Remote Accessibility to Diabetes Management and Therapy in Operational Healthcare Networks

REACTION (FP7 248590)

ID3-8 Test and Calibration of Glucose Sensors

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1 Executive summary

Two different CGM sensor types are followed within REACTION, a fluorescence life-time spectroscopy based sensor applied on an insulin infusion catheter and an IR difference spectroscopy sensor, so far operated with microdialysis.

The fluorescence life-time based sensors are applied onto insulin infusion catheters, thus creating a single-port system that allows continuous glucose measurements and insulin infusion in subcutaneous tissue. The dose response characteristic of the sensors is linear in the range of 45 to 360 mg/dl. As the intercept of the characteristic is different from zero, a two point calibration was necessary. The reason is that the first prototype fluorometer was equipped with only one LED and one photodetector, therefore the phase angle of the glucose sensor could not be trimmed to zero at a glucose concentration of 0 mg/dl. In the currently finalized new prototype fluorometer this drawback was eliminated and a one-point calibration of the sensor will be possible. The sensor drift of the fluorescence life-time based sensors was tested in in-vitro experiments over 50h and was found to be -0.38%/h at low glucose concentrations of 45 mg/dl and 0.13%/h at high glucose concentrations of 360 mg/dl. In vivo performance of the sensors was evaluated in domestic pigs. The accuracy of the sensors was assessed by calculation of mean and median ARE (absolute relative error), and the PRESS (predictive error sum of squares) value. The average median ARE value for the sensors tested (n=18) was approx. 20% although the sensors were read out with the first prototype fluorometer.

The IR CGM sensor combined with microdialysis is based on difference spectroscopy method, comparing the transmission through a cell containing the glucose sample and a cell containing just the carrier liquid (perfusate). Since this measuring technique is not an absolute one, at least a one-point calibration is required, associating a glucose value to a difference voltage, when the range of voltage change as a function of glucose concentration is known. In case that a drift is superimposed to the signal level, a two-point calibration is required, taking into account the relative change of sensor measured and reference glucose level. In-vivo tests on blood plasma samples revealed that the sensor signal follows the reference glucose level nicely within the accuracy of the reference measuring device (Super GL Compact) which is better than about +/-10%. In-vivo measurements will be performed within a clinical study at the Medical University Graz in January/February 2013 and reveal accuracy under real patient conditions.

2 Purpose, context and scope of this deliverable

This deliverable describes briefly the two basic concepts of continuous glucose sensors that are followed during the REACTION project, their operating principles and the way of calibrating the sensors for practical application. In the second part in-vitro and in-vivo results for the sensors are described, although no in-vivo data so for the IMM IR CGM sensor is available. This data will be collected during a study at the medical university in Graz, taking place from mid of January 2013 till mid of February 2013 and presented in the report deliverable D3-9 Sensors for Glucose Measurement for first Field Trials and second generation ePatch (due March 2013).

3 Glucose sensor concepts

3.1 Fluorescence life time based sensor

Joanneum Research (JR) developed a prototype single-port system that can perform simultaneously in-vivo glucose monitoring and insulin infusion in subcutaneous adipose tissue. Our system, which we term the I-Cath system, is based on a glucose sensor applied as a thin coating onto a commercial insulin infusion catheter (see Figure 1).

The glucose oxidase reaction presents a good opportunity for optical sensing because the reaction consumes oxygen, which can easily be quantified by luminescence measurements. There are many fluorophores that are quenched selectively by oxygen, thereby allowing oxygen levels to be determined by measuring phosphorescence intensity, phase shift or decay time.

The I-Cath concept uses a commercial insulin infusion catheter as a substrate for the glucose sensor. There are two active layers deposited onto the catheter to provide glucose-dependent sensor signals. The first is a layer of glucose oxidase (GOx) as glucose-specific receptor, which is then covered by the luminescent dye platinum(II)-*meso*-tetra(4-fluorophenyl)-tetrabenzoporphyrin (PtTPTBPF), which exhibits absorption and emission maxima at 635 and 860 nm, respectively. As radiation with wavelengths in the near infrared (NIR) region can easily penetrate tissue, the sensor can be interrogated by an optical reader from outside the body. For biocompatibility reasons, and to limit glucose diffusion to the enzyme layer, the dye is incorporated into a hydrogel matrix. Due to their excellent ability to take up water, polyethylene glycol hydrogels and similar gels have been reported to improve the biocompatibility of implanted sensors. As the high water content makes hydrogels very permeable for glucose, the hydrogel is blended with ethyl cellulose to achieve the desired range of permeability of the layer. Poly(*tert*-butyl-styrene) particles stained with the luminescent dye are suspended in the hydrogel matrix prior to its application onto the insulin infusion catheter.

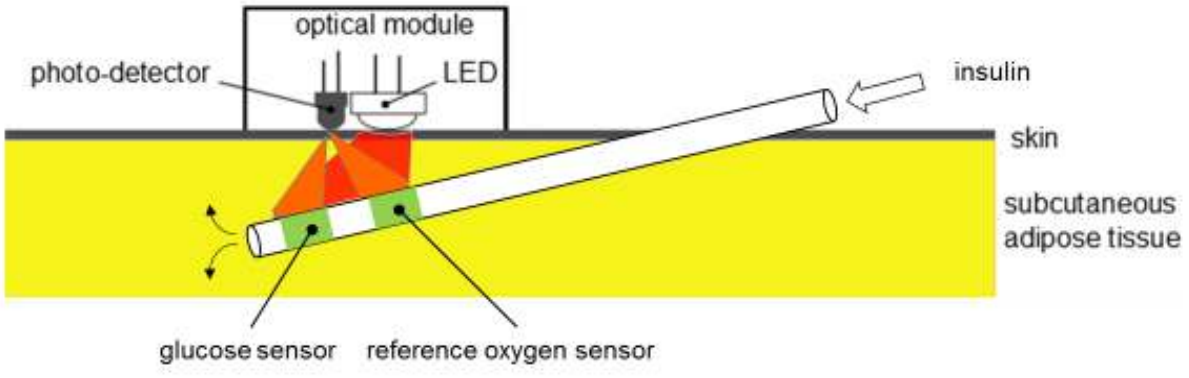

Figure 1: Scheme of the i-Cath concept: glucose and oxygen optical sensors applied onto an insulin infusion catheter and read out transcutaneously by an optical module placed onto the skin.

In order to measure tissue oxygen levels, a second dye is used; platinum(II)-6-aza-13,20,27-triphenyltetra(*tert*-butylbenzo)porphyrin (tbutPtNTBP), which has absorption and emission maxima at 617 and 768 nm respectively. The similar absorption properties of the luminescent dyes allow their simultaneous excitation, while the good separation between their emission maxima facilitates detection of the discrete signals.

The measuring system uses a miniaturized fluorometer with a mobile measuring head that houses a light-emitting diode (LED) for transcutaneous excitation of the luminescent dye and also a photo-detector for detection of the emitted light. The excitation of the luminescent dye is carried out at two different frequencies, which allows discrimination of the background signal (e.g. skin autoluminescence) and the glucose-related signal emitted by the sensor. The use of two different modulation frequencies also allows for correction of fast temporal artefacts that may arise due to the movement of the sensor within the tissue. The measuring system displays relevant data like signal intensity and shift of the phase angle. As the enzyme consumes oxygen, and the luminescence is quenched by oxygen, glucose concentrations are reflected in the phase shift of the modulated luminescent signal.

As tissue oxygen levels can vary over time, the device is designed to include a second (reference) sensor to detect local oxygen levels. In order to accurately compensate for the influence of oxygen fluctuations on the glucose sensor, the reference oxygen sensor should be located near the glucose sensor because oxygen concentrations in subcutaneous tissue are strongly dependent on local vascularization and therefore also vary spatially. In our case, however, the prototype fluorometer had only one LED and one photo-detector integrated in the measuring system, which meant that the glucose and oxygen sensors had to be on separate catheters as only one parameter could be read out with the fluorometer. Thus, oxygen levels were recorded to gain information about the magnitude of possible oxygen fluctuations but the information could not be used to compensate the influence of oxygen variations on the glucose sensor signals.

3.2 IR spectroscopy based sensor

The basic concept of the disposable chip based infrared optical absorption spectroscopy sensor combined with microdialysis is shown Figure 2.

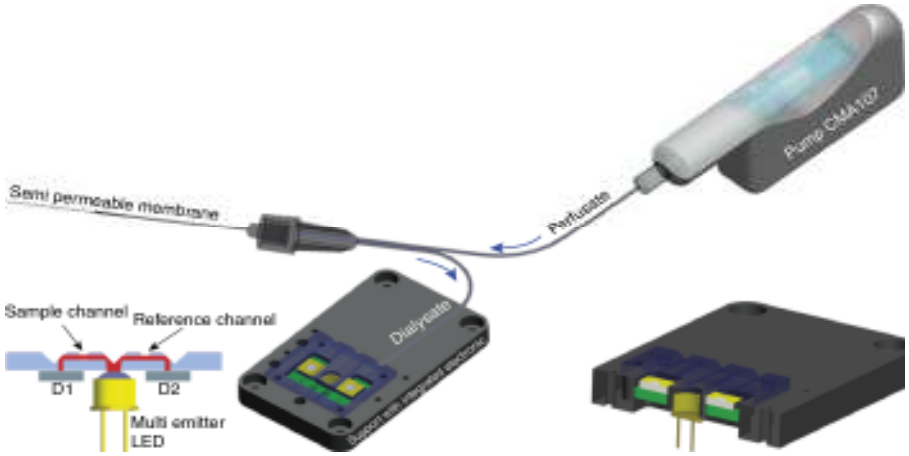

Figure 2: Schematic of the chip based infrared optical absorption spectroscopy sensor combined with microdialysis (displayed in red are the IR light beams passing through the chip).

The system is divided into two major parts, first a medically approved microdialysis catheter, combined with a medically approved perfusion pump and second a disposable polymer chip with microfluidic channels and optically functionalized surfaces which is connected to an electronics driving the light sources (LEDs) and detectors (InGaAs-photodetectors) for optical transmission spectroscopy, performed within the polymer chip.

The microdialysis catheter represents the body interface, being applied either subcutaneously or intravenously. It consists of a biocompatible polymer needle which at its front tip is equipped with a semi-permeable membrane that allows passing through molecules with < 20,000 Dalton. The polymer needle consists of an inner and an outer tube, both connected to a separate tube via a Luer-connector or similar connector. The inner tube is connected to the perfusion pump, delivering the perfusion solution and is ending in the region of the membrane. The perfusion solution (perfusate) is interacting with the body fluid under application via diffusion processes through the membrane and then directed back between the inner and the outer tube of the catheter into the outlet tube (dialysate).

The dialysate is guided through the measuring channel on the disposable polymer chip. The reference channel on the disposable polymer chip is either filled with a reference liquid (usually the perfusion solution) or operated in flow-through via an additional tube connected to a perfusion pump. After passing the polymer chip, each liquid (dialysate and reference perfusate) is guided into a separate waste. The polymer chip is realized as a disposable for one-time usage (on only one patient for a time period of several days), to avoid the risk of infection.

The disposable chip is placed on an electronic board, containing one multi-emitter LED and two InGaAs-photodetectors. The light emitted from the LEDs is split up into two partial beams at a beam splitter integrated in the polymer chip and deflected sideways by 90°. After passing through the reference and measuring channels, the light beams are deflected downwards again by 90°, onto the photodetectors. For each light source (wavelength, respectively) the optical transmission in the measuring and reference cell is measured and a difference signal is generated via electronic processing. The wavelengths have been selected in a way that a significant change of absorption

appears in the measuring channel if glucose is present, resulting in a change of the difference signal, correlated to the glucose level in the dialysate.

The accuracy of the sensor can be influenced by two major factors, serving for the need of sensor calibration, these are:

a) Spectral interference in the NIR 1st overtone and combination band

b) Drift of the difference signal (e.g. caused by temperature difference in the optical cells but also by effects during microdialysis, like recovery change over time, caused by e.g. membrane clogging)

In the following, paragraphs the different factors are described:

a) Spectral interference

Since the measurement is done in the first overtone and combination band of the NIR spectrum, it is not 100% specific to glucose, cross sensitivities might occur during the measurements that might require an individual calibration of the sensor system. The most relevant substance in that field is lactate. Also Bilirubin which does not form OH-bridges in water and Acetaminophen, a substance becoming relevant during medication with e.g. Paracetamol, might be interfering but those have not been investigated.

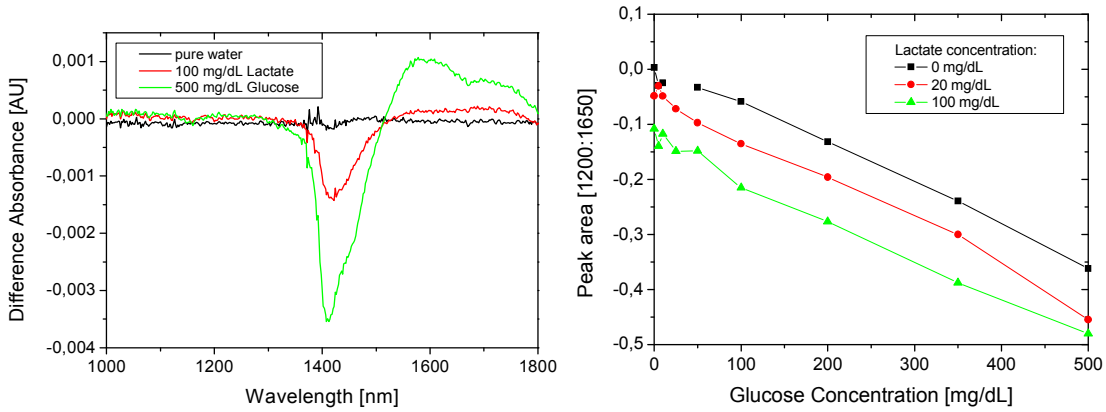

Figure 3: Difference absorption spectra in the 1st overtone band region for pure water, 100 mg/dL lactate and 500 mg/dL glucose (left) and peak area of difference absorption spectra as a function of glucose concentration with varying lactate concentrations (right).

When glucose samples are spiked with different lactate concentrations and glucose concentration is varied, the integrated peak area under the difference spectrum as a function of glucose still shows a linear behaviour with identical slopes. However, the lines are shifted to lower peak areas with increasing lactate concentration (Figure 3).

In a sensor application this effect would deliver too high glucose levels when lactate concentration is high, since the lower peak areas usually appear for the larger glucose concentrations (risk of running into a hypoglycaemia in case of insulin dosage). Therefore, great care has to be taken for correction of large lactate concentrations. However, since the measurement principle is a Null-Measurement (difference spectroscopy), only changes in concentration of possibly interfering substances are of relevance, not absolute concentration values.

b) Signal drift

Signal drift can be caused by a lot different reasons, all related to a differential change of measuring conditions in the optical reference and measuring cells. This could be caused e.g. by a different thermal heating of the liquids in the fluidic compartments because the LED is not placed symmetrically to the beam splitter (possibly also caused by a mechanical drift of the chip over time) or the flow rates differ slightly from each other or the temporal behaviour of the two photo-detectors is not identical or a change of the glucose recovery through the membrane of the body interface occurs. However, it is extremely difficult to determine the exact reason for difference signal drift. A typical example of a signal drift is shown in Figure 4.

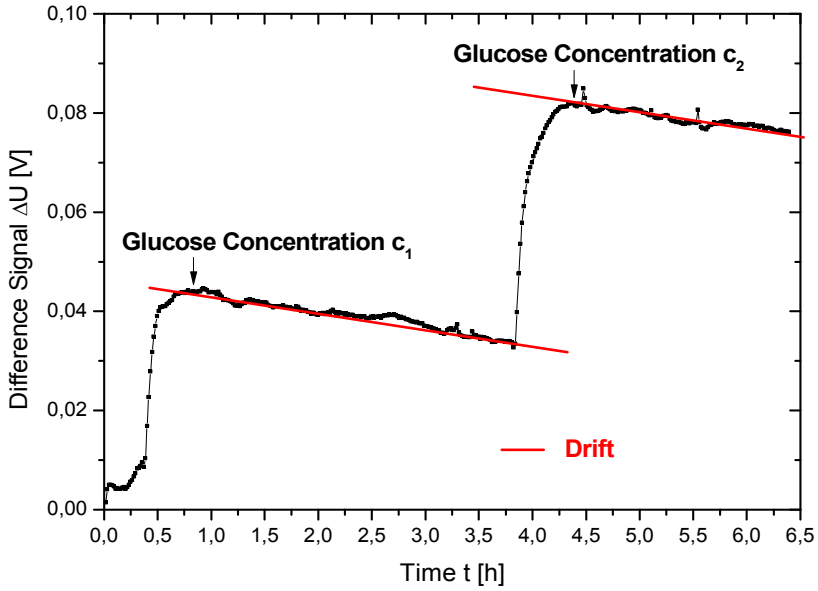

Figure 4: Difference signal of IR glucose sensor as a function of time, for two different glucose concentrations with overlaid drift, marked in red.

In general the drift is constant as a function of time, indicating that a continuous process is involved. A constant drift can be corrected by a two-point-calibration which is equivalent to a linear baseline correction of the signal. Non-linear drift can be introduced by effects like thermalization processes or changes of the membrane recovery. This could be overcome only by a frequent re-calibration based on reference measurements to control the side-effect causing the non-linear drift.

4 Glucose sensor calibration

4.1 Fluorescence life time based sensor

A one point calibration as it will be necessary for use in diabetic patients is not feasible with the first prototype since the mobile measuring head is equipped with only one LED and one photo-detector. This allows the read out of only 1 sensor, either the glucose sensor or the oxygen reference sensor. As can be seen in the dose-response characteristics of the glucose sensor (Figure 8) the intercept of the sensor characteristic is different from zero.

The currently finalized new prototype of the miniaturized fluorometer is equipped with two LEDs and two photo-detectors, which allows a simultaneous read out of the glucose sensor and the reference oxygen sensor. The sensor signal of the glucose sensor is trimmed to be zero at 0 mg/dl glucose by calibration with the oxygen sensors in vitro. In this setup the intercept of the glucose sensor is zero and only the slope has to be calibrated with a blood reference value of the patient.

The in-vivo glucose profiles measured in the preclinical trial with the fluorescence life time based sensor proved that there is no need to recalibrate the sensor within 8 hours. One calibration per day may be sufficient, assuming that the in-vivo drift is similar to the data derived from the in-vitro experiment.

4.2 IR spectroscopy based sensor

The IR CGM sensor calibration is made within three steps:

1) LED/Detector calibration
2) Signal scale calibration
3) Drift compensation calibration

In the following the three calibration steps are introduced and described in detail. To achieve an optimum performance of the IR CGM sensor, all three calibration steps have to be performed.

4.2.1 LED/Detector calibration

Due to the chip fabrication tolerances as well as the error of chip positioning above the multi-emitter LED it cannot be ensured an exact 50%:50% splitting of the light onto the two channels of measuring cell and reference cell, respectively. Therefore, slight changes of the light intensity, combined also with different responses of the two photo-detectors, a change of the difference signal could be introduced although the conditions in the two transmission cells are constant as a function of time. To cope with this problem, in a first step the sensor system is operated with identical liquids in the measuring and reference cell and varied LED operation currents (Figure 5).

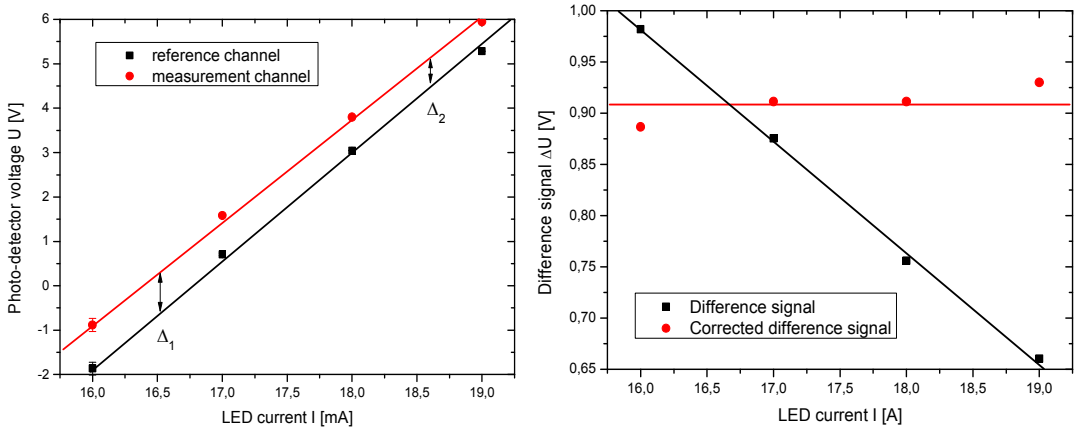

Figure 5: Signal level as a function of LED current for the measurement and the reference cell (left), as well as the corresponding difference signal and the corrected difference signal (right).

Variation of the LED current shows that in general the measured photo-voltage corresponding to the reference and measurement channel, behaves slightly different, resulting in different slopes of the U/I-curves. The difference signal therefore in general is not constant but rather a linear function of the LED current. This problem can be overcome by introducing a correction factor being the ratio of the two slopes and correcting one of the linear curves with this factor before difference forming, resulting in a constant difference level.

The LED/Detector calibration accounts for correction of the difference signal level change caused by intensity changes of the LED but not for the absolute signal level offset which has to be calibrated separately by the signal level calibration.

4.2.2 Signal level offset calibration

During signal level calibration an offset correction is performed, via a two-point calibration. Since the overall composition of the body liquid under investigation (either ISF or blood) can vary from patient to patient and also the composition of the perfusate as carrier medium can vary, the absolute difference signal level will also varied in general. From the in-vitro experiments it is known that there is a linear relationship between the glucose concentration in the perfusate (body liquid) and the difference signal level change. For the above mentioned reasons, usually different slopes appear for the linear functions. Therefore, during the measurement a two-point calibration has to be performed, as indicated in Figure 6.

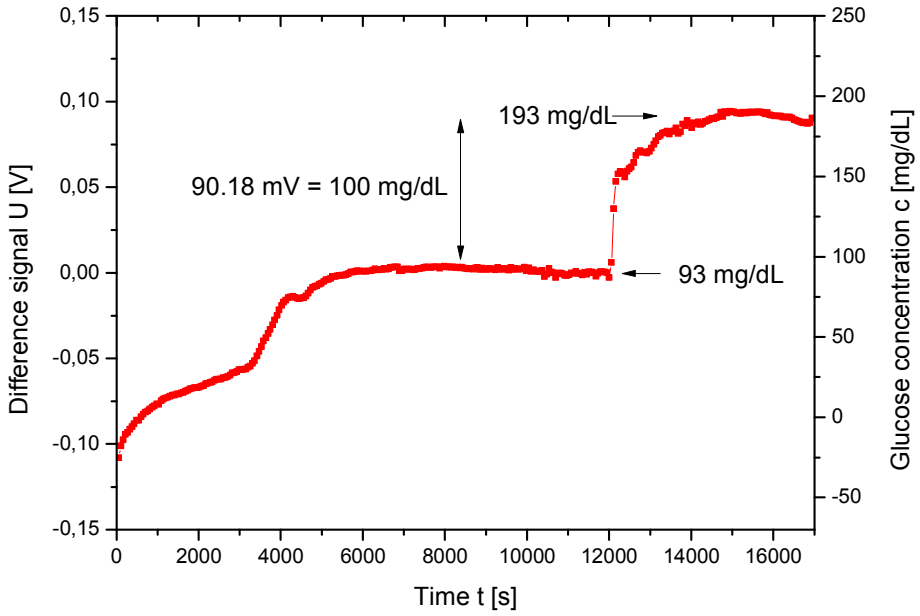

Figure 6: IR CGM sensor signal, as measured with the microdialysis for two different glucose concentrations in blood plasma at constant lactate level. A difference signal voltage of 90.18 mV corresponds to a change of glucose concentration of 100 mg/dL.

During the two-point calibration at time t_1 a reference value of the glucose c_1 is taken and compared to the actual difference signal voltage level U_1 and at a later time t_2 a second reference glucose value c_2 is taken and also compared to the corresponding difference signal voltage U_2. The glucose concentration can then be calculated from the difference voltage signal by:

$$c = c_1 + \frac{c_2 - c_1}{U_2 - U_1} \cdot (U - U_1) \quad \text{(Eq. 1)}$$

In practice, typically one of the two reference points corresponds to zero glucose concentration where in case of microdialysis only the pure perfusate is measured. In general this kind of calibration would be sufficient in case that no temporal drift is implied on the sensor signal. If a temporal drift is present, also a drift compensation calibration has to be performed.

4.2.3 Drift compensation calibration

In case that a long term drift is implied on the sensor signal, an additional error is introduced, since the difference signal voltage change is reduced by the signal drift (Figure 7). In that case a drift compensation calibration should be applied by a two-point baseline correction. For that two reference measurements had to be made with a temporal distance of a few hours.

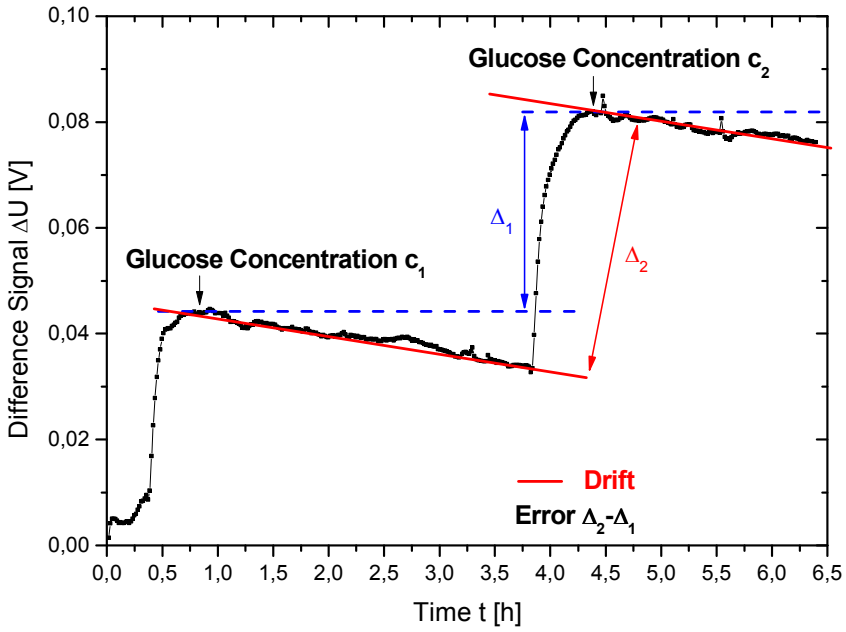

Figure 7: Influence of drift on the voltage range of two reference glucose measurements c_1 and c_2.

For drift compensation the calibrated sensor glucose signal is compared to two reference measurements of glucose concentration. The slope of the linear change of the reference glucose levels is then compared to the slope of sensor glucose levels in the same time interval. If the two slopes differ from each other, a drift is implied on the sensor signal. For correction of the linear drift, the sensor signal has to be corrected by:

$$c_{real} = c_{meas} + f_{corr} \cdot t \qquad \text{(Eq. 2)}$$

where

$$f_{corr} = \frac{\Delta c_2(t_2) - \Delta c_1(t_1)}{t_2 - t_1} \qquad \text{(Eq. 3)}$$

The Δc are the differences of reference and sensor measured concentrations at times t_2 and t_1 with $t_2 > t_1$.

The drift compensation calibration is only valid in case of a linear constant drift over time and corresponds to a baseline correction. In case on non-linear drift, frequent recalibration of the sensor would be required, in defined time intervals. However, so far drift observed with the IR CGM sensor typically is linear. In practice signal level offset and drift compensation calibration will be done by a two-point calibration, taking into account that the measured difference voltage curve is congruent to the reference glucose curve. Then at time t_1 the voltage curve is projected onto the glucose curve by multiplying it with a calibration factor k and at time t_2 reference and measured glucose are compared and in case of drift corrected, as given in Eq. 2, where $\Delta c_1 = 0$ in this case.

It might be possible that over longer time periods a change of the microdialysis recovery, caused by clogging of the needle membrane, leads to a non-linear drift which then only can be compensated by a control measurement of the recovery.

5 Glucose sensor test results

5.1 Fluorescence life time based sensor

5.1.1 In vitro tests

In vitro tests of the optical measurement system were carried out to evaluate the linear measuring range and sensor drift. We used a custom-built flow-through cell of dimensions, 1 mm × 1 mm × 100 mm (height × width × length). Five extensions held the glucose-sensitive part of the catheters in the centre of the measuring channel. Thus, five sensors could be characterized in parallel with this setup. The excitation of the sensor and the optical readout were performed with the mobile measuring head of the optical module. Test solutions were prepared containing 0, 45, 90, 180 and 360 mg/dl glucose in air-saturated phosphate buffered saline solution, pH = 7.4. The flow rate of the glucose test solutions was 5 ml/min, which was fast enough to ensure that there was no depletion of the analyte due to the glucose consumption of the sensors.

In the first two hours of the in-vitro tests the glucose concentration was increased stepwise from 0 to 360 mg/dl and then decreased again to 0 mg/dl. Each glucose concentration was maintained for 15 min to ensure a complete flushing of the measuring cell with the new solution and to allow two readings to be made with each sensor. This “glucose concentration pyramid” was used to generate the dose-response characteristics of the sensors (Figure 8). Error bars indicate the standard deviation of the 5 sensors used for the test.

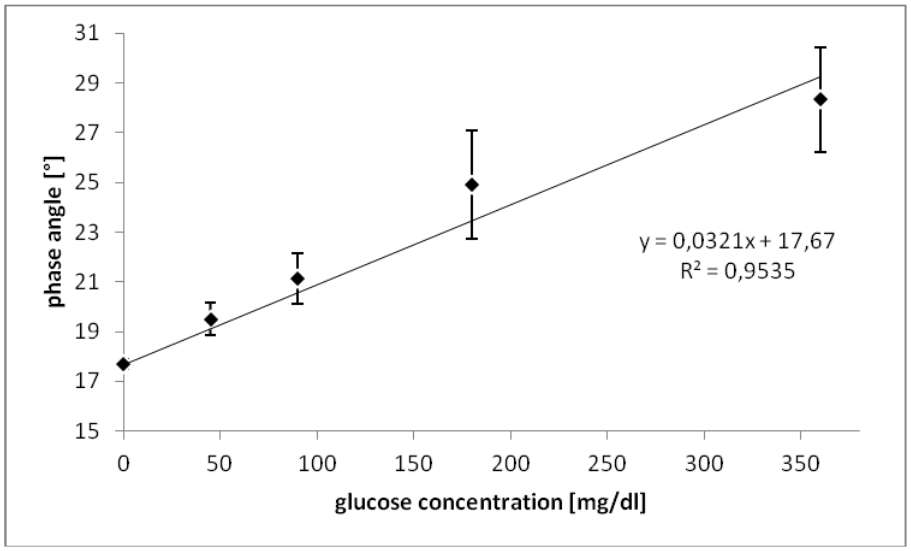

Figure 8: Dose-response characteristics of the sensors.

After completion of the concentration pyramid the glucose concentration was alternated between 45 and 360 mg/dl to identify any sensor drift at high and low glucose concentrations. The sensor drift is calculated as the difference between the first and last sensor reading divided by the time between the measurements. The drift at high and low concentration was found to be +0.13%/h for the 360 mg/dl values and -0.38%/h for the 45 mg/dl values respectively (Figure 9).

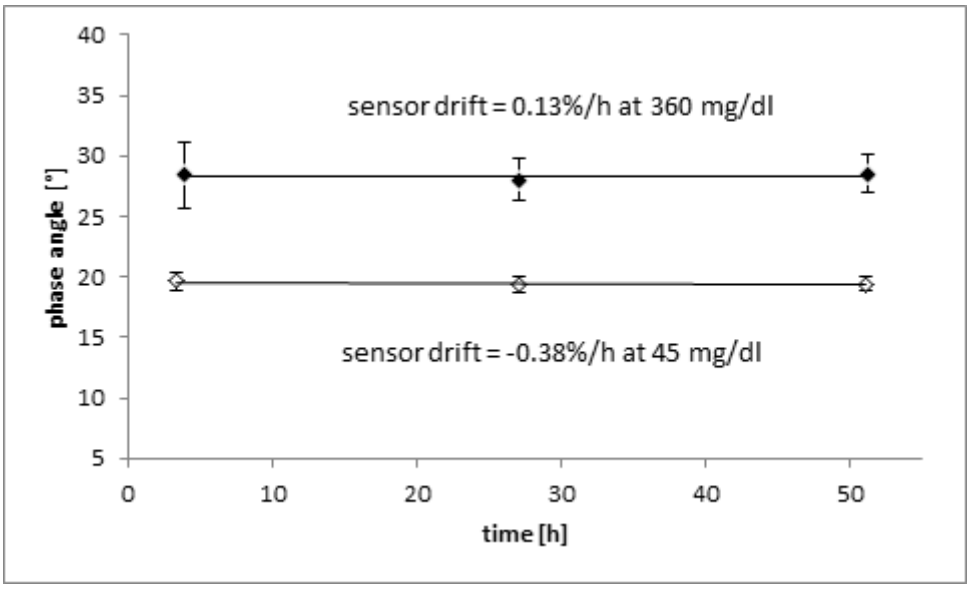

Figure 9: Sensor-drift at glucose concentrations of 45 mg/dl (empty diamonds) and 360 mg/dl (solid diamonds) over 50 h.

5.1.2 In-vivo tests

The animal experiments were approved by the Austrian federal government (number of permission: BMWF-66.010/0117-II/3b/2011) and were performed in consent with Directive 2010/63/EU on the protection of animals used for scientific purposes. The sensor tests were performed in domestic pigs of an age of 2 months and a weight of 29 ± 2 kg. The animals were anaesthetized with a bolus of 3 mg/kg propofol 1% before the start of the experiments and were kept anaesthetized over the whole duration of the experiments of 10 hours by infusion of 6-10 mg/kg/h propofol 1%. At the end of the experiments the animals were sacrificed by intravenous administration of 200 mg propofol 1% and 60 mval potassium-chloride. Each of the animals had six glucose-sensitive insulin infusion catheters as well as two oxygen sensors inserted in their abdominal subcutaneous adipose tissue. Of the glucose-sensitive catheters, two were used for insulin infusion and two for the infusion of physiological NaCl solution, while two had no infusion.

The catheters were implanted by using stainless steel cannulas (Venflon I.V. Canule, BD) as follows: A cannula containing a catheter is inserted under the skin of the pig. The cannula is then removed, leaving the catheter in place under the skin. The catheter's position is then fixed with adhesive tape to prevent accidental movement. The tubings for insulin delivery are primed with insulin (Novorapid 100U/mL, Novo Nordisk) and then connected to the catheters. The mobile measuring head is positioned above the inserted catheters to determine the maximum phosphorescence. When the maximum is found, the position of the measuring head is marked on the skin so that the position can be restored when the measuring head is moved from catheter to catheter. Reference blood-glucose values were determined by analyzing blood samples with a Super GL2 (HITADO GmbH, Germany).

The blood-glucose levels were clamped according a predetermined profile starting at normoglycaemia with 100 mg/dL glucose. After a baseline of 45 min, the blood-glucose concentration was decreased to a hypoglycaemic level of 40 mg/dL, maintained at this level for 45 min, and then increased to a hyperglycaemic level of 250 mg/dL where it was then maintained for 45 min. These hypo- and hyperglycaemic excursions were each performed twice. At the end of the experiment, a normoglycaemic level of 100 mg/dL glucose was maintained. During the clamp experiment, the glucose concentration was controlled by constant subcutaneous insulin delivery at 2.4 U/h, which was infused through two glucose-sensitive catheters, resulting in an infusion rate of 1.2 U/h per catheter, and variable intravenous glucose infusion. Insulin and sodium chloride solution (0.9%; Fresenius, Austria) were infused by syringe pump (SP230iw, World Precision Instruments, UK). Further sodium chloride solution was infused through another two catheters with a flow-rate of 12 µl/h per catheter to achieve the same volume flow as in the insulin infusion. Two further sensors were used for monitoring reference glucose values without any infusion. Subcutaneous glucose measurements with the I-Cath system were synchronized with the blood withdrawal for reference glucose values and were carried out every 5 min. Two additional sensors were used to monitor tissue-oxygen levels.

5.1.3 Statistics

The mean absolute relative error (mean ARE) and the median absolute relative error (median ARE) are measures of the accuracy of a method for calculating fitted time series values, specifically in trend estimations. The difference between bg_i and cm_i is divided by the actual bg_i value again. The absolute value in this calculation is summed for every sensor or reference point in time and divided again by the number of fitted points n. Multiplying by 100 makes it a percentage error. They express accuracy as a percentage value and are defined by the following formulas where bg_i is the actual blood glucose value and cm_i is the corresponding glucose concentration calculated from the sensor readout.

$$mean\ ARE = \sum \left| \frac{bg_i - cm_i}{bg_i} \right| \cdot \frac{100}{n} \qquad \text{(Eq. 4)}$$

$$median\ ARE = median \left| \frac{bg_i - cm_i}{bg_i} \right| \cdot 100 \qquad \text{(Eq. 5)}$$

Accuracy is also given as the predictive error sum of squares (PRESS) which is calculated as follows:

$$PRESS = \sqrt{\frac{\sum (cm_i - bg_i)^2}{\sum bg_i^2}} \cdot 100 \qquad \text{(Eq. 6)}$$

The mean ARE, median ARE and PRESS values were calculated for each sensor and average and standard deviation were calculated for each sensor group.

Profiles of measured tissue glucose concentrations are shown in Figure 10, Figure 11 and Figure 12 for one sensor from each group. The profiles are calculated from a retrospective calibration using a linear regression of the measured phase shift and the reference blood-glucose values. The first two hours of the glucose profile are not included in the calculation due to the run-in time required: implanting the catheter can cause a small amount of tissue trauma and possible bleeding, which can alter the physiology of the tissue surrounding the sensor. We observed a variation in the run-in time among the 3 sensor groups: sensors with no infusion appear to have shorter run-in times than those with infusion. The maximum run-in time of 2 hours was observed for the insulin infusion sensor group. This is probably due to the effect of insulin, which lowers local glucose concentrations at the start of the infusion. No correction for the physiological time lag between blood and subcutaneous tissue was performed. Nor could a compensation of variations in tissue oxygen levels be performed because, as discussed above, the glucose and oxygen sensors were on separate catheters.

As can be seen from Figure 10, Figure 11 and Figure 12, glucose profiles from the three sensor groups show good correlation of the sensor readouts with the reference glucose values; the average median ARE value is in the range of 20 %, and there is little deviation between the sensor groups with different types of infusion.

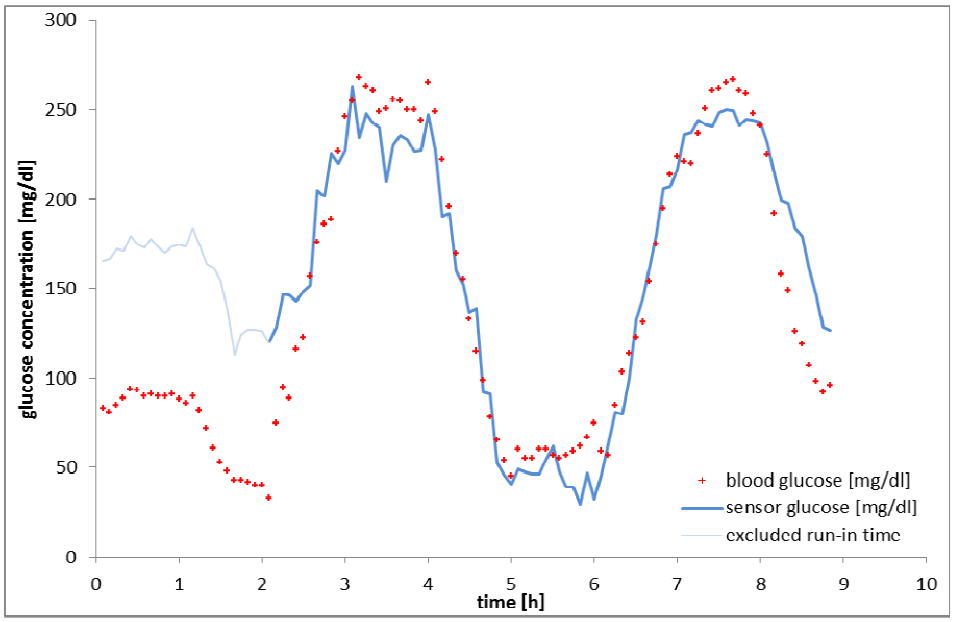

Figure 10: Glucose profile derived from a sensor with insulin infusion.

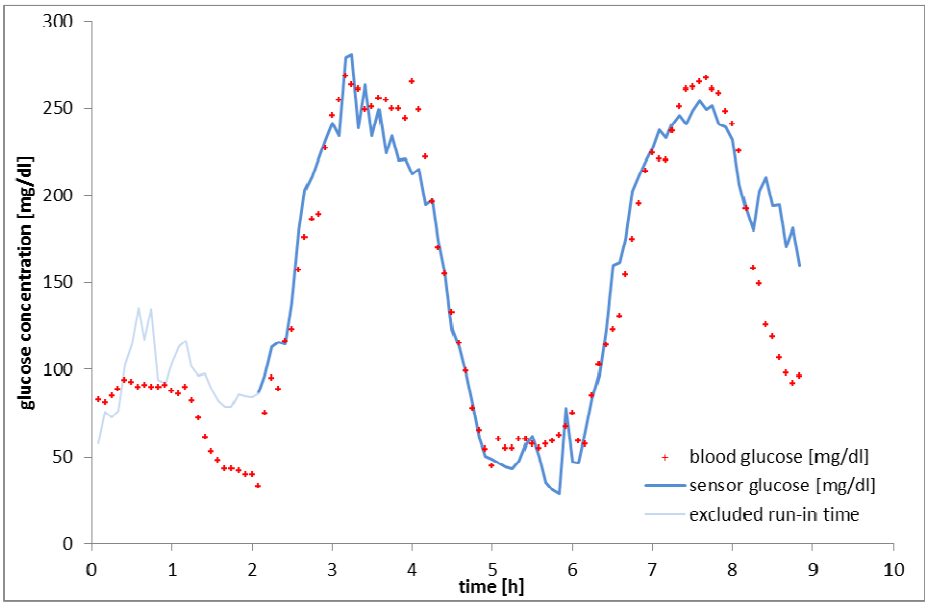

Figure 11: Glucose profile derived from a sensor with sodium chloride infusion.

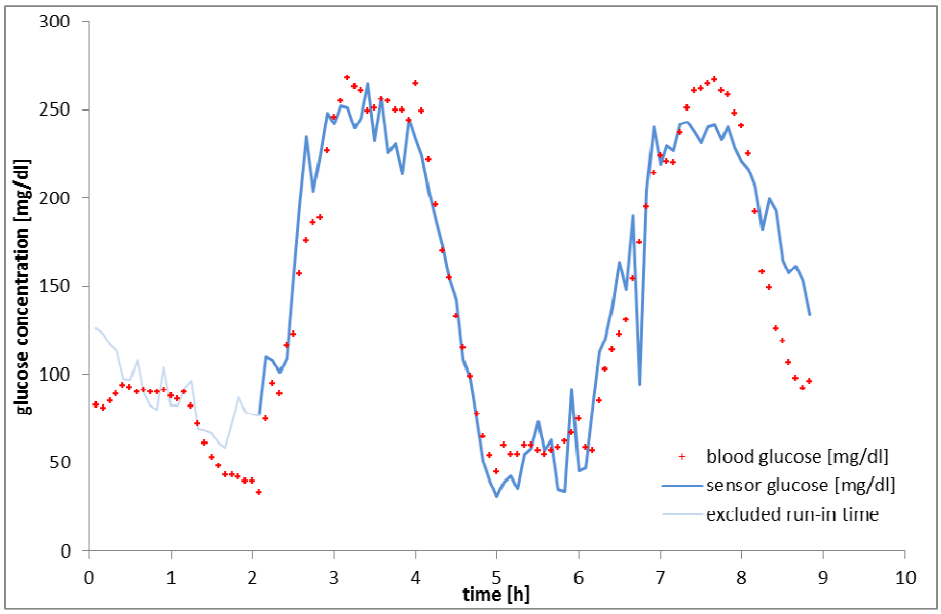

Figure 12: Glucose profile derived from a sensor without any infusion

The predictive error sum of squares (%PRESS) was calculated for each sensor, according to Equation 6, and the average %PRESS values were calculated for each sensor group (see Table 1).

Sensor	Infusion	Mean ARE	Median ARE	PRESS
1	Insulin infusion 1.2 U/h	46.7%	42.5%	33.8%
2		31.0%	16.4%	20.7%
3		17.5%	8.5%	14.4%
4		22.8%	15.5%	20.2%
5		35.6%	22.6%	29.8%
6		40.2%	24.4%	30.3%
mean ± SD		32.3% ± 8.3%	21.6% ± 5.7%	24.9% ± 6.3%
1	0.9% NaCl infusion 12 µl/h	27.3%	18.5%	19.7%
2		41.5%	20.4%	25.4%
3		27.7%	17.1%	25.4%
4		13.8%	8.1%	15.8%
5		51.5%	24.5%	36.3%
6		40.1%	20.3%	25.8%
mean ± SD		33.6% ± 13.0%	18.1% ± 5.8%	24.8% ± 6.6%
1	no infusion	33.2%	22.7%	23.2%
2		63.2%	29.8%	34.1%
3		16.7%	9.8%	15.1%
4		19.3%	12.8%	16.9%
5		33.1%	18.0%	26.7%
6		41.3%	22.3%	28.8%
mean ± SD		34.5% ± 18.8%	19.2% ± 7.9%	24.1% ± 8.1%

Table 1: Mean ARE, median ARE and PRESS values for the fluorescence spectroscopy sensor, as determined during the in-vivo animal tests.

5.2 IR spectroscopy based sensor

5.2.1 In-vitro tests

In-vitro tests of the IMM IR CGM sensor have been made with citrated human blood plasma and microdialysis, using a CMA60 catheter with ELO-MEL perfusion and a flow rate of 0.5 µl/min.

Reference measurements on the blood plasma as well as the dialysate were measured with a *Super GL Compact* from Dr. Müller, which exhibits an accuracy of about ±10%.

At first the microdialysis was dipped in vials of 2.5 ml total sample volume, exhibiting different concentrations of glucose (different plasma sample with different glucose concentration and lactate concentration) to determine the signal level changes as a function of glucose concentration (Figure 13).

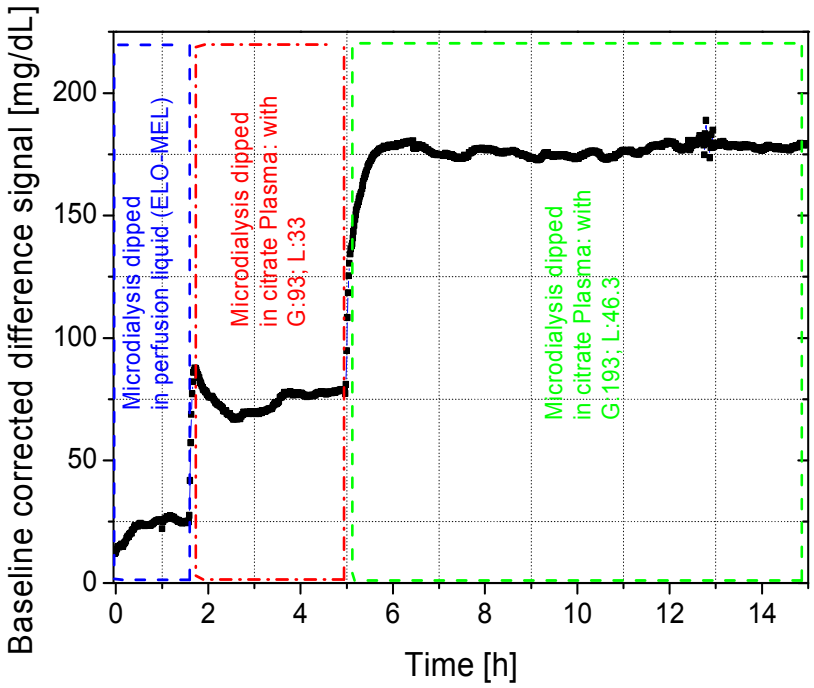

Figure 13: Time shifted and baseline corrected IR CGM sensor signal as a function of time during microdialysis in three different samples (pure ELO-MEL, blood plasma 1 and blood plasma 2).

After drift and time shift compensation a typical signal response, as shown in Figure 13, is achieved:

1) Warm-up phase (mainly LED), approximately 1.5 hours, during which the signal level reaches saturation (pure ELO-MEL is in the measuring and reference channel)
2) Signal jump (1.5 hours delay is corrected) after dipping the microdialysis catheter into the first blood plasma sample. The delay is caused by the finite flow rate of 0.5 µl/min and the length of the tubing as well as the volume of the optical flow through cells.
3) Signal jump (1.5 hours delay is corrected) after dipping the microdialysis catheter into the second blood plasma sample.

Obviously by setting the glucose zero level after about 1 hour for pure ELO-MEL to zero, does not give a correct absolute glucose scale for the sensor. This is due to the fact that lactate also creates part of the signal level (interference problem) and has to be taken into account during calibration. Since the lactate level changes in blood sample #2 compared to blood sample #1, only the difference between the two measured glucose levels is given correctly but not the absolute scale. In fact, changes of lactate in the blood of real patients always will give rise to an error of glucose measurement, if not corrected by a recalibration (how strong the measurement will be affected has to be clarified during the clinical trials).

To investigate if the sensor signal runs stable over a longer time period at constant sample conditions and if the dialysate shows the same values as the sensor itself, a measurement on a blood sample (60 mL) with about 100 mg/dL glucose and 35 mg/dL (citrated human blood plasma) lactate was sampled with a CMA60 microdialysis catheter (0.5 µl/min) and frequent analysis of the sample glucose and lactate concentrations, as well as the dialysate glucose and lactate concentrations (Figure 14).

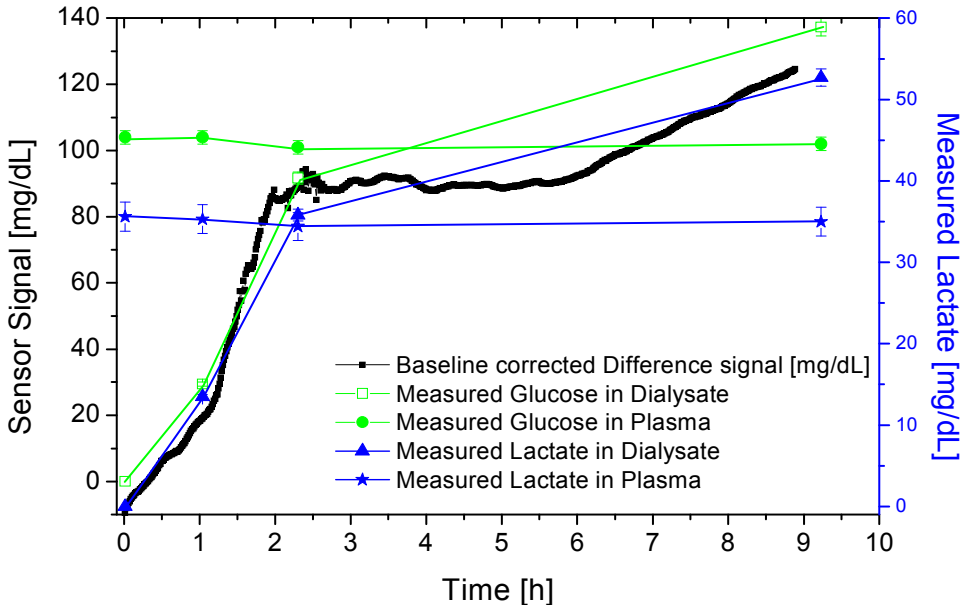

Figure 14: Glucose (green) and lactate (blue) concentrations, as measured by the IR CGM sensor via microdialysis and a Super GL compact in the blood plasma and dialysate, respectively. Glucose concentration in the sample vial remained constant over time.

Evidently the sensor signal shows a good temporal correlation with the blood glucose level measured in the dialysate after a level calibration at time t=0 (calibrated on the dialysate level). The glucose level measured via the microdialysis (either by the sensor or by the laboratory device in the dialysate) is always a little lower than that measured in the blood sample directly, which is attributed to slightly lower recovery than 100 % of the microdialysis. Again, due to the delay caused by the microdialysis tubings, the signal level increases during the first 2 hours to a constant level. After about 6 hours the sensor signal, as well as the reference signal in the dialysate, start rising, although the concentration in the blood sample stays constant. This effect was attributed to loss of perfusion solution by evaporation through the tubings of the chip connections which were made of silicone rubber, leading to an increase of glucose concentration in the optical measuring cell of the chip as well as in the dialysate container behind the chip. To avoid such effects, the tubings are replaced by medically approved *Tygon S-54 HL* tubes, avoiding liquid leakage by evaporation through the tube walls.

To investigate how the IR CGM sensor reacts on glucose concentration changes, microdialysis was performed, as in the previous section (60 mL sample volume, citrated blood plasma with constant lactate, CMA60 microdialysis catheter at 0.5 µl/min) but glucose concentration was changed after 4 hours by adding concentrated plasma of the same sample to the original sample, increasing the glucose concentration from 115 mg/dL to 145 mg/dL (Figure 15).

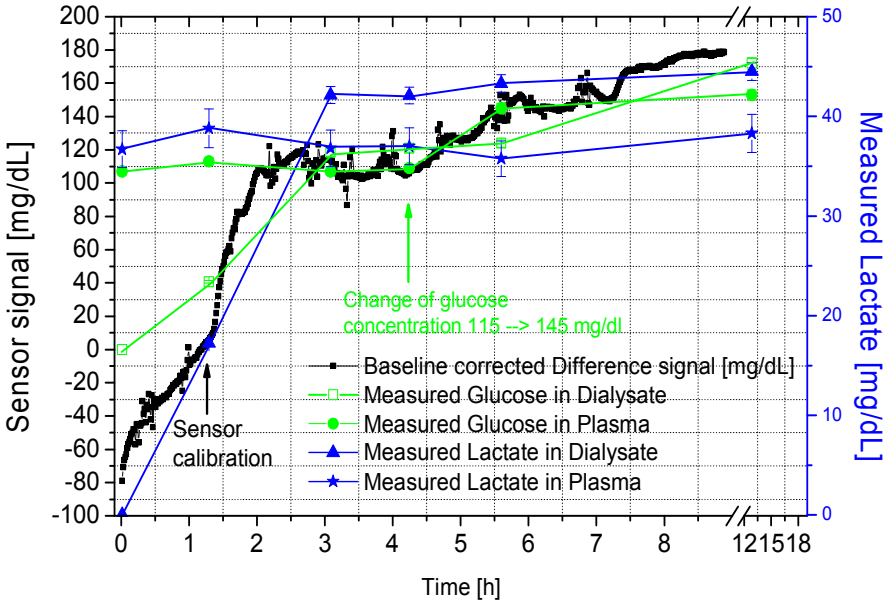

Figure 15: Glucose (green) and lactate (blue) concentrations, as measured by the IR CGM sensor via microdialysis and a Super GL Compact in the blood plasma and dialysate, respectively. Glucose concentration was changed from 115 mg/dL to 145 mg/dL after about 4 h measuring time.

Again, the sensor signal reaches the level corresponding to the actual glucose concentration only after about 2 hours delay time, caused by the microdialysis tubings. After about 4 hours after the start of the measurement, the glucose concentration in the sample vial is changed from 115 mg/dL to 145 mg/dL. The sensor signal after calibration and baseline correction follows nicely the glucose level measured with the reference device (black symbols → sensor data, closed red symbols → reference data). The change of glucose level at the sensor is visible again only after about 2 hours delay, after glucose concentration has been changed in the reservoir. The reference data in the dialysate (open red symbols) also follows the sensor trend quiet good. The lactate values in the dialysate are systematically slightly higher than those measured in the sample vial which is attributed to contamination effects, although the deviation lies within the error tolerance of the reference device.

Further qualification measurements are currently under way and also clinical in-vivo trials are going to be performed in January/February 2013, the results of which are given in the report of deliverable D3-9 Sensors for Glucose Measurement for first Field Trials and second generation ePatch (due March 2013).