In-vitro spectrometric analysis of hyperlactatemia and lactic acidosis in buffer relating to sepsis

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Abstract

The normal range for pH in the human body is 7.35-7.45. When pH falls below 7.3, it is considered as severe acidemia. Acidemia, together with increased blood lactate concentrations (hyperlactatemia) constitute a severe threat to life, which is often referred to as lactic acidosis. The feasibility of near infrared transmission/reflectance spectroscopy as a tool to determine lactate concentration levels and pH, independently, has been well established. However, the effects on spectral features arising from simultaneous variations in pH and lactate are not fully understood. Hence, this paper reports on a spectroscopic study of 37 different lactate concentrations that were prepared at three different pH levels (7.4, 7.0 and 6.5). Near infrared spectra were acquired in the range 800-2500 nm, and were later divided into four spectral ranges. Further investigations were carried out on various wavelengths within each spectral range and sample set. Furthermore, partial least squares regression with cross-validation was performed on all data sets. The results showed a clear interdependence and overlapping spectral behavior between blood lactate concentrations and pH. The findings from this study suggest that for an accurate estimation of blood lactate using this technique, the pH of the sample must be previously known.

Keywords

Hyperlactatemia, lactic acidosis, near infrared spectroscopy, sepsis, lactate

Introduction

Lactate is produced in the human body by an enzyme known as lactate dehydrogenase (LDH), which reduces pyruvate during anaerobic glycolysis. The formation and disposal of lactate in the body are naturally balanced, such that lactate concentrations in blood are maintained at around $\leq 2\,\text{mmol}\,\text{L}^{-1}$.[1] The human body produces lactate at an approximate rate of $0.8\,\text{mmol}\,\text{kg}^{-1}$ body weight hour^{-1}, and of this, around $0.6\,\text{mmol}\ \text{L}^{-1}$ is typically found in arterial blood and $0.9\,\text{mmol}\ \text{L}^{-1}$ in venous blood. However, under conditions of hypoperfusion and hypoxia, the entry of pyruvate into the Krebs cycle of aerobic respiration is reduced, which in turn, leads to increased levels of lactate in blood.[2]

Elevated blood lactate levels, termed hyperlactatemia, is considered an important biomarker of morbidity and mortality in critically-ill patients.[3–7] For this reason, routine measurement of blood lactate concentration is important in various patient groups,[8] including patients with severe injury,[9] post-operative patients,[10] and those with severe sepsis.[11,12] The Surviving Sepsis Campaign (SSC), in their 'SSC bundle: 2018 update',[13] have mentioned that sepsis is a medical emergency and if the initial admission lactate level is $\geq 2\,\text{mmol}\ \text{L}^{-1}$, resuscitation and management should be started immediately. Lactate concentrations should be re-measured within two hours and again until they are stabilized. Blood lactate concentration can be regarded as an indirect measure of tissue hypoperfusion. However, studies have also shown that hyperlactatemia, together with the degree of acidosis, is a better mortality predictor in critical care patients with sepsis.[14–16] The metabolic acidosis (pH ≤ 7.35) which may be associated with sepsis (pathogenesis) and hyperlactatemia is due to the influence of lactate production and ionization

leading to a strong ionic compositional difference.[17] Another important aspect to consider is that the time between the inception of inadequate tissue perfusion and death is very short, potentially less than 1 hour.[18] The most recent study on this subject was carried out in 2017,[19] and reported a worldwide incidence of sepsis at around 48.9 million people between 1990 to 2017, and the number of global sepsis-related deaths at 11 million people (19·7% of global deaths). This data has seen a recent spike in numbers following the global pandemic of coronavirus disease 2019 (COVID-19). According to a study from Wuhan, China, published in March 2020,[20] sepsis was the ubiquitous symptom in all 191 patients considered in the study.

A prompt and adequate hemodynamic resuscitation protocol that incorporates continuous lactate monitoring is thus, necessary. In present clinical care settings, lactate is commonly measured by an add on capability to arterial blood gas (ABG) analysers that requires sampling of arterial blood, or *via* venipuncture (sampling of venous blood). Also, measurements performed using photometric or amperometric techniques, which are the most commonly used methods in critical care, may be subjected to chemical interference and oxygen limitation.

Transmittance/reflectance spectroscopy has been previously investigated as a means of identifying clear and unique absorption peaks for lactate across the NIR spectrum, both in-vitro and in-vivo. Many of such studies were conducted by Lafrance et al.,[21–23] where the optical regions of 1540–1740 nm and 2050–2400 nm were utilised for lactate measurements in human plasma and whole blood. These studies were performed on a group of exercising individuals using reflectance spectrometers, while blood sampling was undertaken simultaneously. The motivation for this study was to establish the feasibility and prediction capability of non-invasive determination of lactate concentration in varying localized muscle lactate. The results of these studies provides the necessary confidence that NIR transmission/reflectance spectroscopy could be used to estimate lactate concentrations in-vitro and in-vivo, though further investigation is required to understand the effects of additional parameters in the spectra. As this study only considers lactate levels within a healthy range, further investigations are also necessary into the interaction of lactate with the NIR spectrum at levels seen in critical care patients.

Previous studies have also reported spectrometric investigations of lactate changes and varying pH separately,[24–26] as well as the feasibility of NIR as a potential tool for detection of these parameters. Nevertheless, the effects of simultaneous variations in these parameters are not yet fully understood, and hence for the first time, this study provides an analysis of the combined effects of hyperlactatemia and lactic acidosis in an in-vitro set-up using NIR spectroscopy in buffer solutions.

Reagents and materials

In order to see the effects of lactic acidosis on spectroscopic measurement in an *in-vitro* set up, three pH buffer solutions (6.5, 7.0 and 7.4) were prepared. A lactate stock solution of 600 mmol L^{-1} was prepared separately in de-ionized water and isotonic phosphate buffer solution (PBS). Both Na-L-Lactate and salts for the PBS were in dry form (analytical grade), and obtained from Thermo Fisher Scientific (Waltham, MA, USA). Isotonic PBS was used as a buffer to maintain a steady initial pH of 7.4 ± 0.02 at 24° C, replicating physiological values. The pH of the buffer was changed by adding drops of HCl into the solution. Thereby, thirty seven samples of varying lactate concentrations for each set of pH buffer were prepared by mixing aliquots of buffer and lactate stock solution. The pH and temperature of all the solutions were measured by an Orion Star A211 Advanced pH Benchtop Meter Kit, from Thermo Fisher Scientific, (Waltham, MA, USA), immediately prior to measurement.

The concentrations of the thirty seven samples of varying lactate concentration for each set of pH were: 0–5 mmol L^{-1} with an iteration of 0.25 mmol L^{-1} in between each sample and then from 5-20 mmol L^{-1} with an iteration of 1.0 mmol L^{-1}. The first set of concentrations (0–5 mmol L^{-1}) emulates lactate concentration levels in critical care. However, the latter set (5–20 mmol L^{-1}) reflects the localized lactate level concentrations in muscle during strenuous physical activity. An LM5 Lactate Analyzer from Analox Instrument Limited, (Stourbridge, UK) was used to test the concentration of each sample before collecting the spectra. The reported range from the manufacturer for a linear dependency for lactate is 0–10 mmol L^{-1}.

NIR spectroscopy

A Lambda 1050 dual beam spectrometer from Perkin Elmer (Waltham, MA, USA), was used to collect three spectra from each sample in the region 800-2600 nm (NIR) with step increments of 1 nm. Thus, a total of 333 spectra were collected. These spectra were then averaged and a total of 111 spectra were used in subsequent analysis. The samples were chosen at random from each set to prevent bias. The light source used was a halogen-tungsten lamp. The detector settings are as shown in Table 1. These were selected to prevent oversaturation of all detectors due to high –OH absorbance in this region. In addition, the reference beam

Table 1. Detector settings for NIR spectroscopy.

Detectors	Wavelength range (nm)	Slit Size	Gain	Response time (s)
InGaAs	800–1800	Servo Mode	5	0.2
PbS	1800–2600	Servo Mode	1	0.2

was set at 1% attenuation for the purpose of noise reduction at high absorbance values.

Baseline correction of 100% Transmittance/0% Absorption was also added in the spectrophotometer to reduce "background noise" or peaks arising from the ambient environment. The samples were placed in the sample holder of the spectrophotometer quartz cuvettes (Hellma GmbH & Co. KG, Müllheim, Germany) of 1 mm path length.

Spectral analysis

The spectral viewing and analysis tasks were executed on MATLAB R2020b, MathWorks™ (Natwick, MA, USA).

Pre-processing of all the three sets of spectra was performed separately to understand the effects of pH on concentration changes of lactate. The first step was to perform a spectral difference of the blank (0 mmol L^{-1} concentration of lactate) from all other spectra. This step was followed by application of Multiplicative Scatter Correction (MSC) on the resulting spectra in the three sets independently to minimize any multiplicative effects and to enhance any spectral features. To further intensify and emphasize the spectral absorption features and to reduce noise, a Savitzky-Golay derivation (SG) was performed on the three sets, separately. The values of the polynomial order, derivative and window length of the SG filter were maintained at 2, 1 and 71 respectively. These values were decided as a trade-off between noise suppression and feature enhancement in the plots. The window size was decided after trial and testing of different values (7, 31, 51 and 71).

A 2 D correlation analysis was then performed on the pre-processed spectra which provided an understanding of the systematic variations in spectral intensities (both absorbance and wavelength) for different pH sets. These variations could be seen as a result of the induced changes in the spectra caused by the changes in the concentration of lactate at each pH. A partial least square (PLS) regression analysis with leave-one-out cross-validation was carried out independently for each data-set.

The predictions for each cycle was performed by extracting orthogonal factors (also known as latent variables (LVs)) from the training set for the best fit of the model. The optimal number of LVs were determined by Predicted Residual Sum of Squares (PRESS) for each set. Since this is a clear example of a random effect model, the smallest values of PRESS were used for each set. The accuracy of the predictive models were measured using regression analysis for the predicted concentration of lactate vs actual (or known) concentration of lactate. The parameters, coefficient of determination, (R^2), derived from the sum of squares, together with the root mean squared error of cross validation (RMSECV) for each set were used as indicators for the regression models.

This process was also repeated and the data-sets were chosen iteratively in a random manner, where, two sets were used as calibration sets, in order to predict the remaining set for testing the predictive performance as the hold-out set.

Results and discussion

The three sets of raw and pre-processed absorption spectra appeared similar to typical aqueous NIR spectra and could be seen in Figure 1, where the first data-set of pH 7.4 is depicted.

Since all samples contained a high degree of water, absorption bands/peaks related to O-H bonds were clearly evident in all acquired sets of measurement. Three substantial water overtone bands could be seen at around 1450 nm ($a\nu_s + b\nu_{as}$, $a + b = 2$), 1950 nm ($a\nu_s + \delta_s + b\nu_{as}$, $a + b = 1$), and 2500 nm

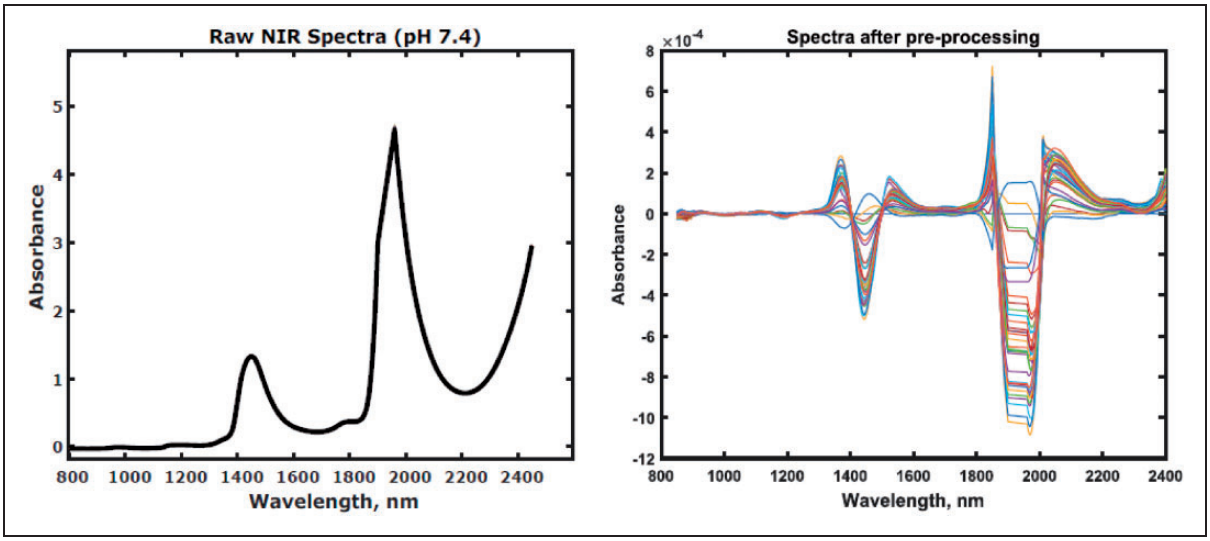

Figure 1. Raw spectra (left) and pre-processed NIR spectra of 37 lactate concentrations in PBS at pH 7.4.

(ν_{as} where, a & b are integers $\geq$0),[27,28] where, ν_s is symmetrical stretching, ν_{as} is asymmetrical stretching and δ_s is the symmetrical in-plane bending. Also, minor –OH Absorbance peaks could be seen at wavelengths 1490 nm, 1540 nm, 1820 nm, 2070 nm and 2100 nm.[29]

As shown in the previous study,[30] all bands of -OH absorption experience linear hyperchromic shifts (p-values ≤ 0.05) when pH is varied and the concentration of lactate is kept constant. However, as seen in Table 2, with the exception of wavelengths at 1820 nm, 1490 nm and 1540 nm, p-values were not significant across all three sets. Hence, these wavelengths/bands could potentially act as windows for NIR data-sets with changes in pH (lactic acidosis) only without changes in lactate.

For the purpose of identifying wavelengths of interest which relate to variations in lactate concentration, 2 D synchronous contour plots were constructed for all three sets. Minute highlights could be seen in the regions between 1100–1350 nm, 1700–1760 nm and 2200–2400 nm. These highlighted changes were in between –OH absorption bands/peaks for water. Linear Regression was performed on the absorption values corresponding to the wavelengths for interest, in order to understand the statistical significance of the lactate concentration changes at each pH data-set.[31]

In the first spectral range, 1100–1350 nm, two wavelengths, 1142 nm and 1233 nm were investigated. At both the wavelengths, 1142 nm and 1233 nm, linear correlations were observed for pH data-sets 7.4, 7.0 and 6.5.

The second spectral range 1700–1760 nm could be identified in the spectra as a result of C–H stretch (ν) 1st overtone. Again, linear regression was performed on two wavelengths, 1710 nm and 1750 nm. Except for pH 7.4, they showed no significance in any of the other sets.

Finally, in the last spectral region, 2200–2400 nm, three wavelengths 2205 nm, 2319 nm and 2341 nm were taken into consideration. The p-values of these wavelengths suggested that they were linear for pH data-sets 7.4 and 7.0.

Yano et al.[32] performed similar experiments with lactate concentration changes (100-300 g L^{-1}) in peritoneal dialysis solutions (usually with an acidic pH of 5.2–5.5). They assigned the wavelengths, 1268 nm and 1688 nm to the lactate molecule. These wavelengths, as expected, did not correlate linearly in any of the data-sets, as the pH of the medium was acidic compared to those which were considered here. Lafrance et al.[21–23] performed a series of experiments in different media, and considered different wavelength ranges in each study. The studies were mainly focused on higher concentrations of lactate and pH, varying from 7.44–7.66 in whole blood and in 7.2 buffered solution which was then mixed in serum to increase the number of samples. Hence, the attributed wavelengths were not expected to be linear across all four data-sets. While wavelengths 1675 nm, 1690 nm, 2166 nm and 2254 nm were linearly correlated ($p \leq 0.05$) for data-sets of pH 7.0 and 6.5, the wavelengths 1730 nm and 2292 nm were linear for the data-set of pH 7.4, as well. Finally, in 2017, Kossowski et al.,[33] attributed wavelengths 823 nm, 923 nm and 1047 nm in the NIR region to lactic acid from their in-vivo experiment with six LEDs (light source) and a silicon pin diode (detector). The wavelengths 823 nm and 923 nm were linear for the pH data-sets 7.4 and 7.0, while, the wavelength 1047 nm was linear for the pH data-sets 7.0 and 6.5, respectively.

Hence, it may be assumed by the results displayed in Table 3, that wavelengths pertinent to lactate are largely dependent on the pH of the solution sample. Previous studies have shown that the wavelengths, 1233 nm, 1710 nm, 1750 nm, 2205 nm, 2319 nm and 2341 nm vary in absorbance with respect to changes in pH. These changes could be attributed to

Table 2. p-values of the linear regression done on the -OH Absorption wavelengths (nm) on data sets of pH 7.4, pH 6.5 and pH 7.0 with varying concentrations of lactate. Significant p-values ($\leq$ 0.05) are highlighted.

NIR	Wavelength (nm)						
Data Sets	970	1450	1490	1540	1820	2070	2100
pH 7.4	0.67	0.15	**0.040**	0.36	**0.034**	0.92	0.96
pH 7.0	0.88	0.0514	0.155	**0.038**	**0.035**	0.09	0.11
pH 6.5	0.66	0.79	**1.86E-03**	0.25	**4.48E-03**	0.42	0.66

Table 3. p-values of the linear regression done on wavelengths (nm) for data sets of pH 7.4, pH 6.5 and pH 7.0 with varying concentrations of lactate. Significant p-values $\leq$ 0.05 are highlighted.

NIR	Wavelength (nm)						
Data Sets	1142	1233	1710	1750	2205	2319	2341
pH 7.4	**1.45E-05**	**1.39E-05**	**0.027**	**1.19E-05**	**5.41E-07**	**0.0016**	**0.032**
pH 7.0	**5.63E-08**	**0.008**	0.69	0.34	**6.52E-05**	**0.0081**	**0.0020**
pH 6.5	**0.0030**	**4.66E-04**	0.854	0.23	**0.044**	0.56	0.22

ionization/de-protonation of lactic acid to lactate ($CH_3CH(OH)COO^-$).[30,34] Similarly, the changes in lactate concentration within each pH set would also ionize/de-protonate minute amounts of lactic acid to lactate ion. However, these changes are infinitesimal and thus, unlikely to appear in the acquired spectra. This justifies the use of multivariate analysis based on linear regression, using the whole spectral range, to further understand the relevant spectral changes in all three sets of measurements. For this purpose, Partial Least Square regression analysis was performed on individual data-sets to understand the effects of lactate concentration variation or hyperlactatemia.

Figure 2 shows the Prediction Error Sum of Squares results for three sets of acquired data. These results were used to determine the number of Latent Variables (LVs) to build prediction models for dimentionality reduction.[35] Therefore, predictive models were built for all three data-sets using LVs 6, 5 and 8 for the three pH data-sets 7.4, 7.0 and 6.5, respectively.

Figure 3, plots actual concentration of lactate vs predicted concentrations by the three predictive PLS models that were built using leave-one-out cross-validation analysis. The coefficients of determination R^2 for each of the three sets are displayed in the figure.

As seen from Figure 3, R^2, for the three data-sets relating to pH 7.4, 7.0 and 6.5 were 0.94, 0.83 and 0.95, respectively. The root mean square error of cross validation for the linear regression models of observed vs the predicted concentrations of lactate for the same data-sets were 0.58 mmol L^{-1}, 0.46 mmol L^{-1} and 0.44 mmol L^{-1}, respectively. The high RMSECV values could be an indication that lactate concentration at higher values can be predicted accurately. However, due to a lower number of samples in the three sets for the higher concentrations of lactate, cross-validation was not performed. These results are in-line with Lafrance et al.[23]

Moreover, to investigate the generalizability of predictions into sets with different pH values, a leave-one-pH (data-set)-out test set validation was carried out, where pairs of pH sets were used for training and the remaining set for testing. This led to very poor predictive performance on all cases. For example, when pH data-sets 7.4 and 6.5 were used to build the models and predict the test set 6.5, the R^2 values were negative and the root mean squared error of prediction (RMSEP) value was 56.32 mmol L^{-1}. This means that the changes in pH values influence the optical absorbance in a

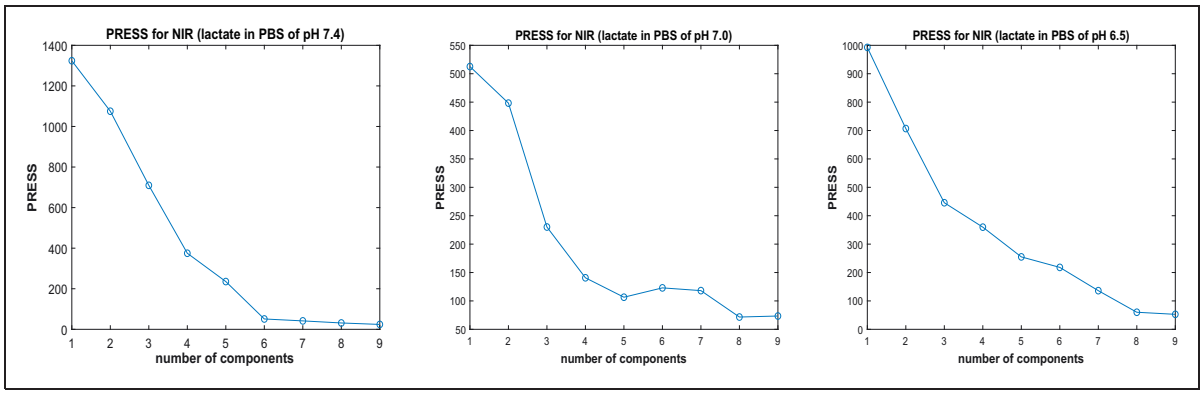

Figure 2. Prediction Error Sum of Squares (PRESS) versus number of latent variables (LV). (left) The number of LVs in the NIR spectral region for pH 7.4 used was 6; (center) The number of LVs in the NIR spectral region for pH 7.0 used was 5; (right) The number of LVs in the NIR spectral region for pH 6.5 used was 8.

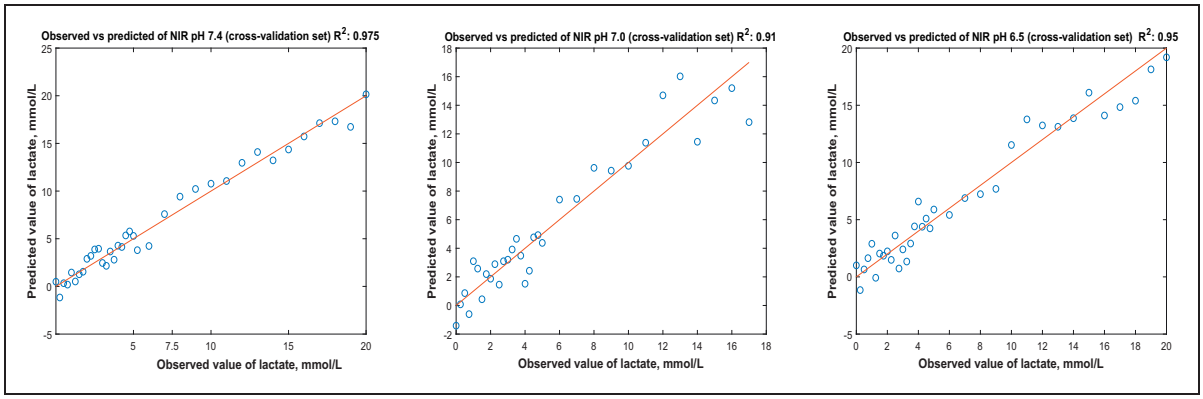

Figure 3. Predicted vs Actual plots for lactate concentration of 37 PBS samples.

non-linear manner, hence, such interrelationship cannot be modelled using PLS and leave-one-pH (dataset)-out test set validation. This is what was expected from our previous analytical investigation, where a change in pH from 6-8, while keeping the lactate concentration constant at 2 mmol L^{-1} showed non-linear characteristics in the NIR spectral region,[30] unlike the MIR spectral region.[31]

Conclusion

In conclusion, the results of this work indicate that there exists an influence of pH on the absorption spectrum of lactate, which could be seen in the spectral features. In order to identify spectral changes occurring as a result of lactate concentration changes (or hyperlactatemia), which could be a potential reflection of the ionization of lactic acid to lactate in different regions of the NIR spectra were investigated. This led to the assumption that the absorption bands of water exhibit non-linear correlations with changes in lactate concentration, and this is true for all acquired pH data-sets. In turn, this indicates that these peaks could be a marker for pH (or lactic acidosis) determination alone. Nevertheless, specific wavelengths within the NIR spectral region exhibit a linear correlation in relation to the pH data-sets. Variations in lactate concentration could be predicted with more than 90% accuracy for each pH data-set independently, but when combining multiple pH data sets into the model, predictions results were non-linear. Hence, it could be concluded that lactate concentration changes in NIR spectra could only be predicted accurately, when the pH of the sample is also known. However, similar studies should also be produced in complex biological fluids like serum and whole blood in order to verify the observations. These media might pose challenges because of the presence of scatterers, which also absorb in the NIR spectral region. The inclusion of more data-sets with different pH values and media together with nonlinear models (such as Support Vector Regression (SVR)) might address these potential challenges.

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