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Portable device for continuous fractionated blood sampling and continuous *ex vivo* blood glucose monitoring

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Abstract: The objective of the study was to develop and evaluate a portable device for continuous fractionated blood sampling and continuous *ex vivo* monitoring of blood glucose. The inner lumen of a double lumen catheter (18 gauge × 45 mm) was placed in a peripheral vein and perfused with heparin solution (1·4 U min^{-1}). The outer lumen was used to collect heparinized blood into 48 vacuum tubes at programmable sample volumes and time intervals (0·2–2 ml in 2·5–30 min). A sensor flow chamber with an internal volume of 1 mm^3 incorporating a miniaturized thin-film amperometric glucose sensor was placed in the sampling line for continuous *ex vivo* blood glucose monitoring. Blood glucose and plasma insulin were measured during a frequently sampled intravenous glucose tolerance test (250 mg kg^{-1}) and a subsequent oral glucose tolerance test (150 g) over 6 h in eight healthy volunteers (BMI 24·5 ± 3·2 kg m^{-2}). Additionally, in four experiments blood glucose was measured on-line using the glucose sensors. The overall correlation coefficients for whole blood glucose and plasma insulin between the manually drawn samples and the vacuum tubes were 0·73 and 0·87, respectively ($p < 0·001$). The miniaturized glucose sensor exhibited a linear measuring range of 25 $mmol^{-1}$ glucose concentration and 95% response times of less than 30 s. Sensor readings and laboratory analyser results for the blood glucose measurement correlated between 0·93 and 0·98 ($p < 0·001$). In summary, continuous fractionated blood sampling and *ex vivo* blood glucose monitoring in ambulatory subjects is possible with a portable device.
Keywords: blood glucose measurement, glucose sensors, biochemical monitoring

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INTRODUCTION

For many routine or research investigations in medicine, especially in endocrinology, physiology and pharmacology, repeated blood samples of ambulatory subjects or animals are necessary for obtaining hormonal or metabolic profiles. However, the necessity of the presence of skilled medical staff for drawing the blood samples, restricted movement or disturbed sleep of the subject under investigation can be limiting factors in these studies. Moreover, in some cases continuous blood sampling is desirable in order to obtain an integrated profile (area under the curve) of the substances of interest. A portable device with preset or programmable sample volumes and/or sample times for continuous blood sampling would therefore be of great benefit. Additionally, a blood sampling device which also enables on-line blood glucose measurement could be advantageous in a number of clinical situations where permanent glucose control is required, like intensive care monitoring or hypoglycaemia studies. In 1974, Albisser *et al.* (Albisser *et al.*, 1974) presented an artificial endocrine pancreas which used a double lumen catheter and extracorporeal heparinization for blood glucose analysis. A portable device for continuous extracorporeal blood glucose measurement has also been developed and successfully applied (Brückel *et al.*, 1990). However, due to the specific purpose and consequent design-based restrictions like small sampling volumes and one waste container without cooling, these devices are not adequate for blood sampling and off-line analysis of glucose, hormones or metabolites.

The objective of the present study was twofold. First, to develop and evaluate a portable device for continuous fractionated blood sampling in ambulatory subjects (continuous ambulatory blood sampler (CABS)). Second, to investigate the possibility of extending the function of this device to continuous *ex vivo* blood glucose monitoring using flow chamber with amperometric glucose sensors in the sampling line.

SUBJECTS, MATERIALS AND METHODS

Continuous ambulatory blood sampler—CABS

Figure 1 shows the architecture of the continuous ambulatory sampler. The sampling method is based on a double lumen catheter for extracorporeal blood heparization and an automatic fluid sampler. The catheter (18 gauge × 45 mm, B. Braun Melsungen AG, Melsungen, Germany) was placed in a peripheral vein. The inner lumen of the double lumen catheter was connected to a 10 ml syringe containing heparin solution (2000 U Novo Nordisk, Bagsvaerd, Denmark). Heparin was infused at a rate of 5·5 U min^{-1} by adjusting the speed of the piston pump (Fig. 1) at 22·2 μl min^{-1}. For pharmacological studies two additional syringes were built to enable drug infusion at freely progammable speed either *via* the double lumen catheter or *via* a second catheter. The outer lumen of the double lumen catheter was connected to a roller pump with programmable speed. A needle which can be moved in two directions supplied 56 vacuum tubes (2 ml tubes in three rows in a rotating sample cartridge) with the collected blood. The vacuum tubes (Vacutainer, Becton Dickinson, Rutherford, NJ, USA) were prefilled with 2 mg NaFl per 2 ml blood dissolved in 0·1 ml 0·9% NaCl. The sampling volume and sampling time for 48 tubes were adjustable between 0·2 and 2 ml and between 2·5 and 30 min, respectively (Table 1). Any other equidistant sampling time within the range could be set by program downloading from a personal computer. Eight additional tubes were reserved for special events

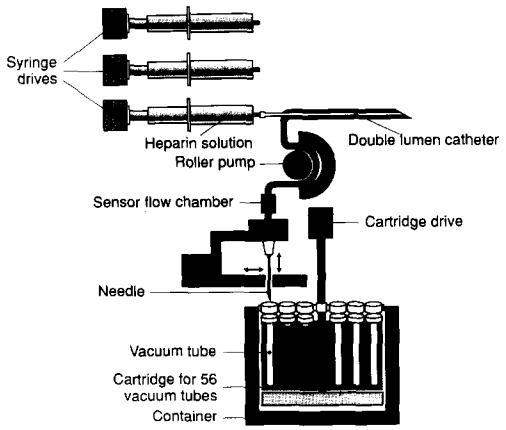

Fig. 1. Schematic representation of the portable continuous ambulatory blood sampling device (CABS). The heparin solution is transported to the tip of the inner lumen of the catheter and the heparinized blood is collected through the outer lumen. The speed of the roller pump is programmable between 0·0067 and 0·8 ml min^{-1}.

TABLE 1 Preset programs for the continuous ambulatory blood sampler (CABS).

Number	*Sample times (min)*	*Sample volumes (ml)*
1	2·5	0·2, 0·5, 1, 1·5, 2
2	7·7	0·2, 0·5, 1, 1·5, 2
3	15	0·2, 0·5, 1, 1·5, 2
4	30	0·2, 0·5, 1, 1·5, 2

and could be filled immediately on demand using a fixed pump rate (2 ml in 2·5 min). The roller pump was programmed to operate continuously at any rate except for the short period of 10 s which was set to enable the movement of the needle to the next vacuum tube.

The sample cartridge was placed in a container which was precooled with a cool pack and which kept the temperature at 4°C for at least 24 h. In order to control the temperature during the operation of the device a temperature sensor was placed in the container. An analogue digital converter (ADC) enabled sensor readings of the temperature sensor and of an additional sensor with a sampling time of 1 min over 24 h. Both sensors were displayed on an LCD. An RS 232 serial interface was used to download additional programs to the sampler by an IBM compatible personal computer and to upload the sensor data. The display, the serial interface, the ADC, the drives of the syringes, of the needle feeding the vacuum tubes, of the rotating cartridge and of the roller pump were all controlled by a built-in microcontroller.

The complete device including the cooling, the battery (9·6 V 100 mAh) and the cartridge weighs 3 kg and measures 30 × 15 × 20 cm.

Miniaturized thin-film glucose sensor

A scheme of the glucose sensors with integrated flow cell is given in Fig. 2. The internal volume in the chamber was 1 mm^3. The miniaturized thin-film glucose sensor used in this study has been described previously (Urban *et al.*, 1992). Briefly, an electrochemical glucose sensor was produced on a glass carrier by means of thin-film technology. The glucose sensor is based on the measurement of H_2O_2 which is produced by the membrane-entrapped enzyme glucose oxidase (GOD). In order to minimize electrochemical

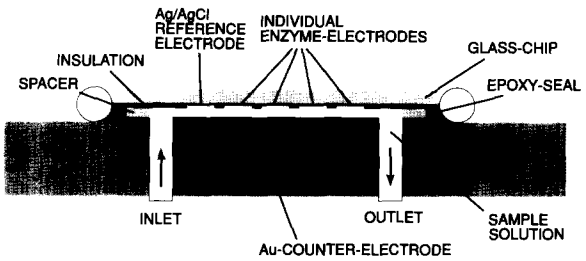

Fig. 2. The sensor flow chamber (dimension 5 × 1 ×0·1 mm) which was serially placed in the sampling line of the CABS. Up to four enzyme electrodes (each 0·5 × 0·5 mm) can be used within the flow chamber.

interferences an electrode configuration was designed to perform differential measurements. A triple layered pHEMA (poly 2-hydroxyethyl methacrylate) membrane setup was realized comprising a GOD membrane, a diffusion limiting membrane and an outer membrane containing catalase. This enzyme decomposed the H_2O_2, which was not consumed by a Pt working electrode, into O_2. The enzymes were immobilized by physical entrapment in these photolithographically patterned hydrogels.

The electrode structure consists of four working electrodes (area 0·25 mm^2 each) and an Ag/AgCl reference electrode. This sensor chip is assembled with a printed circuit board comprising a gold counter electrode and a 200 μm thick seal to form the flow chamber (Fig. 2). The Pt working electrode was polarized to +500 mV *vs.* the Ag/AgCl electrode. An analogue circuit was used to transform and amplify the sensor signals. In order to enable sampling with a higher frequency, the outputs of this circuit were connected to an ADC placed in a portable personal computer. The signals were sampled with a sampling frequency of 0·1 Hz and filtered by a second order Butterworth filter with a cut-off frequency of 0·02 Hz.

Subjects and protocols

The study group consisted of eight young healthy male subjects (age 25·6 ± 2·2 years, body mass index 24·5 ± 3·2 kg m^{-2}, means ± SD). The study was approved by the local ethical committee and informed consent was given by all subjects. After a 12 h overnight fast the subjects assumed the supine position over 6·5 h and a frequently sampled intravenous glucose tolerance test (FSIVGTT) followed by an oral glucose tolerance

test (OGTT) was then performed. After a 30 min rest, at time −15 min the double lumen catheter was inserted in the left antecubital vein and was connected to the CABS device. An 18 gauge cannula was then placed into the contralateral antecubial vein for manually drawing blood samples. The patency of the cannula was maintained with a controlled saline infusion throughout the study. A butterfly needle (22 gauge) was inserted in the left dorsal hand vein for the injection of glucose and insulin. Two hundred and fifty milligrams per kilogram of glucose was injected as 40% solution at time zero and 0·02 U kg^{-1} regular insulin (Actrapid MC, Novo Nordisk, Bagsvaerd, Denmark) was injected 20 min later. At time 180 min, 150 g glucose was given orally. During the FSIVGTT 2 ml blood samples were drawn according to a published protocol (Welch *et al.*, 1990) and during the OGTT every 20 min. In all experiments the sampling volume and interval of the CABS device were adjusted to 1·5 ml and 7·5 min. The delay time due to the tubing system at this flow rate (0·2 ml min^{-1}) was 30 s. The sampling time for the sensor reading using the external ADC was 10 s.

In all experiments whole blood glucose was measured from the manually drawn samples and from the vacuum tubes using glucose dehydrogenase (Granutest 250, Diagnostika Merck, Darmstadt, Germany) whereas plasma insulin was measured using radioimmunoassay (Pharmacia Insulin RIA, Kabi Pharmacia Diagnostics AB, Uppsala, Sweden) from the manually drawn samples. In four experiments additional measurements of plasma insulin in the vacuum tubes and of blood glucose using the glucose sensors were made. Before each measurement the glucose sensors were calibrated in protein free calibrating solutions. Since the sensor measurements corresponded to plasma glucose and plasma glucose is 10% higher than whole blood glucose, the sensor values were divided by a factor of 1·10 as shown in a recent study (Hashiguchi *et al.*, 1994). The filtered and corrected values from the glucose sensors in each experiment were considered for the calculations.

Statistical analysis

Results are expressed as means ± SD. Linear regression analysis was performed by the method of least squares. The relationship between variables were estimated with Pearsons's correlation coefficients. The clinical significance of the estimation of glucose concentration measured by the glucose sensors was examined by using the Error Grid Analysis proposed by Clarke *et al.* (1984). Briefly, the grid is divided into five zones representing clinically based different degrees of accuracy. Values in zone A are accurate, in zone B acceptable, and in zone C, D or E unacceptable. All calculations were carried out on an IBM compatible personal computer using MATLAB (Mathworks Inc., Natick, MA, USA).

RESULTS

Figures 3 and 4 show whole blood glucose and plasma insulin concentrations measured from the manually drawn samples and from the vacuum tubes, respectively. The fasting glucose concentration averaged (±SD) 4·5 ± 0·5 mmol l^{-1}. After rapid intravenous injection the total glucose concentration rose to 13·0 ± 2·1 mmol l^{-1} in the manually drawn samples and to 9·5 ± 1·4 mmol l^{-1} in the CABS samples. The overall correlation coefficients for whole blood glucose and plasma insulin between the manually drawn samples and the CABS samples were 0·73 and

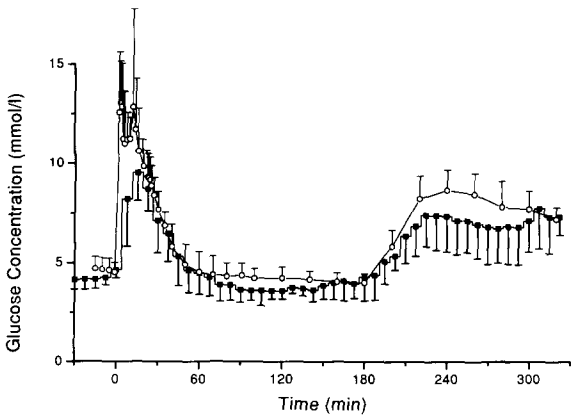

Fig. 3. Blood glucose concentration measured in healthy subjects using the CABS device during an intravenous glucose tolerance test and an oral glucose tolerance test. At time zero 250 mg kg^{-1} glucose and at time 20 min 0·02 U kg^{-1} insulin were injected intravenously. One hundred and fifty grams of glucose were given orally at 180 min. (○) Manually drawn samples (n = 8); (■) CABS vacuum tubes (n = 8). Results are expressed as means ± SD.

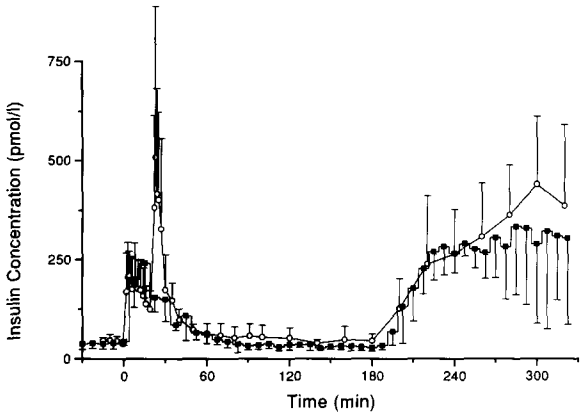

Fig. 4. Plasma insulin concentration measured in healthy subjects (n = 4) *using the CABS device during an intravenous glucose tolerance test and an oral glucose tolerance test. At time zero 250 mg* kg^{-1} *glucose and at time 20 min 0·02 U* kg^{-1} *insulin were injected intravenously. One hundred and fifty grams of glucose were given orally at 180 min. (○) Manually drawn samples; (■) CABS vacuum tubes. Results are expressed as means ± SD.*

0·87, respectively ($p < 0·001$). The parameters of the linear regression analysis for glucose and insulin between the manually drawn samples and the vacuum tubes for each individual experiment are given in Tables 2 and 3, respectively.

The calibration lines of a glucose sensor used in one experiment (experiment no. 4) are given

TABLE 2 Parameters of linear regression between glucose concentrations in the manually drawn samples and in the vacuum tubes*.

Experiment no.	*Slope* a	*Intercept* b	*Correlation coefficient* r†
1	0·78	1·08	0·99
2	0·66	1.45	0·86
3	0·88	0·62	0·97
4	1·00	0·18	0·83
5	0·78	0·40	0·93
6	0·62	0·90	0·83
7	0·85	0·17	0·96
8	1·08	0·30	0·98

*The relationship between glucose concentration in the manually drawn samples (x) and in the vacuum tubes (y) is assumed to have the form $y = ax + b$, where a is the slope and b is the intercept.
†$p < 0·001$.

TABLE 3 Parameters of linear regression between insulin concentrations in the manually drawn samples and in the vacuum tubes*.

Experiment no.	*Slope* a	*Intercept* b	*Correlation coefficient* r†
1	0·97	15·83	0·84
2	0·84	13.47	0·89
3	0·69	3·61	0·96
4	1·00	28·33	0·82

*The relationship between insulin concentration in the manually drawn samples (x) and in the vacuum tubes (y) is assumed to have the form $y = ax + b$, where a is the slope and b is the intercept.
†$p < 0·001$.

in Fig. 5. The miniaturized glucose sensors exhibited a linear measuring range of 0–25 mmol l^{-1} glucose concentration and 95% response times of less than 30 s (Urban *et al.*, 1992, data not shown). Blood glucose concen-

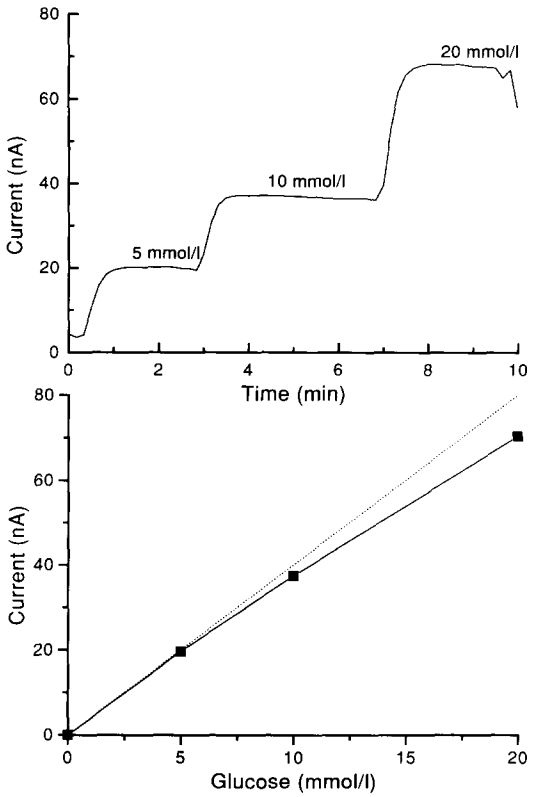

Fig. 5. In vitro *calibration curves of a glucose sensor used in experiment no. 4.*

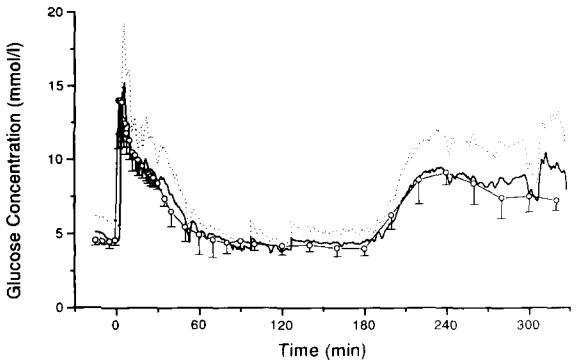

Fig. 6. Blood glucose concentration measured in healthy subjects using the glucose sensors in the CABS sampling line during an intravenous glucose tolerance test and an oral glucose tolerance test. At time zero 250 mg kg^{-1} glucose and at time 20 min 0·02 U kg^{-1} insulin were injected intravenously. One hundred and fifty grams of glucose were given orally at 180 min. Solid line: mean of the glucose sensor measurements. Dotted line: SD of the glucose sensor measurement. (○) Manually drawn samples, mean ± SD (n = 4).

tration measured off-line from the manually drawn samples and the on-line sensor measurement are given in Fig. 6. The total glucose concentration measured by the glucose sensors rose to 15·2 ± 5·7 mmol l^{-1}. The results of the linear regression between glucose concentration and sensor measurements and the correlation coefficients for each subject are given in Table 4. Sensor reading and laboratory analyser results for the blood glucose measurement correlated between 0·93 and 0·98 ($p < 0·001$). Analysis of the results through the Error Grid Analysis of the glucose sensor measurements for each experiment are shown in Table 5. Figure 7 represents the results of the Error Grid Analysis in all experiments. Ninety point four per cent of the blood glucose estimations by the glucose sensors were in zone A or B (accurate or acceptable) and 9·6% in zones C, D or E (unacceptable).

TABLE 4 Parameters of linear regression between manually drawn glucose concentrations and sensor signals*.

Experiment no.	*Slope* a	*Intercept* b	*Correlation coefficient* r†
1	0·67	1·10	0·95
2	1·00	0.69	0·97
3	1·61	−0·28	0·93
4	0·61	1·38	0·98

*The relationship between glucose concentration (x) and sensor signal (y) is assumed to have the form $y = ax + b$, where a is the slope and b is the intercept. †$p < 0·001$.

DISCUSSION

A fully automatic continuous blood sampling device would be advantageous not only in diabetological investigations, but also in a variety of other clinical situations. In order to be able to investigate a greater number of subjects under normal and pathological conditions, such an apparatus should fulfil several prerequisites. Ideally, it should be robust, reliable, easy to use and portable. In this study, a device for continuous blood sampling which meets these requirements and which can be furthermore extended for continuous blood glucose monitoring is presented.

Briefly, the device combines a double-lumen catheter which is perfused with heparin solution and a rotating sample cartridge for collecting blood in vacuum tubes. For continuous blood glucose monitoring a sensor flow chamber with amperometric thin-film sensors can be inserted in the sampling line. The whole system was evaluated in healthy subjects during an intravenous and an oral glucose tolerance test. The results of this study indicate the following.

First, continuous blood sampling in ambulatory subjects is feasible with a portable device. As shown in Figs. 3 and 4, the measured glucose and insulin concentration in the vacuum tubes closely followed the manually drawn values. However, it should be pointed out that the sampling interval of the CABS device in these experiments was adjusted to 7·5 min in order to achieve equidistant sampling times over 6 h. Thus, the rapid changes of glucose and insulin after intravenous injection could not be followed by the device. In investigations where rapid changes are of interest, one can use either smaller sampling times (Table 1), or a custom-defined non-equidistant sampling which can be easily obtained by reprogramming the built-in microcontroller. Nevertheless, the chosen sampling intervals and volumes are suitable for the majority of standard clinical applications. For example, the maximum blood volume which can be collected

TABLE 5 Error Grid Analysis of the four individual experiments in which blood glucose was continuously measured using glucose sensors*.

Experiment no.	*Zone A*	*Zone B*	*Zone C*	*Zone D*	*Zone E*
1	71·8	28·2	0	0	0
2	89·8	5·1	0	5·1	0
3	10·3	58·9	7·7	23·1	0
4	74·3	23·1	0	2·6	0
Mean ± SD	61·5 ± 35·1	28·8 ± 22·4	1·9 ± 3·8	7·7 ± 10·5	0

*The percentage of points (n = 39) lying in the corresponding zones is indicated.

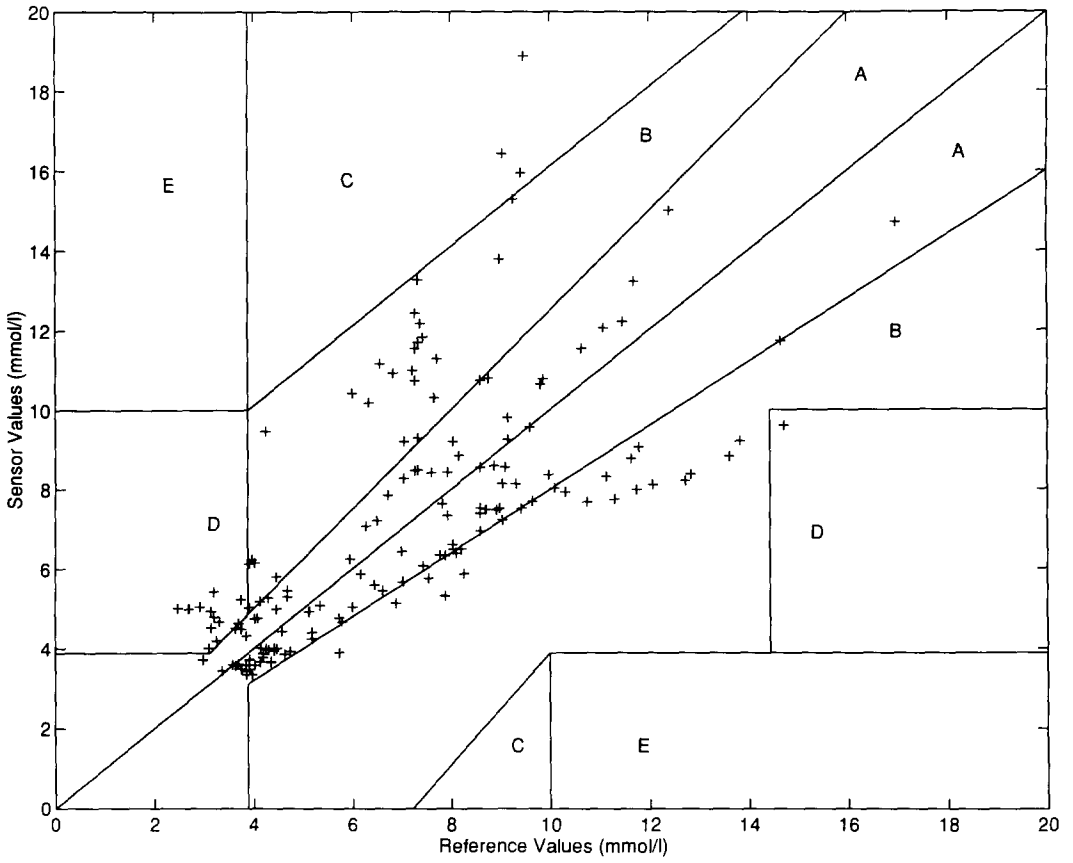

Fig. 7. Error Grid Analysis of the sensor data in four experiments. The corresponding sensor readings are plotted against the actual blood glucose measured from the manually drawn samples. In total, 61·6% of the points were in zone A (accurate), 28·8% in zone B (acceptable) and 1·9, 7·7 and 0% in zones C, D and E (unacceptable), respectively.

is sufficient for the analysis of glucose and all hormones of interest in diabetological studies (insulin, glucagon, epinephrine, norepinephrine, cortisol and growth hormone) from a single vacuum tube. However, in studies in which complete insulin and counter-regulation hormone profiles are needed, the vacuum tubes must be prefilled with EDTA and trasylol (Bayer Austria Ges.m.b.H, Vienna, Austria) in order to prevent hormone degradation (Eisentraut *et al.*, 1968). Note, the error associated with blood dilution due to the heparin infusion is rather small. In

the worst case, assuming that the complete heparin solution amount is recollected in the vacuum tubes the dilution is less than 2·8% for the experiments performed (blood sampling rate of 0·2 ml min^{-1} and infusion rate of the heparin solution of 22·2 μl min^{-1}).

The major difficulties which can occur during blood sampling are due to catheter problems. To prevent obstruction of the catheter, the inner lumen of the double-lumen catheter should be manufactured precisely so that the tip of the inner lumen ends short of the end of the outer lumen catheter. In experiments in which the length of the inner lumen was not suitable (data not shown), the double-lumen catheter had to be removed and replaced. It is also necessary to insert carefully the inner lumen and to avoid bending or twisting. However, once carefully inserted, the catheter can be used for continuous sampling for up to 24 h, which was observed in addition *in vivo* experiments.

Second, blood glucose can be monitored *ex vivo* in a continuous manner using the system presented. The design of the miniaturized glucose sensors enables fast response times and a wide linear measuring range (Urban *et al.*, 1992). These glucose sensors typically show base currents of less than 1 nA and sensitivities of 4 nA $mmol^{-1}$ glucose (Urban *et al.*, 1994). Variations in flow velocity did not cause signal variations. However, due to the small internal volume of the sensor chamber, for flow velocities greater than 0·2 ml min^{-1} haemolysis was observed. Since insulin measurement is influenced by haemolysis (Ziegler *et al.*, 1972) in this case, it would be necessary to use a shunt line with a waste container in order to reduce the flow rate. Another advantage of a system with a shunt line is that insertion of other flow chambers or recalibration of the sensors without interrupting the operation of the CABS device would be possible.

In the clinically oriented data analysis proposed by Clarke *et al.* (1984), the majority of the points lying in unacceptable regions were measured in one experiment (experiment no. 3, Table 5). Since in this experiment a constant deviation of 2·2 mmol l^{-1} between sensor values and manually drawn samples was observed in steady state, and previous *in vitro* experiments demonstrated excellent performance of the glucose sensors in buffer solution, plasma and whole blood (Urban *et al.*, 1992), the inaccuracy in this case is probably due to a calibration error. After subtracting 2·2 mmol l^{-1} from the sensor readings 59·0, 38·4 and 2·6% of the points were in zone A, B and C, respectively. Hence 97·5% of the points in all experiments were in zones A and B (accurate and acceptable). This indicates that the presented *ex vivo* monitoring system enables a reliable on-line estimation of blood glucose. Although in this study only glucose sensors were used, the sensor flow chamber enables the insertion of other miniaturized sensors. For example, lactate, glucose, glutamate, pH, pO_2, and pCO_2 sensors (Urban *et al.*, 1994) can be used in the same chamber, thus enabling a continuous multi-parametric *ex vivo* monitoring in blood. In applications in which only on-line measurement using biosensors is required, the sampling volume can be adjusted to the minimal value (Table 1) to avoid concomitant loss of blood cells and proteins.

In conclusion, this study describes a novel device for continuous blood sampling and continuous *ex vivo* blood glucose monitoring. The presented device could probably be a valuable tool in routine clinical applications as well as in various research investigations in which on-line biochemical monitoring or off-line analysis of substrates, metabolites or hormones is required.

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