ABSTRACT
Recently, enormous interest has been turned to quasi-continuous analysis of glycaemia in the intensive care discipline. For patients under critically ill conditions, the intensive insulin therapy treatment requires reliable assays for blood glucose. Micro-dialysis in combination with infrared spectrometric detection renders a system that can function reagent- and calibration-free over an extended period. A programmable fluidics system has been set-up and tested which allows regular spectral sample and reference measurements. Such instrumentation suits the low pressure conditions necessary for microdialysis and the high pressure for transport flow to the flow-through infrared micro-cell. First assay results are presented.

KEY WORDS
glucose, infrared spectrometry, micro-dialysis, continuous monitoring

1. Introduction
For clinical research, in-vivo blood glucose monitoring is an ongoing, important topic to improve glycemic control in patients with inadequate blood sugar regulation. In particular, for patients under critically ill conditions, the intensive insulin therapy treatment cuts mortality rates by 42% compared to standard therapy for intensive care indication [1]. Despite the existence of commercially available, mainly amperometric biosensors for glucose monitoring [2], there is continued interest in spectroscopic techniques for reagent-free glucose measurements, which avoid frequent recalibration of the sensor. As shown previously, infrared (IR) spectroscopy of body fluids is a convenient and sensitive technique for glucose determination in whole blood or plasma [3, 4] and blood dialysates [5]. Even nanoliter sample measurements have recently been presented [6, 7].

Infrared transmission spectroscopy was chosen, which is less sensitive to inside-cell adsorption of biofluid proteins, which can affect stable long-term performance of sensors based on attenuated total reflection. For such operation, a switch-flow system was developed that enables quasi-continuous glucose monitoring within a patient-monitoring set-up including a micro-dialysis probe. One problem, when previously using micro-dialysis probes in-line with flow-through transmission micro-cells, is the high backpressure resulting from the infrared cuvette dimensions (cell window spacing \( \approx 35 \mu m \)) and the low inner diameter of the tubings for avoiding large dead volumes within the fluidic system. This leads to perfusate losses through the dialysis probe membrane. The benefit of the new system is the complete decoupling of the dialysis flow-stream from the fluidics which incorporates the infrared cuvette.

2. Experimental
IR-spectra were recorded using an FT-IR spectrometer (model Vector 22) from Bruker Optik GmbH, Ettlingen, Germany. The spectra were measured using a custom-made flow-through transmission micro-cell with a 3 mm diameter aperture and 1 mm thick CaF\(_2\) windows (see Fig. 1). Spectral resolution was 16 cm\(^{-1}\) and 50 interferogram scans (measurement time 15 s) were averaged for spectrum recording. CMA-60 micro-dialysis probes certified for use in humans were supplied by CMA Microdialysis, Solna, Sweden.

Fig. 1 Schematics of the infrared micro-cell.

Our first approach by coupling the dialysis probe directly in-line with the IR-cuvette using PEEK tubings of 50 \( \mu m \) inner diameter led to significant leakage of perfusate through the dialysis probe membrane as mentioned above. To cope with this condition, the following switch-flow system was used (Fig. 2). Two syringe pumps were used for the delivery of perfusate to the dialysis probe and the IR-spectrometer cell. A computer-controlled 6-port
injection valve equipped with a 5 µl sample loop was used for the injection of the dialysate sample from the micro-dialysis probe to the detection flow-stream.

Fig. 2 Flow diagram for quasi-continuous blood glucose monitoring using microdialysis and infrared spectrometry.

pumps are equipped with a serial RS232 port connected to the USB-port with two USB-to-RS232 cables. The first syringe-pump is equipped with the standard RS232 interface from TSE-systems. The second pump is controlled by a custom-made microcontroller-based circuit. The magnetic switching valve (MSV) is also connected to a RS232 port.

Fig. 4 Time-dependent infrared-spectra for injected glucose solution (75 mg/dL, sample volume 5 µL) using the switch-flow operation with spectral recording at a flow rate of 1 µL/min.

2.3 Software
The system is controlled by a custom-made C-program, which is coded with the 'lcc' c-compiler (http://www.cs.virginia.edu/~lcc-win32, free for non-commercial usage). It enables the set up and control of the two syringe pumps, the spectrometer and the MSV. The spectrometer is controlled via the spectrometer software package (OPUS, Bruker Optics). Measured spectra are directly forwarded to a Matlab™ (Mathworks) script, which uses a pre-calculated calibration model for online-prediction of the glucose concentration values. Communication with Matlab™ is established using dynamic data exchange (DDE).

3. Measurements for optimisation of quasi-continuous on-line monitoring

Example spectra recorded with the flow-through microcell are shown in Fig. 3. A protocol for the fluidics automation was established. For the measurements, the sample loop (internal volume of 5 µL) of the injection valve was filled with the sample fluid. The perfusate flow (Ringer’s solution) to the IR-spectrometer started, for example, at a flow-rate of 5 µL/min. After recording the background spectrum using the pure perfusate filled cell the flow protocol was started, beginning with the injection of the sample. IR-spectra were recorded every 15 s. After 60 s the sample plug reached the cell, and the flow-rate of
5 µL/min was reduced to 1 µL/min in order to have a longer time window at the maximum concentration of the injected sample from the sample loop for IR-measurement. After 2.5 min the flow-rate was switched to 20 µL/min to remove any possible residues of the injected sample. An example of the continuously recorded spectra is shown in Fig. 4. Following this scheme, a quasi-continuous monitoring of an in-vivo, changing glucose concentration profile is possible. In addition, there is much flexibility gained with regard to tubing length for connecting the micro-dialysis probe to the IR-sensor system.

Looking at the glucose band maximum at 1034 cm\(^{-1}\) in Fig. 4, obtained with baseline offset correction at 1180 cm\(^{-1}\), one can clearly observe that after a measurement time of 1.0 min after sample injection a plateau is reached. For the presentations of our further investigations only the absorbance band maximum at 1034 cm\(^{-1}\) was exploited, but one should keep in mind, that the whole spectral information for multivariate calibration with more complex sample compositions can be used if necessary (e.g., in our PLS regressions the spectral range from 950 to 1180 cm\(^{-1}\) was exploited).

![Absorbance spectra at 1034 cm\(^{-1}\) with dialysis probe](image1)

**Fig. 5 A** Reproducible infrared measurements with no memory effects from sample transfer using the fluidics protocol. B univariate regression results using glucose absorption-band maximum (open triangles refer to outliers).

The performance of the novel switch-flow set-up with regard to reliability and reproducibility was tested by consecutive injections from the sample loop, filled with aqueous standard solutions containing different glucose concentrations. The flow protocol was the same as mentioned above, but reducing the flushing flow to 5 µL/min at 4.5 min, recording the next background spectrum at 5.75 min and restarting the protocol after 6.0 min with another glucose solution. Fig. 5A shows examples of the time dependent IR-spectra of such injected glucose solutions.

The regression line in Figure 5B shows excellent precision and linearity of the signal from 37.5 mg/dL up to 450 mg/dL glucose, whereas the smaller sloped regression line (using the micro-dialysis probe immersed in corresponding aqueous glucose solutions; see abscissa values) shows increasing deviations at higher glucose concentrations which are not attributed to a decreasing recovery rate (about 70 % based on measurement results marked with solid triangles), but more to the reliability of the probe itself (major membrane or fluidics fault). In the future, this will be further investigated to prevent such extreme performance variations.

As shown already in Fig. 5B focusing on the absorption band maximum at 1034 cm\(^{-1}\), the absorbance spectra show excellent reproducibility and sensitivity for the changing glucose concentrations. Due to the flow-scheme with subsequent cell flushing, a spectral background measurement is possible after each sample measurement to enable highest stability. This aspect is important even in the light of slight changes in spectrometer measurement conditions, which is essential for reliable continuous operation within the critical care unit environment.

### 4. Measurements of blood plasma and ultrafiltrate samples

For simulating the influence of low-molecular mass components in the dialysates of real samples, the dialysis probe was immersed into a pool-plasma sample and operated under a perfusate flow rate of 2 µL/min. For the resulting dialysate spectrum, see Fig. 3B. The spectrum shows the unique glucose absorption band structure which can be used for quantification.

The effective use of multivariate calibration using Partial Least-Squares (PLS) regression (for details, e.g., see Ref. [4]) has been effectively demonstrated using transmission spectra of ultrafiltrates produced with a 30 kDalton cut-off, similar in composition to the micro-dialysates, but with a cut-off of 20 kDalton. The aqueous sample spectra were measured with the same micro-cell.

An ultrafiltrated sample was measured as a dry-film in transmission, showing the full mid-infrared spectrum and illustrating its whole chemical composition. In comparison to the ultrafiltrate the dry-film spectrum of a reference serum is also shown in Fig. 6A, which is dominated by absorption bands of the serum proteins...
Fig. 6 A Dry-film spectra of a reference serum and an ultrafiltrate, respectively; B scatter plot of IR-predicted glucose concentrations using cross-validation versus reference concentrations (the inset shows part of the spectral calibration data).

above 1500 cm\(^{-1}\) (amide I and II bands) and around 3250 cm\(^{-1}\). The ultrafiltrate spectrum shows impurities of glycerol (see absorption bands within the interval of 1150 – 950 cm\(^{-1}\)), which stems from the centrifugal filter device, that was soaked with the solvent for preventing from a dry-out during storage. In part B, the scatter plot of IR-spectrometrically predicted versus reference concentrations obtained with the Hexokinase method is presented. The inset shows part of the spectral calibration data obtained using the flow-system with the custom-made IR-microcuvette. The calibration results in an excellent prediction uncertainty of 1.5 mg/dL for glucose concentrations using the leave-one-out cross-validation strategy.

5. Conclusions

The application of intensive insulin therapy to critically ill patients in order to keep their blood glucose level between 4.5 and 6.5 mmol/L (80 - 120 mg/dL) leads to increased research activities especially for minimal-invasive monitoring techniques. The developed sensor system based on micro-dialysis and IR-spectrometry is most effective with a programmable fluidics system, which can be further miniaturized with micro-fluidic elements currently under development at different companies and research groups.

6. Acknowledgements

Financial support by the European Commission with the CLINICIP project (contract no. 506965 within the 6\(^{th}\) Framework Programme) is gratefully acknowledged. We appreciate the effective collaboration with partners from our consortium, in particular from the participating research institution, Joanneum Research, in Graz (Austria), who provided us with ultrafiltrates from whole blood. We are also grateful for continued financial support from the Ministerium für Wissenschaft und Forschung des Landes NRW and the Bundesministerium für Bildung und Forschung.

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