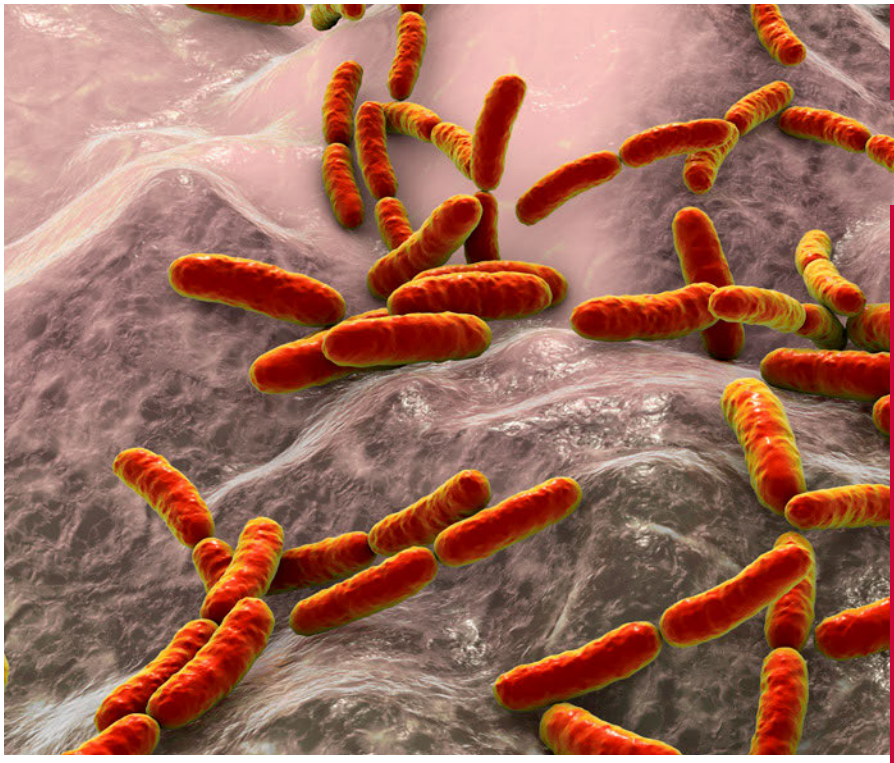

APPLICATION NOTE

UV/VIS Spectroscopy

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Quantification of Lactate in a Multicomponent System

Introduction

Lactate as a reaction product of lactic acid fermentation is not only found in muscles and blood, but has also different regulatory functions for wound development depending on the concentration present. It has been shown that the amount of lactate in wound fluid correlates with the inflammatory or infectious status of a wound.[1]

It is known from publications that the wavelength range 2000 nm to 2400 nm can be used for the identification and quantification of lactate,[2,3] because lactate has two distinct absorption bands in this range. This wavelength range is flanked by two major water absorption bands centered at 2670 nm (3750 cm^{-1}) and 1920 nm (5200 cm^{-1}). However, spectral overlaps occur with other components of wound fluid such as glucose, albumin (protein), triacetin (model substance for triglycerides), and urea, because the chemical information within the combination range (2000 nm - 2500 nm) corresponds to the combination of bending and stretching vibrations associated with CH, NH, and OH molecular groups.

In order to assess the possibilities for an *in-vivo* and noninvasive determination of the lactate content in the near-surface wound layer (wound fluid), spectroscopic investigations were carried out to identify and quantify the metabolite lactate concentration in model mixtures corresponding to the physiological range. Due to the spectral overlap of lactate with other relevant substances in the wavelength range 2000 nm to 2400 nm, a multivariate (multi-wavelength) algorithm was applied for the evaluation.

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Information on the substances contained in the wound fluid of diabetic ulcers in different healing status with their typical physiologically relevant concentration ranges is known from[4,5] and is arranged in Table 1.

Table 1. Physiological concentration range of 5 components of wound fluids.

Wound Fluid Range	Lactate	Glucose	Albumin	Triacetin	Urea
c [mmol/L]	5.4 - 16.7	0.6 - 5.9	0.20 - 0.41	0.4 - 4.6	2.5 - 22.6
c [mg/dL]	48.6 - 150.4	10.8 - 106.3	1400 - 2800	35 - 400	15.0 - 135.7

Transmittance Measurements in the NIR-Region

Measurements were performed with the substances lactate, glucose, albumin, triacetin and urea in the concentration ranges physiologically relevant for wound fluid. Phosphate-buffered saline solution (PBS) with a pH value of 7.4 was used as the solution medium for the substances. From stock solutions of the five substances dilutions of the pure substances and mixed samples in various known concentrations were prepared.

Considering the absorption coefficients and the low concentrations of the substances as well as the strong water absorption bands flanking the interesting wavelength range an excellent signal-to-noise performance and baseline stability are required. The high sensitivity Peltier cooled InGaAs NIR detector (800 nm - 2600 nm) as one part of the three-detector module of a PerkinElmer Lambda® 1050+ WB UV/Vis/NIR spectrometer allows measurements at high resolution and reproducibility in the NIR region (Figure 1).

The correction/baseline files at 0A / 100% T were taken with 1 mm-cells (OH free quartz) filled with PBS buffer in the sample and reference beam. Because of very small differences in the absorption spectra of the two cells always the same cell was used for the reference and sample solutions respectively. The wavelength range for the measurements were chosen from 1000 nm to 2480 nm, but for the calculation only the range of the strongest absorption between 2000 nm and 2320 nm was used.

The parameters of the measurement method are shown in Figure 2 and Table 2.

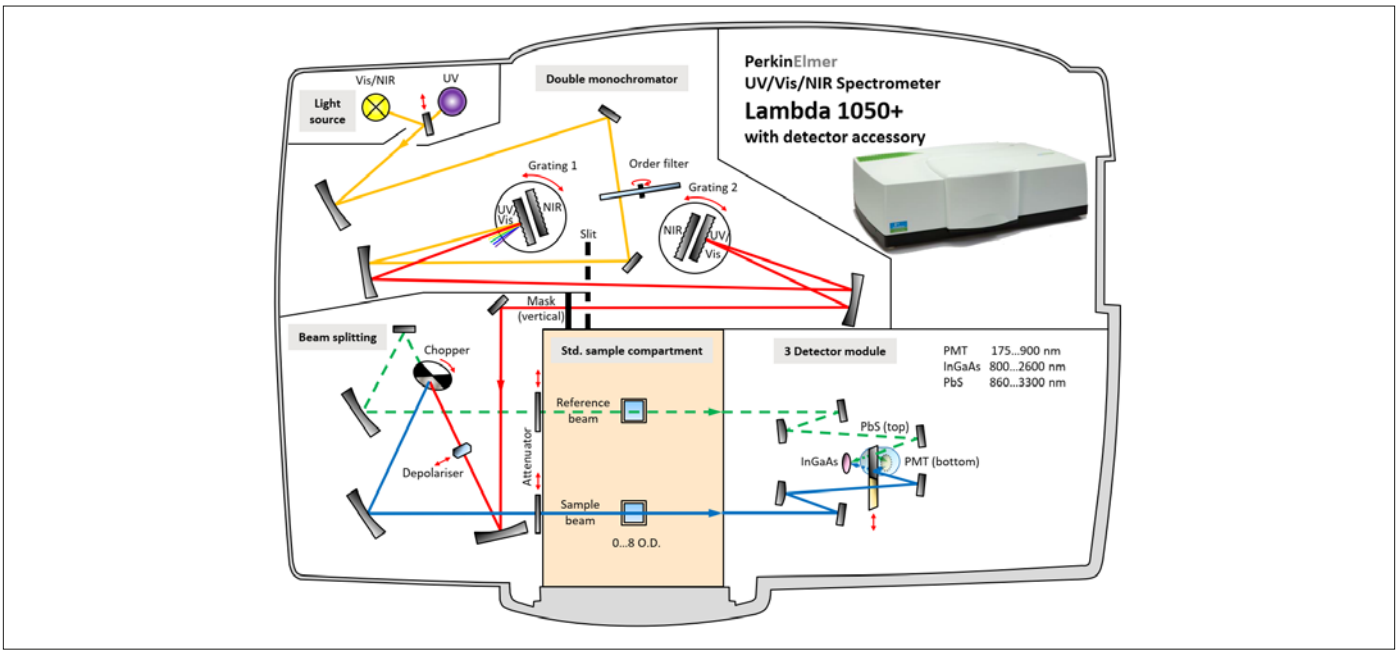

Figure 1. Optical diagram of the instrument with 3-detector module.

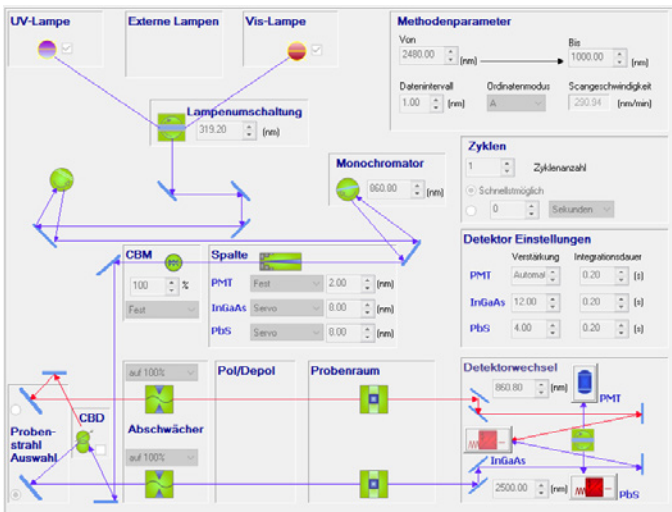

Figure 2. UVWinLab™ Software setup.

Table 2. Measurement parameters.

Wavelength Range	1000-2480 nm
Data Interval	1 nm
Slit (Spectral Band Width)	NIR (InGaAs): servo /Gain 12
Integration Time	0.2 s
Common Beam Mask	100%
Common Beam Depolarizer	Off
Detector And Monochromator Change	860.8 nm
Additional 0%T-Baseline	Yes

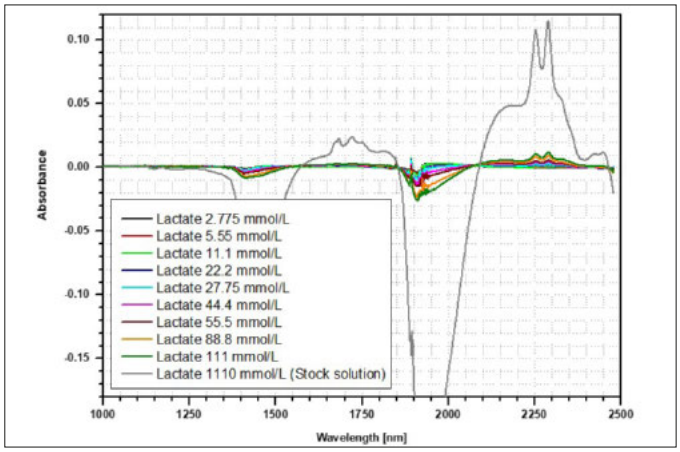

Figure 3. Absorption spectra of lactate in different concentrations.

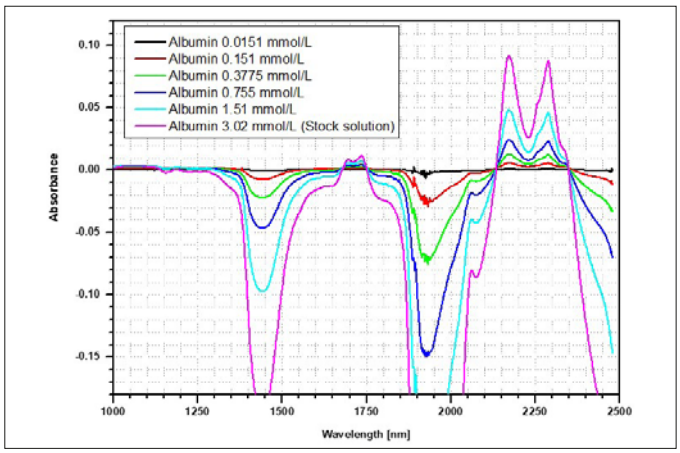

Figure 4. Absorption spectra of albumin in different concentrations.

The measurements of the pure substances within the physiological concentration range are exemplified by the absorption spectra of Lactate (Figure 3) and Albumin (Figure 4). The pure spectrum of the PBS buffer in a 1 mm-cell (Figure 5) shows the strong absorption bands from water in the NIR region (2670 nm, 1920 nm, and 1440 nm), assigned to the combination modes and overtones of the three OH fundamental vibrations. A dilution of water (buffer) with an analyte leads to negative absorptions at the water bands when referenced against pure water (buffer). There also should be considered a strong dependence of the band position and intensity on temperature, pH value and addition of electrolytes.[6] With knowledge of these dependencies, the unusual appearance of the spectra of the pure substances and the mixtures can be classified.

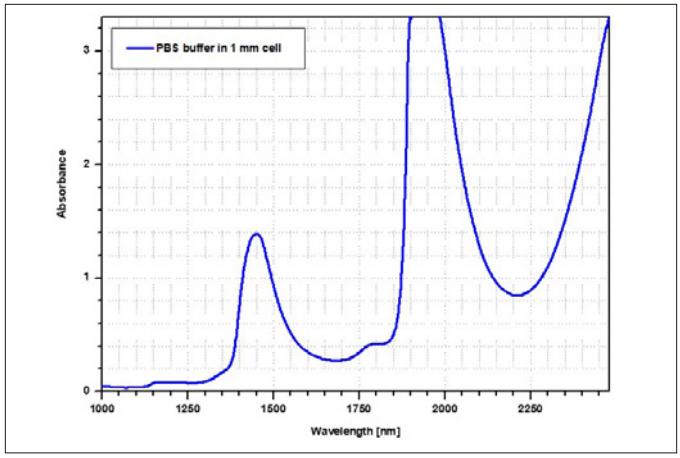

Figure 5. Absorption spectrum of PBS buffer in a 1 mm-cell (OH free quartz).

From the measurements of the pure substances, it can be concluded that the spectrum of the mixed five substances is determined primarily by Albumin and secondarily by Triacetin, Lactate and Urea. The normalized spectra of the substances taken from the stock solution concentrations (Table 3) are shown in Figure 6.

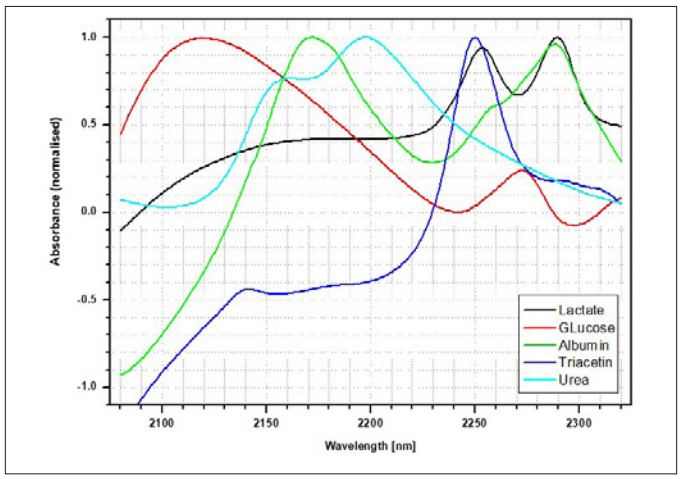

Figure 6. Normalized spectra of the pure substances in PBS buffered solution.

Table 3. Concentration of stock solutions of the pure substances.

Substance	Stock Solution Concentration	
	mg/dL	mmol/L
Lactate	10000	1110
Glucose	5000	277.5
Albumin	20000	3.02
Triacetin	5000	57
Urea	6000	1002

Multivariate (Multi-Wavelength) Algorithm

The concentration (c) of substances contained in a solution can be calculated spectroscopically from the absorbance ($A\lambda$) and the irradiated layer thickness (d) under appropriate conditions according to the Beer-Lambert law [Eq. 1]. The corresponding extinction coefficients ($\varepsilon\lambda$) can be calculated by means of a calibration and then concentrations of the substances in solutions of unknown compositions can be determined from Eq. 2.

$$A\lambda = \varepsilon\lambda \cdot c \cdot d \qquad \text{Eq. 1}$$

$$c = A\lambda \cdot \frac{1}{\varepsilon\lambda \cdot d} \qquad \text{Eq. 2}$$

For multi-component systems with strongly overlapping spectra, multivariate calibration methods are required. In the case of wound fluid as a biological matrix, where unknown substances also provide signal contributions, the quantification of single substances is possible by using the inverse least squares method.[7] From a given set of spectral data this method can mathematically formulated as a matrix compact form:

$$C = A \cdot P + E \qquad \text{Eq. 3}$$

where C represents the matrix of the reference concentrations, A is the spectral data matrix, and P is the matrix of the calibration coefficients (Eq. 4). P represents the correlation between the spectral matrix A and the concentration matrix C:

$$P = \frac{1}{\varepsilon\lambda \cdot d} \qquad \text{Eq. 4}$$

and can be computed by solving:

$$P = C \cdot A^{-1} \qquad \text{Eq. 5}$$

E is the matrix of the concentration errors and includes the measurement uncertainties as well as the spectral contributions of unknown substances. With 15 calibration mixtures from five substances A is not square and with this the pseudo inverse (calculated with the transposed form of the A-matrix, A^T) must be used instead.

$$\hat{P} = (A^T \cdot A)^{-1} \cdot A^T \cdot C \qquad \text{Eq. 6}$$

The concentration matrix of the validation or unknown mixtures than can be determined by the following equation.

$$C = \hat{P} \cdot A \qquad \text{Eq. 7}$$

Results

15 calibration samples with varying mixture ratios (Table 4), and 10 validation samples of known mixture ratios (Table 5) for verification were prepared, and the NIR absorption spectra were measured (Figure 7 and Figure 8 respectively).

Table 4. Concentrations of the calibration mixtures .

Calibration Mixtures	Lactate c [mmol/L]	Glucose c [mmol/L]	Albumin c [mmol/L]	Triacetin c [mmol/L]	Urea c [mmol/L]
KM 01	2.775	0.555	0.3775	0.285	25.05
KM 02	2.775	5.55	0.151	5.7	2.505
KM 03	5.55	2.775	0.151	5.7	10.02
KM 04	5.55	0.555	0.755	0.285	25.05
KM 05	11.1	5.55	0.755	0.57	2.505
KM 06	11.1	5.55	0.3775	0.57	10.02
KM 07	22.2	0.555	0.3775	5.7	2.505
KM 08	22.2	2.775	0.755	0.285	25.05
KM 09	27.75	0.555	0.151	0.285	25.05
KM 10	27.75	2.775	0.755	5.7	10.02
KM 11	44.4	5.55	0.151	0.57	25.05
KM 12	55.5	0.555	0.151	0.285	10.02
KM 13	55.5	5.55	0.3775	0.57	2.505
KM 14	88.8	2.775	0.755	0.57	2.505
KM 15	111	2.775	0.3775	5.7	10.02

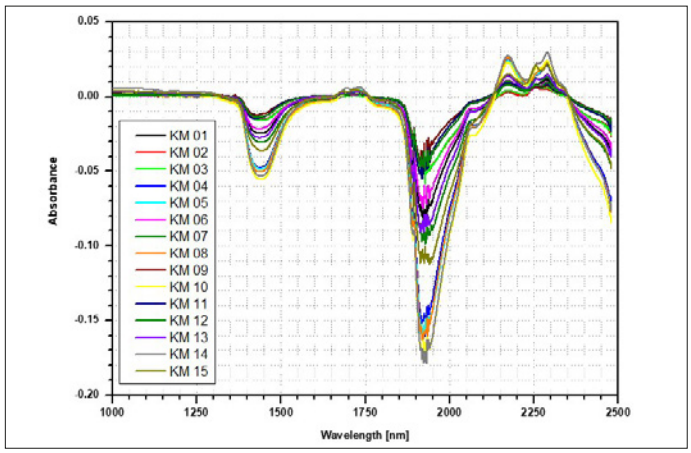

Figure 7. Spectra of the calibration mixtures .

Table 5. Concentrations of the validation mixtures.

Validation Mixtures	Lactate c [mmol/L]	Glucose c [mmol/L]	Albumin c [mmol/L]	Triacetin c [mmol/L]	Urea c [mmol/L]
VM 01	2.775	5.55	0.755	0.285	10.02
VM 02	2.775	0.555	0.151	5.7	2.505
VM 03	5.55	0.555	0.3775	0.285	25.05
VM 04	5.55	2.775	0.755	0.57	2.0505
VM 05	11.1	2.775	0.755	0.57	2.505
VM 06	11.1	0.555	0.151	0.285	25.05
VM 07	22.2	5.55	0.151	0.285	25.05
VM 08	27.75	0.555	0.3775	0.57	10.02
VM 09	55.5	5.55	0.3775	5.7	2.505
VM 10	88.8	0.555	0.151	5.7	10.02

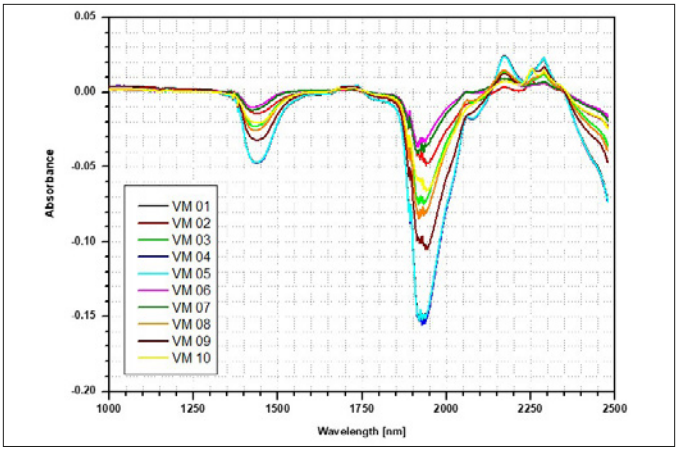

Figure 8. Spectra of the mixtures for validation.

For simplification of the computing processes the spectra were reduced to the absorbance values from five selected wavelength. The selection of the wavelengths is made due to the criteria of strong and small absorptions of each substance and regions of small collinearities. For this example, the wavelengths 2120 nm, 2170 nm, 2200 nm, 2250 nm, and 2290 nm were selected. With the data from the calibration mixtures the $\hat{P}$-matrix (Table 6) was calculated. This $\hat{P}$-matrix was used to calculate the concentrations of lactate from the measured validation spectra and as well from the calibration spectra. These calculations were carried out using Microsoft Excel.

Table 6. $\hat{P}$-matrix calculated from the calibration spectra.

Wavelength [nm]	$\hat{P}$ Regression Coefficients				
	Lactate	Glucose	Albumin	Triacetin	Urea
2120	8067.481	2925.851	-14.526	-235.827	-803.981
2170	-9084.629	7546.306	57.317	789.875	-889.225
2200	4311.404	-6490.523	-39.885	-885.640	8764.909
2250	1468.295	3398.648	1.160	1197.868	3834.496
2290	8836.374	-4626.654	-5.629	-955.062	-6757.681

A comparison of the predicted and the real lactate concentrations in calibration and validation mixtures are shown in Figure 9. With ideal data the predicted and real data should be of the same value and the linear regression equation is expected in the form y = Ax + B with A = 1 and B = 0. For the lactate concentrations the regression coefficient is near one but there is a gap between the calibration and the validation data observable. With higher absorbance values there is a much better match of the calibration and validation data, as shown for albumin in Figure 10. For the measurement of lactate concentration, it can be inferred that with this measurement conditions the physiologically relevant wound fluid concentrations are close to the detection limit. Maybe a temperature control can improve the achievement of better results.

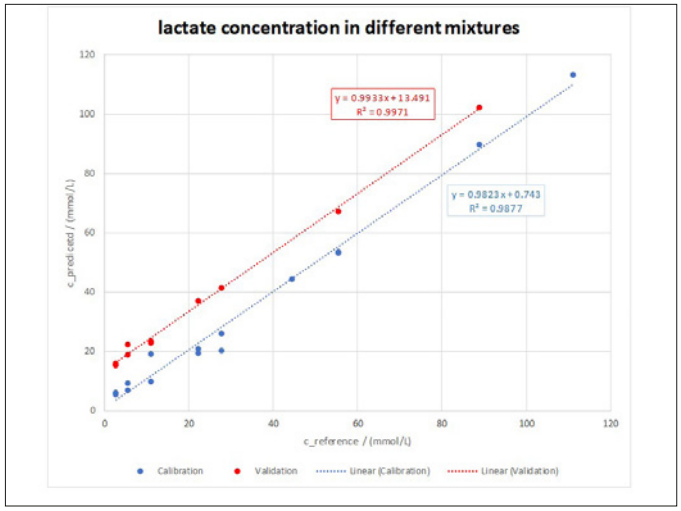

Figure 9. Comparison of predicted and real lactate concentrations in calibration and validation mixtures.

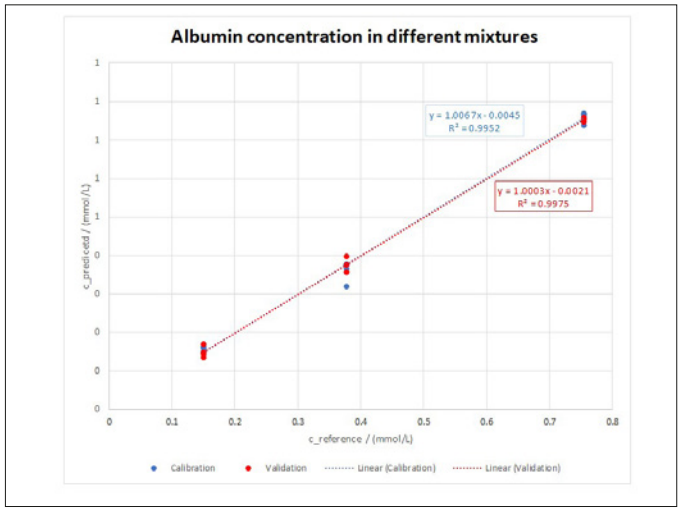

Figure 10. Comparison of predicted and real albumin concentrations in calibration and validation mixtures.

Conclusion

With high-end spectroscopic devices and appropriate chemometric methods it is possible, to characterize the concentrations of lactate in complex systems in physiologically relevant amounts. The described procedure has a good potential to be applied in future noninvasive NIR lactate monitoring devices.

The PerkinElmer LAMBDA 1050+ spectrometer in combination with the three-detector wide band InGaAs module provides an excellent platform for the spectral characterization of biological relevant substances and their multivariate interpretation. With high stability, accuracy, and signal-to-noise ratio in the near infrared region there are good opportunities to characterize complex matrices even in the presence of a large proportion of water.

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