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A novel automated discontinuous venous blood monitoring system for ex vivo glucose determination in humans

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ABSTRACT

Intensive insulin therapy reduces mortality and morbidity in critically ill patients but imposes great demands on medical staff who must take frequent blood samples for the determination of glucose levels. A solution to this resourcing problem would be provided by an automated blood monitoring system. The aim of the present clinical study was to evaluate such a system comprising an automatic blood sampling unit linked to a glucose biosensor. Our approach was to determine the correlation and system error of the sampling unit alone and of the combined system with respect to reference levels over 12 h in humans. Two venous cannulae were inserted to connect the automatic and reference systems to the subjects. Blood samples were taken at 15 and 30 min intervals. The median Pearson coefficient of correlation between manually and automatically withdrawn blood samples was 0.982 for the sampling unit alone and 0.950 for the complete system. The biosensor had a linear range up to 20 mmol l^{-1} and a 95% response time of <2 min. Clark Error Grid analysis showed that 96.93% of the data (228 data pairs) was in zone A and 3.07% in zone B. Insulin Titration Error Grid analysis suggested an acceptable treatment in 99.56% of cases. Implementation of a "Keep Vein Open" saline infusion into the automated blood sampling system reduced blood withdrawal failures through occluded catheters fourfold. In summary, automated blood sampling from a peripheral vein coupled with automatic glucose determination is a promising alternative to frequent manual blood sampling.

1. Introduction

Critically ill patients undergoing intensive medical treatment may develop hyperglycaemia and insulin resistance despite the lack of a previous history of diabetes (McCowen et al., 2001). The use of intensive insulin therapy is very helpful for such patients as illustrated by a 12-month study involving 1548 critically ill surgical patients (Berghe et al., 2001). In this study, maintenance of blood glucose levels within a range of 4.4–6.1 mmol l^{-1} was shown to reduce the rate of mortality from 8.0% (conventional treatment) to 4.6%, an apparent risk reduction of around 42%. The risk of complications and the consumption of intensive care resources were moreover also reduced.

To maintain euglycaemic glucose levels, skilled medical staff must take frequent blood samples. In practice this is often not feasible however, due to the high workload associated with the care of critically ill patients. Reproducible manual blood sampling is moreover technically challenging. The quality of the sample obtained from the fingertip with a lancet is affected by factors such as the location and depth of puncture and dilution by ethanol used for surface sterilisation. On the other hand, sampling from a peripheral vein can be problematic owing to a lack of suitable veins. These technical challenges are rendered more acute by the general lack of available time and the need to minimize patient discomfort. Finally, the complicated logistics of sample processing can potentially lead to delayed results or even sample mix-ups.

Fully automated blood sampling and real time glucose analysis would elegantly avoid these problems and moreover provide valuable trend information (direction and rate of change). Armed with this, and freed from the need to perform routine blood sampling and await the analysis results, an intensive care nurse could more effectively maintain euglycaemia. A prerequisite for the clinical acceptance of such a system would be the avoidance of systemic heparinisation, especially of patients following major surgery.

Systems using double lumen catheters (Weller et al., 1960; Gfrerer et al., 1998) such as the Biostator and Glucostator or ex vivo

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bedside devices employing mid-infrared spectroscopy (Heise et al., 2007) have been successfully used to sample peripheral venous blood. These systems have only gained acceptance for clinical studies however and are in addition more expensive to use than those employing conventional catheters. Via Medical introduced a blood glucose monitoring device using standard venous access but did not supply the European market (Ganesh et al., 2008).

Glucose levels can also be determined in subcutaneous adipose tissue by microdialysis (Jungheim et al., 2001) or by open-flow microperfusion (Trajanoski et al., 1997). Systems using microdialysis and a needle-type sensor include the Abbott Freestyle Navigator (Weinstein et al., 2007), the Dexcom STS sensor (Garg et al., 2006) and the MiniMed CGMS Gold portable system (Mastrototaro, 1999). Non-invasive, continuous measurement systems are also commercially available. These include iontophoresis-based systems such as the Cygnus GlucoWatch (Tierney et al., 2001) or impedance spectroscopy-based devices (Caduff et al., 2003). As well as the above, several glucose sensors have been validated for future use with automatic blood sampling units (Ricci et al., 2007).

The aim of the EU-funded project closed loop insulin infusion for critically ill patients (CLINICIP) was to develop a low-risk, invasive decision support system comprising biosensors and an adaptive control algorithm for the glycaemic control of intensive care patients (Hovorka et al., 2004). Our goal within this project was to develop an automated glucose monitoring system ("AGM-system") comprising an automated blood sampling system ("ABS-system") in combination with an online glucose biosensor ("biosensor"). In the present study, the ability of the resultant system to deliver clinically reliable glucose measurements was determined over a 12-h period in two healthy volunteers using good clinical practice in a purpose built clinical study unit. The glucose values obtained with the AGM-system were compared with those obtained from manually withdrawn blood samples using correlation, system error and error grid analyses. Prior to this, the ABS-system was validated alone in six subjects since this was the most technically complex part of the AGM-system and thus, we reasoned, the most error-prone part (the biosensor was already known to function well over an extended period). The study safety endpoints were the determination of the activated partial thromboplastin time (APTT) as a measure of systemic heparinisation caused by the system and the monitoring of adverse events.

2. Experimental

2.1. Blood sampling

Physicians and nurses conventionally obtain peripheral venous blood samples using a manual procedure employing two stopcocks and two syringes. This procedure was used as the study reference. The overall configuration and a description of the AGM-system are provided in Fig. 1. The following materials were used: single lumen catheters (18 gauge × 45 mm, BBraun, Melsungen, Germany) sterile 0.9% saline solution (Fresenius, Fesenius Kabi, Graz, Austria), peristaltic pumps (Minipuls MP3, Gilson, Cedex, France), heparin (Novo Nordisk 5000 IE ml^{-1}, Bagsvaerd, Denmark), tubing (TYGON® S-50-HL and TYGON® 3350, Saint-Gobain Performance Plastics, Beaverton, France), drop chambers (Infusiomatleitung, BBraun, Melsungen, Germany), dripping sensors (Type: 3450578 A, BBraun, Melsungen, Germany), pinch valves (PM-0815W, Takasago Electric Inc., Japan), check valves (Part No. 11582, Qosina, Edgewood, USA), reusable pressure transducers and disposable pressure domes (SP854, Memscap, Norway) and air bubble sensors (customized sensor, Zevex, Salt Lake City, Utah, USA). The system housing was designed by Solid Works (Solid works, Concord, MA, USA) and

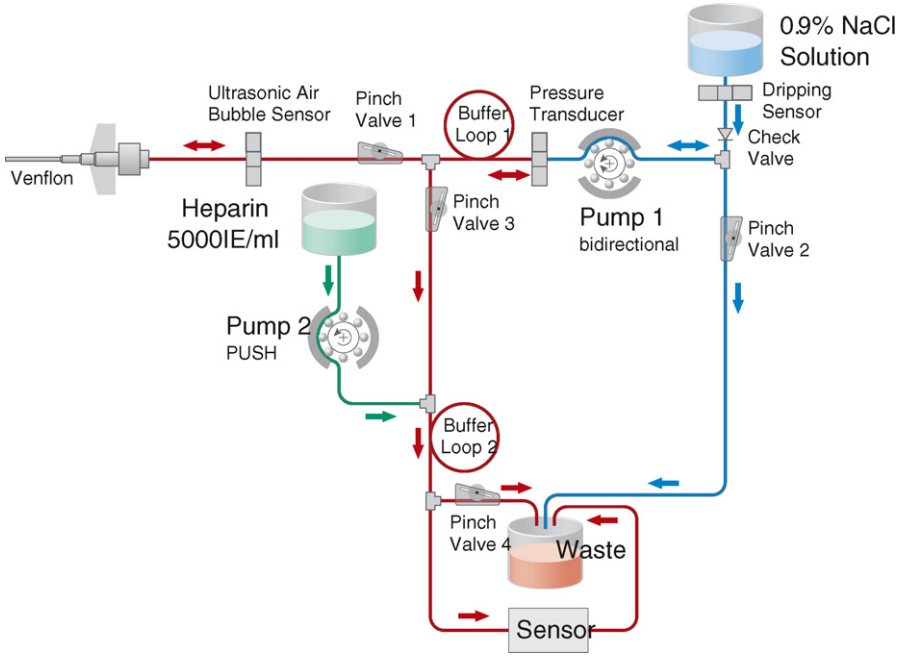

Fig. 1. AGM-system. *Construction*: The in-house produced tubing system was sterilised with ethylene oxide and afterwards completely flushed using saline solution attached to the system using a standard fluid administration set containing a drop chamber and a dripping sensor to monitor the fluid level. Flow was controlled using pinch valves compatible with the tubing used. A check valve was used to prevent reflux and to maintain the sterility of the saline solution. For safety reasons, pressure changes within the accessed vessel were monitored using a reusable pressure transducer and a disposable pressure dome. The maximum allowed pressure was set at 150 kPa which is comparable to the maximum pressure applied by infusion pumps. As well as this, an air bubble sensor was used to avoid accidental air infusion. Alarms were displayed both optically and acoustically. *Operation*: Blood (4.0 ml) was withdrawn at 10 ml min^{-1} using peristaltic pump 1 and stored in buffer loop 1. From this, 1.69 ± 0.09 ml were branched off, heparinised (final concentration 50 IE ml^{-1}) and afterwards transferred to loop 2 for storage and analysis using pump 2. The remaining non-heparinised blood was immediately re-infused into the subject to minimize blood consumption. The line was then flushed to avoid clotting. Closure of a pinch valve prevented the reflux of heparinised blood into the subject. The blood in buffer loop 2 was then uni-directionally pumped through the biosensor for 10 min at 20 μl min^{-1} and immediately discarded. The biosensor current was recorded every 10 s.

rapidly prototyped (FH OOE, Linz, Austria). All materials used (e.g. tubing) are approved for use in humans.

2.2. Glucose biosensor

In fundamental terms, the Senslab glucose biosensor consists of a micro-structured planar flow-through cell and a screen-printed amperometric thick film sensor coated with glucose oxidase (GOD). The sample flows along the elliptic flow channels and is drawn by capillary forces into the gap between the indicating window of the biosensor and the upper embossed area of the flow-through cell. Microair bubbles present in the analyte solution collect in the elliptic flow channels and are thus excluded from the sensitive area of the indicating window. Glucose is detected by a primary indicating reaction mediated by immobilised GOD, which catalyses the oxidation of glucose to glucono delta-lactone and hydrogen peroxide according to Eq. (1):

$$\text{D-(+)-}\beta\,\text{glucose} + O_2 + H_2O \xrightarrow{\text{glucose Oxidase(EC1.1.3.4)}} \text{D-glucono-1, 5-lactone} + H_2O_2 \quad (1)$$

The hydrogen peroxide generated is then quantified by a secondary electrochemical reaction at the amperometric sensor. Oxidation of hydrogen peroxide according to Eq. (2) at the surface of the working electrode caused by the application of a polarisation voltage of +400 mV with respect to the internal silver/silver chloride reference electrode generates an anodic electron flow that is directly proportional to the glucose content of the sample, which is measured by an ampere meter as part of the potentiostat.

$$2H_2O_2 \xrightarrow{\text{working electrode (anode)}} O_2 + 2H^+ + 2H_2O + 2e^- \quad (2)$$

A diffusion-controlled reaction rate was obtained by the application of a diffusion barrier layer.

The biosensor comprises a plastic support (Polycarbonate, ThyssenKrupp Schulte, Duesseldorf, Germany) with outer dimensions of 10 mm × 10 mm × 2 mm ($L \times W \times H$) on which three different polymeric carbon paste conducting paths are printed. In the area of the indicating window, formed later by the dielectric layer, a platinised carbon paste is overprinted on two of these three conducting paths to create working and counter electrodes, respectively. The third electrode is covered by a silver/silver chloride paste to create the reference electrode. In a final screen printing step, an isolating (dielectric) paste is overprinted to define the indicating window of the sensor. The total inner volume of the measuring chamber is 0.5 μl. The three electrode system consists of the working, counter and reference electrodes according to the potentiostatic principle. Approximately 31 units (0.5 μg) of GOD (Serva, Heidelberg, Germany) are immobilised on the surface of the working electrode using polycarbamoyl sulfonate (PCS; Prepolymer of Polycarbomoyl sulfonate, SensLab, Leipzig, Germany) as an entrapping matrix, after which siloprene (Fluka, Schnelldorf), Germany is deposited at the GOD-PCS matrix layer to form a diffusion barrier and protective layer (Fig. 2).

Prior to the present study we evaluated the precision, accuracy and long-term stability of the biosensor using diluted bovine serum containing 10 mmol l^{-1} glucose. Typical values obtained during these pre-investigations (unpublished data) were: accuracy: 5.0%, precision: 5.1% and long-term stability −0.02 mmol l^{-1} per hour (Fig. 1, Supplementary material).

2.3. Data acquisition

Data were acquired with a notebook computer using a DAQ-Card 6036-E 16-bit analogue/digital data acquisition PCMCIA card, an SC-2345 configurable connector and a data acquisition programme based on Lab VIEW® 7.0 (all from National Instruments Inc., Austin, TX, USA). Data were stored in an Excel worksheet (Microsoft Inc., Redmond, WA, USA). Biosensor data (1–100 nA) were acquired at 10 s intervals and stored after signal processing using a custom-made data logger containing a 12-bit analogue/digital converter (Disetronic, Burgdorf, Switzerland). Data were transmitted to a personal computer via an infrared interface, acquired using PC software (Disetronic, Burgdorf, Switzerland) and stored in an ASCII format.

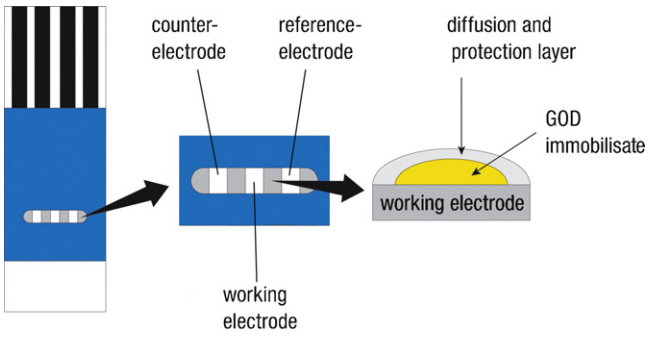

Fig. 2. Assembly of the biosensor showing immobilised GOD and diffusion/protective layer.

2.4. Subjects and protocols

The study, a 12-h open mono-centre trial at the Medical University of Graz, was approved by the local ethics committee and written informed consent was obtained from all participants. Eight suitable healthy volunteers (7 males and 1 female aged 28.88 ± 3.52 years with a body mass index of 25.11 ± 1.49 kg m^{-2}) were recruited and attended the trial centre at 8:00 a.m. in a fasting state. For reference blood sampling a catheter was inserted into a peripheral vein in the left forearm with controlled saline infusion to prevent occlusion. A second catheter was then placed in the same arm and connected to the ABS-system. The first blood sample was then taken manually for determination of the baseline activated partial thromboplastin time (APTT).

The study comprised of two parts. The objective of the first part (subjects 1–6) was to validate the ABS-system through a comparison with the manual sampling technique. In the second part (subjects 7 and 8), the complete AGM-system (ABS-system and online biosensor) was validated. Blood samples were generally withdrawn at 30 min intervals. Following oral administration of 75 g of glucose at 1:00 p.m. to modify the subjects' glucose concentration profiles, the rate of sampling was increased to four samples/hour for 3 h. The theoretical total blood consumption was 79.5 ml per volunteer.

2.5. Laboratory analysis, calibration of the biosensor and system, data analysis and statistics

Manually and automatically drawn blood samples were analysed using a laboratory glucose analyser (Omni S6, Roche, Basel, Switzerland). The ABS-system is designed to be operated without calibration. The online biosensor was calibrated before the start of each experiment using 5, 10, 15 and 20 mmol l^{-1} standard aqueous glucose solutions prepared by spiking ELO-MEL (ELO-MEL, Fresenius Kabi, Graz, Austria) with glucose (D(+)-Glucose, 108337, Merck KGaA, Darmstadt, Germany) according to good laboratory practice (GLP). The online biosensor current signal (95% of the peak value) was recalibrated 1 h after the start of the online measuring procedure using the reference blood glucose values. The agreement between the glucose concentrations obtained by automatic blood

withdrawal (with and without the biosensor) and those obtained with manually withdrawn blood was assessed by calculating the Pearson coefficient of correlation (Bland and Altman, 1986) and the system error (Lodwig and Heinemann, 2003). A tool for assessing the degree of clinical agreement between two methods (Clarke et al., 1987) was applied to the glucose values obtained with automatically sampled blood and those obtained from the corresponding manually drawn samples. The data were also compared using the recently published Insulin Titration Error Grid analysis Fig. 2, Supplementary material (Ellmerer et al., 2006).

3. Results

3.1. General

All eight volunteers successfully completed the study according to the study plan. The catheters were well tolerated throughout with no sign of local infection. For two subjects, the catheters were renewed due to permanent occlusion. From a total of 240 measurements made, only 12 were invalid due to catheter blockage, coagulation, insufficient sample volume or haemolysis (7 for the ABS-system and 5 for the complete AGM-system). The overall median frequency of successful sampling was 96.67 ± 5.33% per subject [range: 86.67–100.00%].

3.2. Calibration

The biosensor responded linearly to increasing glucose concentrations up to 20 mmol l^{-1} during in vitro calibration (Fig. 3). Base current and sensitivity of the sensor were typically 5 nA and 1.8 nA $mmol^{-1}$ glucose, respectively.

3.3. Correlation

The median Pearson coefficient of correlation between glucose levels determined with manually and automatically withdrawn blood samples was 0.982 [range: 0.928–0.997, subjects 1–6]. The corresponding value for manually withdrawn blood samples and glucose levels obtained fully automatically was 0.950 [range: 0.936–0.963, subjects 7 and 8]. Illustrative examples of the comparison of glucose concentration profiles obtained using (i) the ABS-system alone and (ii) the AGM-system with the respective profiles obtained using manually withdrawn blood samples are shown in Fig. 4.

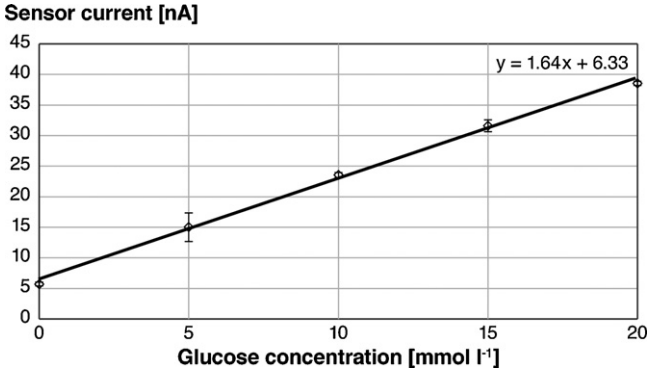

Fig. 3. Biosensor calibration curve. Standard glucose solutions (0, 5, 10, 15 and 20 mmol l^{-1}) were used for the construction of the biosensor calibration curve. The linear equation used was $y = 1.64x + 6.3$, $R = 0.9984$. The 95% response time was less than 2 min.

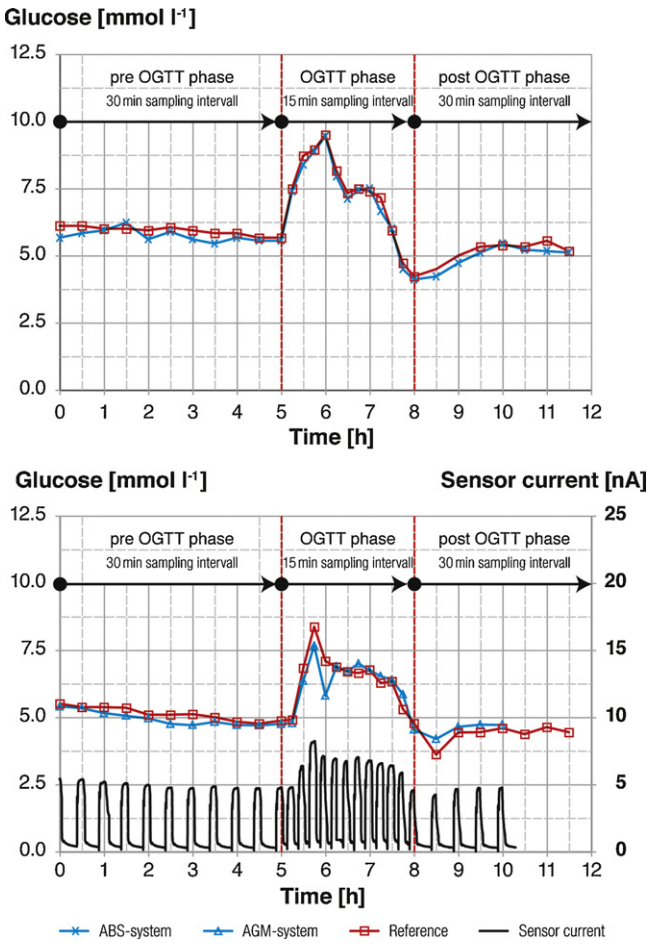

Fig. 4. Top: Comparison of glucose concentration profiles obtained for subject 03 with automatically (crosses) and manually (squares) withdrawn blood. Bottom: Comparison of glucose concentration profiles obtained for subject 7 using the AGM-system (triangles) with manually withdrawn reference blood samples (squares). Also shown are the corresponding chronoamperometric measurements obtained with the biosensor. The sensor current in nA (2nd *y*-axis) for each reading is displayed as a solid line.

3.4. System error

The system error is defined according to the following equation:

$$\text{system error} = \frac{\text{estimated value} - \text{reference value}}{\text{reference value}} \times 100 \qquad (3)$$

Fig. 5 shows the system error for subjects 1–8. The individual and mean system error ± the standard deviations are indicated for each subject. The calculated system error between the ABS-system and reference measurements was −1.66 ± 9.01% (subjects 1–6). For the complete AGM-system this was −1.00 ± 6.61% (subjects 7 and 8). The system error for subjects 7 and 8 was around zero due to the calibration of the ABS-system together with the sensor in the complete AGM-system.

In order to minimize unwanted coagulation, especially when sampling at 30 min intervals, a "Keep Vein Open" saline infusion (2 ml h^{-1}) was implemented into the ABS-system and found to reduce the frequency of blood withdrawal failures due to occluded catheters from 13.33% to 3.33%. The mean volume of saline used to flush the disposable tubing system of the ABS-system after each automatic blood withdrawal was 8.83 ± 0.28 ml. The total amount of flushing fluid used for the complete study was less than 270 ml.

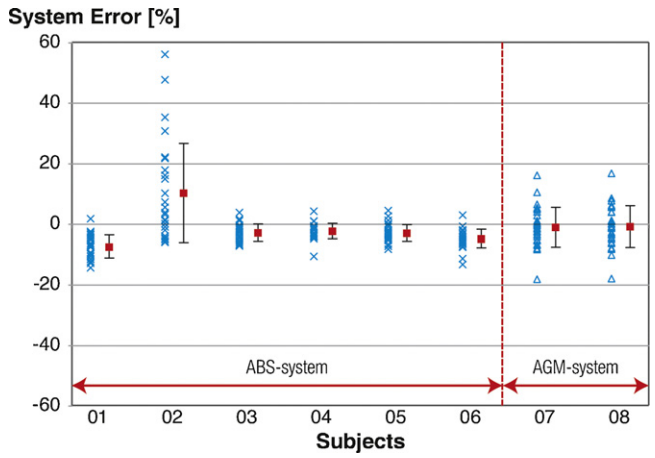

Fig. 5. Error graph for all subjects: subjects 1–6 (ABS); subjects 7 and 8 (ABS + biosensor). Crosses and triangles indicate data pairs of reference vs. ABS-system or reference vs. the complete AGM-system. Squares represent the mean values for each subject and the bars the respective standard deviations.

3.5. Clark Error Grid analysis

Clark Error Grid analysis (EGA) is a clinically oriented graphical method for the comparison of blood glucose data obtained with a novel method and with an established reference system. EGA is conducted by plotting sample data on a graph (estimated blood glucose value on the ordinate and the reference glucose value on the abscissa) divided into five zones, each representing a different degree of clinical acceptability as follows: A (accurate), B (acceptable), C, D and E (not acceptable). EGA thus categorizes individual glucose values obtained with the new method with respect to the corresponding reference values according to the possible clinical consequences of the inaccuracy of the investigated method.

EGA analysis of the study results is shown in Fig. 6. Of the 173 valid data pairs obtained by conventional glucose analysis of blood samples drawn with the ABS-system and with the reference method, 166 (95.95%) were in zone A and only 7 (4.05%) in zone B. Furthermore, of the 55 data pairs obtained for the complete AGM-system (i.e. ABS-system plus biosensor) and corresponding reference measurements, all were located in zone A. Thus, overall a total of 96.93% of the measurements were in zone A ("accurate") and only 3.07% in zone B ("acceptable"). This result is indicative of a high level of accuracy of both the ABS-system alone and in combination with the biosensor with respect to the manual state of the art procedure.

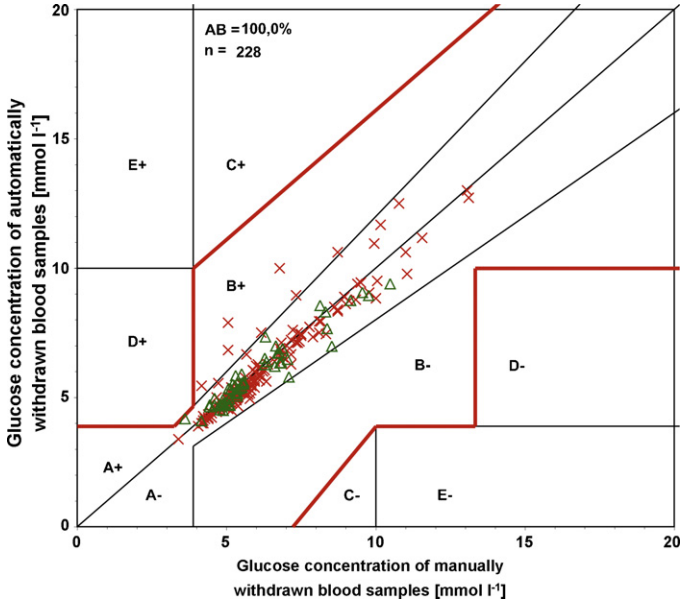

Fig. 6. Clark Error Grid analysis of glucose concentration data obtained using the ABS-system alone (subjects 1–6; crosses) and with the online biosensor (subjects 7 and 8; triangles); ABS: 173 data pairs, zone AB (100%); combined system: 55 data pairs, zone AB (100%).

3.6. Insulin Titration Error Grid analysis

Glucose measurements can also be clinically evaluated by Insulin Titration Error Grid analysis, which also involves the plotting of blood glucose concentrations for the reference method and the method under evaluation on the *x*- and *y*-axis, respectively. Glucose ranges on the *x*- and *y*-axis are related to major treatment interventions as suggested by the Leuven insulin titration guideline (Berghe, 2002). Different zones have been defined on the basis of these treatment actions, according to the severity of violation of the titration guidelines as: "appropriate treatment", "unacceptable violation", "major violation" and "life threatening treatment". A total of 99.56% of the 227 valid data pairs generated by the study were located in the acceptable treatment zone. The single data pair that lay outside of this zone was an erroneous measurement for subject 2 caused by dilution effects (and considered in more detail in Section 4).

3.7. Safety endpoints and adverse events

None of the subjects exhibited a statistically significant increase in APTT levels ($p = 0.05$), from which we conclude that unintended heparinisation of the subjects did not take place and that the use of heparin to avoid coagulation within the AGM-system is both effective and harmless. No adverse trial-related events occurred during the study.

4. Discussion

Intensive insulin therapy has been shown to benefit critically ill patients that develop hyperglycaemia and insulin resistance. The frequent blood sampling that is required can however conflict with other aspects of patient care. We present here a controlled clinical evaluation of a prototype automatic blood glucose monitoring system (the "AGM-system") comprising an automated blood sampling system (the "ABS system") coupled to a glucose biosensor (the "biosensor"). Our goal was to examine the clinical suitability and accuracy of this novel system. Eight volunteers participated in the evaluation. The performance of the ABS-system was studied with six of these and the performance of the complete AGM-system with two subjects.

A key feature of the AGM-system is that sampling from a peripheral vein is carried out using a single lumen catheter that is available in every hospital. Unlike other products which use double lumen catheters (DLC) the AGM-system can thus be used without the need for additional expenditure in this regard. Medical personnel are moreover well acquainted with such catheters whereas a DLC needs special handling, particularly if the inner lumen is inserted or removed. The performance of the ABS-system's catheter was improved fourfold by implementing a Keep Vein Open (KVO) saline infusion at a rate of 2 ml h^{-1}. Despite this however, the peripheral venous catheter might represent a weak link in the system during long-term use due to coagulation. The use of a central venous or arterial catheter could avoid this and so improve the long-term stability of the system and would also be feasible in

intensive care patients with whom central catheters are commonly used.

An excellent degree of correlation was observed between glucose concentrations obtained with manually drawn reference samples and those taken with the ABS-system (median Pearson correlation coefficient 0.982, range 0.928–0.997, system error $1.66 \pm 9.01\%$). Glucose values obtained fully automatically with the AGM-system also exhibited a very high degree of correlation with those obtained by conventional analysis of manually drawn blood samples (median Pearson correlation coefficient 0.950, range 0.936–0.963, system error $1.00 \pm 6.61\%$). On the basis of these correlation data the AGM-system could in principle be used to drive an algorithm for insulin infusion and enable tight, fully automated glycaemic control.

The amount of blood consumed by the ABS-system was consistently low (1.69 ± 0.09 ml per withdrawing procedure). On the basis of a survey performed at the Medical University of Graz that calculated that the maximum amount of blood that can be withdrawn from patients in the intensive care unit (ICU) is 50 ml per day, this would allow on average hourly glucose measurements of ICU patients to be made. More frequent glucose determinations would be enabled by reducing the diameters and lengths of the system's disposable tubing system to reduce the amount of blood consumed per measurement.

A notable feature of the initial results obtained for subject 2 was that the glucose concentrations in the manually drawn reference samples were generally lower than those obtained with automatically sampled blood (see Fig. 5). This was found to be due to the location of the catheters for both manual and automatic blood withdrawal in the same forearm to allow patient's comfort and thus in the same venous plexus. At the beginning samples were taken with the ABS-system before manual withdrawal. We reasoned that because of this, the flushing fluid from the sampling unit was afterwards withdrawn with the manual reference samples, leading to sample dilution. This was confirmed by the finding that after synchronising the withdrawing and reinfusion procedure for both systems from time point 9 h onwards to avoid interaction between them, both system error and correlation improved (see Fig. 5). This modified procedure was used for all remaining subjects.

The clinically oriented and highly visual Clark Error Grid analysis is the most commonly used method for the comparison of glucose values. This revealed that 96.93% (221) of the data points obtained in the study were located in zone A (accurate treatment) and 3.07% (7) in zone B (acceptable treatment). The data were also analysed using Insulin Titration Error Grid analysis which confirmed the conclusion drawn from EGA: 99.56% (227) of the data pairs suggested an acceptable treatment whereas 0.44% (1) suggested an unacceptable violation. The latter data pair was one of those measured in subject 2 where the ABS-system biased the reference measurements by causing dilution of the corresponding manually withdrawn samples.

Taken together, our results thus demonstrate that the AGM-system would be suitable for use in a variety of clinical applications, in particular in ICUs to enable the frequent blood sampling necessary to maintain tight glycaemic control in critically ill hyperglycaemic patients, with significantly less staff effort than is currently required. Although many systems have already been introduced to the market, these have been found to be unsuitable for use in a clinical setting due to their long set-up time. The AGM-system, in contrast, could always be assembled in less than 5 min, which is comparable to the time required to set-up a standard infusion pump. The system moreover incorporates acoustic and optical alarms to alert staff to required interventions. Thus the time saved by the system is not offset by a demanding and extended set-up time and a high level of maintenance.

Finally, the constant APTT levels that were observed throughout the study confirm that the use of the AGM-system did not cause unintended systemic heparinisation and support its use in ICUs.

5. Conclusions

In conclusion, this study demonstrated the feasibility of automated real time ex vivo glucose determination. Following appropriate calibration the amperometric biosensor used to quantify glucose in blood, delivered glucose values closely matching those determined by standard analysis methods. Permanent flushing of the catheter reduced the number of occlusions fourfold. We view the peripheral catheter as the weakest link in the current system however and future work will examine its performance together with a central venous or arterial catheter. Biosensors from other manufacturers should also be examined. The amount of blood consumed by the system per sampling currently imposes a ceiling of on average hourly measurements in ICU patients. A reduction in the dimension of the disposable tubing system will decrease the amount of blood per withdrawal and so allow more frequent measurements.

The AGM-system presented here could be a valuable device in situations in which tight glyceamic control is required, especially in ICUs and/or after major surgery. It could also be a valuable research tool for clinical investigations requiring real time glucose monitoring.

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Thanks to my love Michèle Ammann for supporting me.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.11.029.

References

Berghe, G., Wouters, P., Weekers, F., Verwaest, C., Bruyninckx, F., Schetz, M., Vlasselaers, D., Gerdinande, P., Lauwers, P., Bouillon, R., 2001. N. Engl. J. Med. 345, 1359–1367.
Berghe, G., 2002. Int. J. Obes. 26 (Suppl. 3), 3–8.
Bland, J.M., Altman, D.G., 1986. Lancet 327 (8476), 307–310.
Caduff, A., Hirt, E., Feldman, Y., Ali, Z., Heinemann, L., 2003. Biosens. Bioelectron. 19 (3), 209–217.
Clarke, W.L., Cox, D., Gonder-Frederick, L.A., Carter, W., Pohl, S.L., 1987. Diabetes Care 10 (5), 622–628.
Ellmerer, M., Haluzik, M., Blaha, J., Kremen, J., Svacina, S., Toller, W., Mader, J., Schaupp, L., Plank, J., Pieber, T.R., 2006. Diabetes Care 29 (6), 1275–1281.
Ganesh, A., Hipszer, B., Loomba, N., Simon, B., Torjman, M.C., Joseph, J., 2008. J. Diabetes Sci. Technol. 2 (2), 182–193.
Garg, S., Zisser, H., Schwartz, S., Bailey, T., Kaplan, R., Ellis, S., Jovanovic, L., 2006. Diabetes Care 29 (1), 44–50.
Gfrerer, R.J., Brunner, G.A., Trajanoski, Z., Schaupp, L., Sendlhofer, G., Skrabal, F., Jobst, G., Moser, I., Urban, G., Pieber, T.R., Wach, P., 1998. Biosens. Bioelectron. 13 (12), 1271–1278.
Heise, H.M., Damm, U., Bodenlenz, M., Kondepati, V.R., Köhler, G., Ellmerer, M., 2007. J. Biomed. Opt. 12 (2), 024004.
Hovorka, R., Canonico, V., Chassin, L.J., Haueter, U., Massi-Benedetti, M., Orsini-Federici, M., Pieber, T.R., Schaller, H., Schaupp, L., Vering, T., Wilinska, M.E., 2004. Physiol. Meas. 25 (4), 905–920.
Jungheim, K., Wientjes, K.J., Heinemann, L., Lodwig, V., Koschinsky, T., Schoonen, A.J., 2001. Diabetes Care 24 (12), 2030–2034.
Lodwig, V., Heinemann, L., 2003. Diabetes Technol. Ther. 5 (4), 573–587.
Mastrototaro, J., 1999. J. Pediatr. Endocrinol. Metab. 12 (Suppl. 3), 751–758.
McCowen, K.C., Malhotra, A., Bistrian, B.R., 2001. Crit. Care Clin. 17 (1), 107–124.
Ricci, F., Caprio, F., Poscia, A., Valgimigli, F., Messeri, D., Lepori, E., Dall'Oglio, G., Palleschia, G., Moscone, D., 2007. Biosens. Bioelectron. 22 (9–10), 2032–2039.

Tierney, M.J., Tamada, J.A., Potts, R.O., Jovanovic, L., Garg, S., 2001. Biosens. Bioelectron. 16 (9–12), 621–629.
Trajanoski, Z., Brunner, G.A., Schaupp, L., Ellmerer, M., Wach, P., Pieber, T.R., Kotanko, P., Skrabal, F., 1997. Diabetes Care 20 (7), 1114–1121.
Weinstein, R.L., Schwartz, S.L., Brazg, R.L., Bugler, J.R., Peyser, T.A., McGarraugh, G.V., 2007. Diabetes Care 30 (5), 1125–1130.
Weller, C., Linder, M., Macaulay, A., Ferrari, A., Kessler, G., 1960. Ann. NY Acad. Sci. 87, 658–668.