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Demonstration of an external-cavity quantum cascade laser based blood sensor

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Introduction

A novel approach for a **reagent-free and robust sensor setup** for blood samples based on a pulsed **External Cavity – Quantum Cascade Laser (EC-QCL)** is presented. EC-QCLs offer high intensity mid-infrared radiation (up to several hundreds of mW) combined with broad tuning ranges (up to 600 cm^{-1}). Their tunability renders the simultaneous measurement of several analytes possible by applying chemometric modelling algorithms like Partial Least Squares (PLS) regression analysis. Their high emission power on the other hand, permits the use of **large optical pathlengths (> 100 µm)** improving the robustness of the measurement.

The presented work aimed on the development of a blood sensor which is capable of reagent-free and simultaneous determination of multiple blood parameters, such as glucose, lactate, phosphate, urea and albumin, to name a few. In a previous work [1] we already reported on the determination of glucose and lactate in Ringer solution in the presence of interferents (xylose and maltose).

Here we report on recent results of the measurement of **glucose and lactate in human blood serum** as well as on a proof of concept for the determination of addititional blood parameters, including proteins. Furthermore, **experimental results on the optimal optical pathlength** for glucose measurements in aqueous phase are presented, showing that the theoretically calculated values for the optimal pathlength are not valid in experimental practice.

Sensor setup for measuring biofluids

For the sensor setup a broadly tunable pulsed **External Cavity QCL (EC-QCL)** was implemented. The spectral linewidth was smaller than 1 cm^{-1} and the emission wavenumber could be tuned between 1030 cm^{-1} and 1230 cm^{-1}. The samples are introduced into the beam path by a standard flow cell with calcium-fluoride windows (**135 µm optical pathlength**). The maximum emission power achieved within the tuning range was 350 mW.

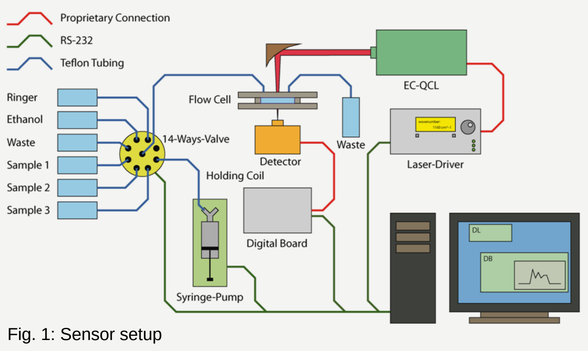
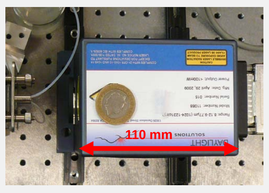


Fig. 1: Sensor setup

The complete setup, including the flow injection system (syringe pump, 14 port injection valve), the signal processing system and the EC-QCL was controlled by the server-client based **ATLAS system** (Advanced Total Lab Automation System) [2].

Dimensions of the External Cavity – Quantum Cascade Laser:

Present size:



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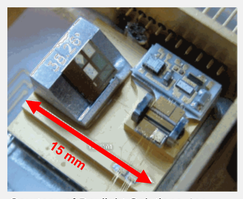

Courtesy of Daylight Solutions, Inc.

The small size of the EC-QCL allows the development of portable mid-infrared sensors such as blood sensors for point-of-care application.

The high spectral emission power of the employed EC-QCL **(> factor 10^4 compared to a FTIR spectrometer**) permits long optical pathlength greater than 130 µm. As a consequence a robust sensor setup operating in transmission mode can be realized which is less prone to clogging. This is of increased importance when doing measurements in complex matrices such as blood or blood serum.

[1] Brandstetter M., Genner A., Anic K., Lendl B., *Tunable external cavity quantum cascade laser for the simultaneous determination of glucose and lactate in aqueous phase*, Analyst, 2010

[2] Wagner C., Genner A., Ramer G., Lendl B., *Labview - Modelling, Programming and Simulations*, Book chapter, published 2011

Proof of concept - multiple blood parameter determination in Ringer solution

The tunabiltity of the employed EC-QCL over 200 cm^{-1} offers the possibility of muliple analyte detection in the liquid phase. Concentrating on the physiologically relevant parameters in blood one can find several important analytes having characteristic absorption bands in the covered spectral region between 1030 cm^{-1} and 1230 cm^{-1}.

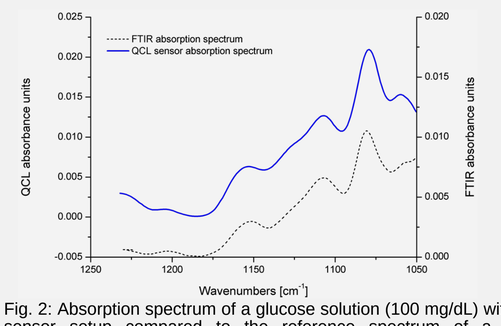

Fig. 2: Absorption spectrum of a glucose solution (100 mg/dL) with the sensor setup compared to the reference spectrum of a FTIR spectrometer

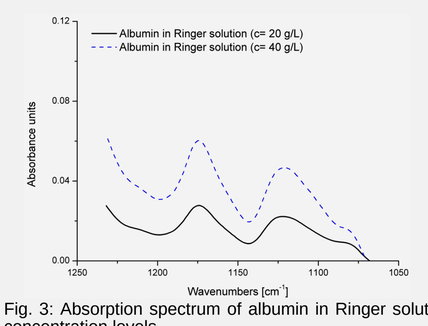

Fig. 3: Absorption spectrum of albumin in Ringer solution at two concentration levels

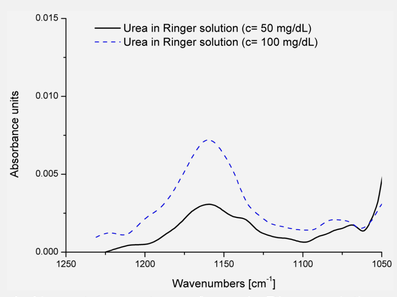

Fig. 4: Absorpton spectrum of urea in Ringer solution

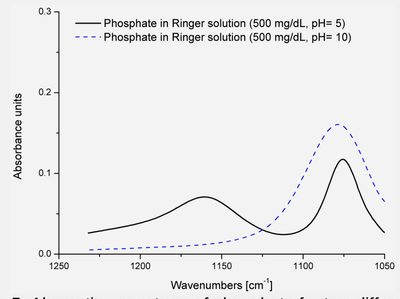

Fig. 5: Absorption spectrum of phosphate for two different pH-levels

Fig. 2 to Fig. 5 show a selection of these analytes which have been measured in Ringer solution at physiologically relevant concentration levels to prove feasibility.

Optimal optical pathlength for glucose measurements

For every chemical solution exists a certain **theoretical optical pathlength which is optimal** in terms of relative absorption caused by the analyte of interest. The calculated value depends on the absorption coefficients of analyte (e.g. glucose) and solution (e.g. Ringer solution).

For aqueous solutions of glucose the optimal pathlength was calculated to be around 16.5 µm [3]. However, in experimental practise the optimal signal-to-noise ratio is achieved at higher optical pathlengths as we could show with a **Variable Pathlength Liquid Cell** (VPLC, Fig. 6) and a FTIR spectrometer.

Fig. 6: Image of the Variable Pathlength Liquid Cell (VPLC)

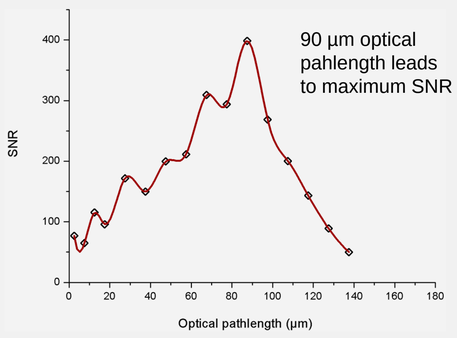

Fig. 7: The optimal signal-to-noise ratio is achieved for 90 µm optical pathlength

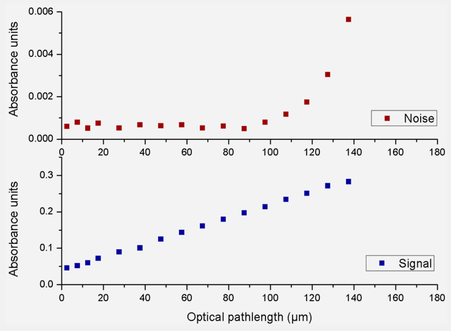

Fig. 8: Signal (bottom) and noise (top) values measured with a VPLC and a FTIR spectrometer

Sample spectra were taken from a **100 mg/dl glucose solution** (dissolved in Ringer solution) and pure Ringer solution served as background. A number of 16 measurements of background and sample spectra were recorded for various optical pathlengths **between 0 µm and 140 µm** adjusted on the VPLC.

Results show that the actual **optimal optical pathlength is located around 90 µm** and therefore much higher than suggested by [3].

[3] Herrmann C., Vrancic C., Formichova A., Gretz N., Hoecker S., Pucci A., Petrich W., *In-vitro characteristics of a mid-infrared, continuous glucose sensor,* SPIE-Proceedings, 2010

Simultaneous determination of glucose and lactate in blood serum

In a previous work the ability of the EC-QCL sensor setup for simultaneous determination of glucose and lactate in Ringer solution in the presence of xylose and maltose as interferents was shown [1]. Now we present first results on spiked human blood serum.

Fig. 10, left shows absorption spectra of blood serum solely spiked with glucose to give an idea of the prevailing absorption values and the signal quality of the EC-QCL blood sensor.

Fig. 9: Blood serum sampls. The sample volume required for a single measurement was 200 µL

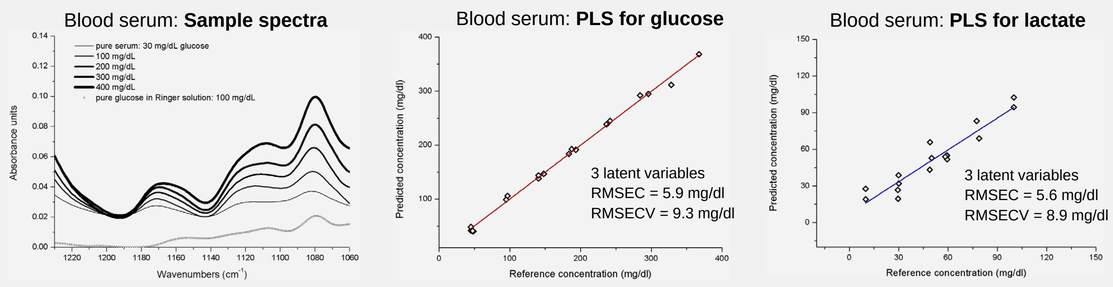

Fig. 10: Absorbance spectra of blood serum solely spiked with glucose (left) and results of the PLS regression analysis for glucose (middle) and lactate (right) on blood serum samples spiked with both glucose and lactate of differenct concentrations

The data set was analyzed by a Partial Least Squares (PLS) regression analysis (PLS toolbox for Matlab, Eigenvector Research Inc.) leading to a chemometric model for the prediction of glucose and lactate in the spiked serum samples (Fig. 10, middle and right plot). Cross-validation was performed by using the leave-one-out algorithm.

Current work concentrates on the application of the QCL-sensor on larger sample sets of blood serum and the inclusion of additional parameters, such as albumin and phosphate.