light interactions in water or aqueous systems is very critical in all biological processes as water forms the basis of all living beings. The NIR region of light has been used extensively to study these systems as it depicts precise water conformations, like dimers, trimers, etc. of water at different wavelengths [9]. In both the data sets of aqueous solutions described above, the primary component was water and hence, the band around 1450 mm could be visually distinguished (Fig 1 (a) & (b)), as expected. This WAB could be assigned to the first overtone of -OH stretching (aν1+ bν3, a+b=2), where, ν1 is symmetric stretching and ν3 is asymmetric stretching of the bonds present in the solution. As seen in Fig 2 (a) & (b), the WAPS showed variations in the pre-treated spectra, which reflects the chemical interactions of the concentration changes in lactate and water conformations in the two media. This region of the NIR spectra had been of special interest, lately and with the help of *"water mirror approach"* in the study of Aquaphotomics, various biomolecular aqueous systems have been studied [16]. The WABS in the NIR region had always been considered as an obstacle as it absorbs most of the light and overshadows the

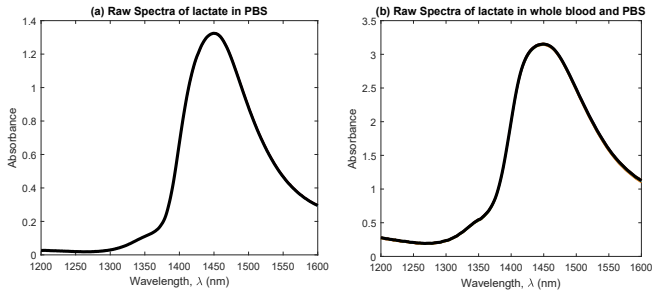

Fig. 1. (a) Raw Near Infrared (NIR) Absorbance Spectra of varying concentrations of lactate in PBS samples from 1200 to 1600 mm and (b) Raw Near Infrared (NIR) Absorbance Spectra of varying concentrations of lactate in whole blood and PBS samples from 1200 to 1600 mm. The NIR region 1200 - 1600 mm is the first Water Absorbance Bands (WABS).

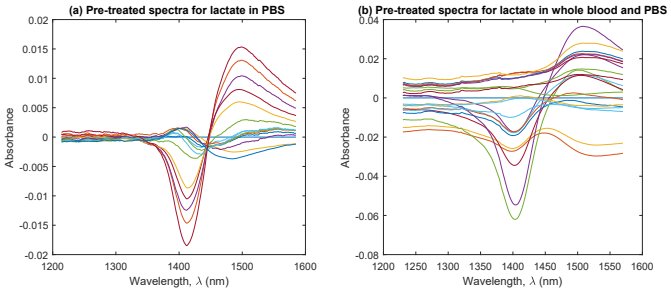

Fig. 2. (a) Spectra after pre-treatment of varying concentrations of lactate in PBS samples from 1200 to 1600 mm and (b) Spectra after pre-treatment of varying concentrations of lactate in whole blood and PBS samples from 1200 to 1600 mm. Pre- treatments include (a) subtraction of base sample, (b) Savitzky- Golay (SG) derivation and (c) Multiplicative Scatter Correction (MSC)). These figures represent the Water Absorbance Spectral Pattern (WAPS) for the above two aqueous systems.

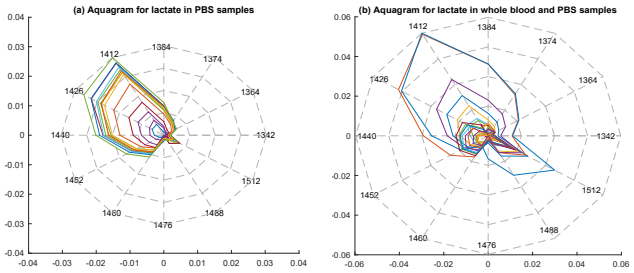

Fig. 3. (a) Aquagrams showing different Water matrix co-ordinates (WAMACS) of varying concentrations of lactate in PBS samples (b) Aquagrams showing different Water matrix co-ordinates (WAMACS) of varying concentrations of lactate in whole blood and PBS samples. The WAMACS: 1342, 1364, 1374, 1412, 1426, 1440, 1452, 1460, 1476, 1488, 1512 represents different water absorbance bands and in this figure depicts molecular conformations which arises due to water-NIR light-lactate molecule interactions.

important information hidden in that region. However, after the development of Aquaphotomics as a discipline, it was found that this region of the spectrum reflects the structural changes of the analyte due to the rearrangement of the bonds in such a system. Careful observation of the changes in molecular structure of water due to different perturbations in an aqueous system has been used as for quantification, detection, and structural analysis of various biomolecular aqueous systems [11].

In the first WAB of the NIR region, 12 wavelengths (WAMACS) at 6-20 mm apart, had been identified which are now designated to different water conformations and are almost consistent for different aqueous systems [9]. These WAMACS reflects the different conformation forms of water; for example, 1398-1418 (which are the shorter wavelengths) reflects more free water or -OH bonds and 1506-1516 (longer wavelengths) indicates bonded water molecules. In the two sets of lactate solution samples considered here for this study, the water absorption patterns (WASP) for these WAMACS were examined by constructing Aquagrams for the two sets (Figure 3 (a) and (b)).

From the Aquagrams (Fig 3(a) & (b)), it was evident that in PBS samples, there existed more free water molecules as the clusters were seen to be confined in the lower wavelength regions. This signifies that there are fewer lactate-water molecular interactions in this aqueous system. It has also been reported that the more number of hydroxyl (-OH) side groups, they tend to form weaker hydrogen bonds amongst

themselves and are hence, affected by the concentration changes of other solutes/analytes present in the solution [17]. Thus, in PBS aqueous set (Fig 3 (a)), the concentration changes of lactate could be more distinctly seen than in whole blood (Fig 3 (b)), due to the presence of more free -OH side groups.

In contrast, molecular clusters in the whole blood samples were seen tending towards longer wavelengths, signifying more bonded water and water-lactate molecular interactions. Whole blood aqueous systems are particularly more polar than PBS systems, hence, molecular interaction increases because of the availability of ionic components are dominant in these type of solvents. Increasing activity is therefore seen in WAMAC 1482-1495 (Fig 3(b)), which represent water-water molecular clusters with 4 hydrogen bonds. Also, an increased number of bound lactate-water bonds could be seen as a cluster in the WAMAC 1506-1516 for whole blood, (Fig 3 (b)) than in the PBS aqueous system (Fig 3 (a)).

In an *in-vivo* set-up for Near Infrared Absorption/Reflectance Spectroscopy, the knowledge from this study could provide a better understanding of the reflected signal obtained in *in-vivo* measurements. NIR light interacts with soft tissues in the subcutaneous level and blood, where water is found either in free and bound states [18]. The depth of penetration and reflection due to scattering of NIR light is mostly affected by the water present in these sites. A more in-depth understanding of free and bound water in particular sites of lactate monitoring pertinent to each application, will further strengthen NIR Absorption/Reflectance Spectroscopy as a possible alternative for non-invasive lactate monitoring in critical care.

VI. CONCLUSIONS

This study has demonstrated for the first time that the water peaks in the NIR region could provide insights of the interactions between lactate concentration changes in different aqueous systems. The results shown by a thorough study of the first overtone water peak in NIR region has depicted clear differences in these interactions, which are otherwise difficult to interpret. This study builds necessary confidence for *in-vivo* NIR Absorption/Reflectance Spectroscopy studies in different sites of the human body to understand the depth of penetration of NIR light and aid in better diagnostic lactate sensors for critical care.

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