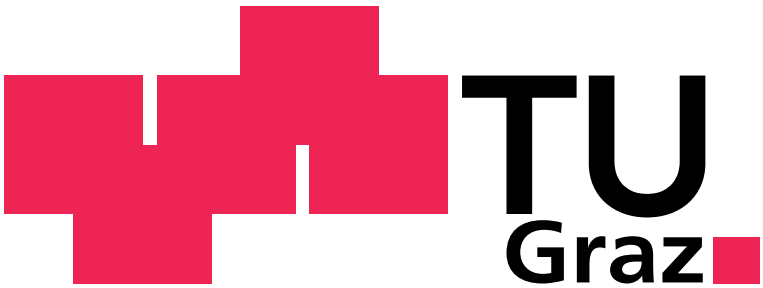
Andreas HUBER

Continuous Blood Glucose Monitoring in Humans combining Intravenous Microdialysis and Ionic Reference Technique

Diploma Thesis



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Abstract

The International Diabetes Federation expects a rise from 366 to 552 million people suffering from diabetes by 2030 [3]. To increase their quality of life, new and more efficacious insulin types are developed. Before these types are being launched on the market, their efficacy to lower blood glucose has to be tested in clinical studies using the glucose clamp technique. To reduce costs caused by these labour- and personal-intensive manual clamp studies, the industry is looking for an automated system. The first stage of such a system is a reliable measurement of plasma glucose. The primary objective of this thesis was to assess if blood glucose can be sampled by means of intravenous microdialysis (ivMD) and corrected applying the ionic reference technique (IRT) in a reproducible manner during an in vivo study in 20 subjects. Results achieved applying the IRT on raw data are promising: In 32 out of all 38 analysed systems (84.2%) the overall performance (correlation) was increased using the IRT. Additionally the Error Grid Analysis showed an increase from 27.8 to 62.3% for estimate values located in zone A and a decrease from 9.7 to 0.8% for estimate values located in the clinical critically zones C, D and E.

Keywords: *diabetes mellitus, glucose, intravenous microdialysis, ionic reference technique, clinical trial*

Zusammenfassung

Die International Diabetes Federation rechnet mit einem Anstieg von 366 auf 552 Millionen Diabetikern bis zum Jahr 2030 [3]. Um deren Lebensqualität zu steigern, werden neue Arten von Insulin entwickelt. Deren Effektivität den Blutzucker zu senken muss vor der Marktzulassung in klinischen Studien mit der Glucose Clamp Methode getestet werden. Um diese arbeitsaufwendigen und personalintensiven manuellen Clamp Studien zukünftig kostengünstiger zu machen, verlangt die Industrie nach einem automatisiertem Clamp-System. Die erste Stufe solch eines Clamp-Systems ist die zuverlässige Messung von Plasma Glukose. Im Zuge einer in vivo Studie mit 20 Probanden sollte ein geeignetes Setup gefunden werden, mit welchem es möglich ist, die Blutzuckerkonzentration mittels intravenöser Mikrodialyse (ivMD) und der Ionen Referenz Methode (IRT) zuverlässig zu ermitteln. Die Ergebnisse welche durch die IRT erreicht wurden sind vielversprechend: In 32 von insgesamt 38 Systemen (84.2%) konnte die Korrelation zwischen Plasmaglukose und Dialysatglukose mittels der IRT gesteigert werden. Zusätzlich konnte die Anzahl der ermittelten Glukosekonzentrationen in Zone A des Error Grids von 27.8 auf 62.3% gesteigert und die Anzahl von Werten in den klinisch kritischen Zonen C, D und E von 9.7 auf 0.8% verringert werden.

Schlüsselwörter: *Diabetes Mellitus, Glukose, intravenöse Mikrodialyse, Ionen Referenz Methode, klinische Studie*

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1 Introduction

1.1 Background

Glucose is the primary energy supplier in the human body and thus the most important molecule of the carbohydrate metabolism. In healthy individuals the glucose concentration is maintained in a narrow range. Blood sugar levels below 60 mg/dl are called hypoglycaemia causing a variety of symptoms and effects ranging from unconsciousness, seizures up to brain damage or death through inadequate supply of glucose to the brain. In contrast to that, consistent blood glucose values higher than 140 mg/dl are called hyperglycaemia. Long term damages associated with these levels affect organs, blood vessels, eyes, kidneys and the nervous system [1].

Elevated blood glucose levels can either be caused by an insufficiency of insulin, an insulin resistance or both. Insulin is a hormone produced in the β-cells of the pancreas, which are located in the islets of Langerhans. It reduces the blood glucose concentration by increasing the permeability of the cell membranes for glucose and raising the enzymatic activity in the cell. If these bodily control mechanisms are disturbed, a chronic, incurable but treatable disease occurs, called diabetes mellitus [2].

Worldwide more than 366 million people or 8.3% of all adults suffer from diabetes and in 2011 an estimated 4.6 million people died from the consequences of high blood glucose levels. The International Diabetes Federation expects a rise to 552 million people who suffer from diabetes by 2030. An additional fact is that more than 80% of all diabetes deaths worldwide occur in low- and middle-income countries, and these countries will also have the highest growth in the next 19 years [3] [4]. Beside gestational diabetes, two types of major diabetes mellitus are distinguished by the World Health Organization (WHO):

Type 1 diabetes is an autoimmune disease, in which the insulin secreting β-cells of the pancreas are destroyed. This leads to a loss of the endogenic insulin production what results in an absolute insulin insufficiency. Thus it is also called the insulin dependent diabetes mellitus (IDDM). The causes of type 1 diabetes are unknown and it is not preventable with current knowledge [5].

Type 2 diabetes is related to an increasing demand of insulin caused by less responding insulin receptors. The β-cells are not able to secrete these increased amount of insulin, which leads to a relative insulin insufficiency. Type 2 is also known as non-insulin dependent diabetes mellitus (NIDDM) and 90% of all diabetic patients suffer from this type [5].

Untreated diabetes mellitus often results in blood glucose levels in the range of 270-450 mg/dl [2]. Since the discovery of insulin in 1921 by Frederick Banting and Charles Best, diabetic patients can apply intensive insulin therapy to reduce the risk of serious complications caused by increased blood glucose levels [6]. The aim of this therapy is to maintain the blood glucose level as close as possible to normal (euglycaemia) by taking extracorporeal insulin doses parenterally. This is either done several times per day by subcutaneous or intramuscular injections (syringe or pen) or continuously by an insulin pump. The dosage of this injection has to be determined by the current blood glucose level, the carbohydrate intake and the level of physical exercise.

Nowadays several types of insulin are available, distinguished by their duration of effect and their time-action profile. There are short-, intermediate- and long-acting insulin types to increase the quality of treatment for the individual requirements of each diabetic patient. Despite the great improvements in the pharmaceutical industry in the last years there is still big potential for developing new more efficacious insulin types.

Before a new anti-diabetic drug (e.g. insulin) is released to the market, it has to be approved by the corresponding health authorities. The effectiveness to lower blood glucose has to be tested in clinical human studies as suggested by the European Medicines Agency using the glucose clamp technique [7]. It is the gold standard for quantifying insulin secretion and resistance in humans of new anti-diabetic drugs [8]. Figure 1 shows the principle of such a clamp.

Throughout a clamp plasma insulin is raised by infusing insulin subcutaneously or into a peripheral vein. Plasma glucose is measured frequently. Based on these measurements a variable glucose infusion is calculated and administered to stabilize the plasma glucose at a target level (clamp level) and to avoid a drop of plasma glucose caused by the given

insulin. The glucose infusion rate (GIR) is used to quantify the glucose lowering effect of the given insulin. Both, the amplitude and the time-action profile of the glucose infusion, give information on the pharmacodynamic of the investigated insulin [9] [10].

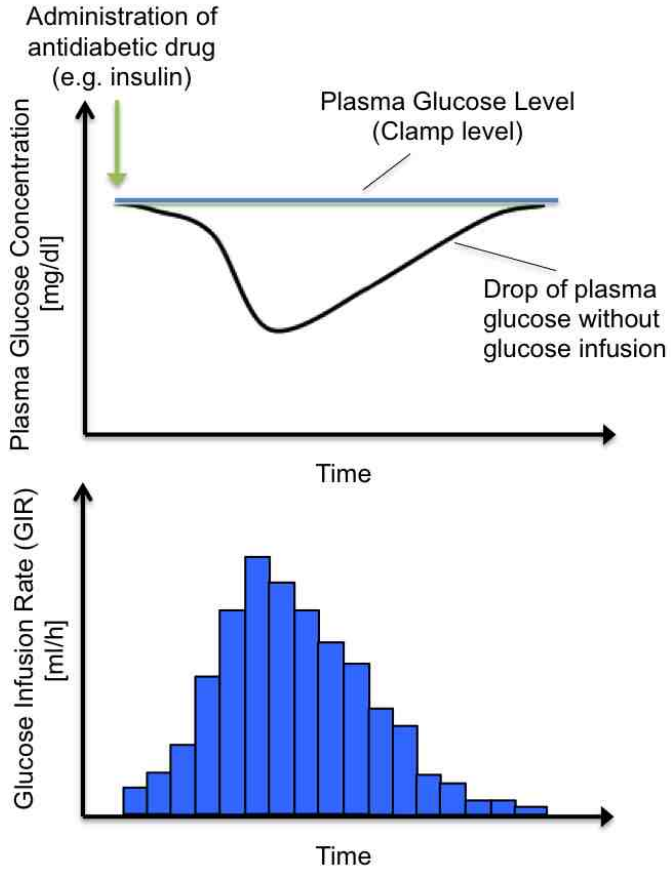


Figure 1: *The principle of a glucose clamp: The glucose infusion rate needed to stabilize blood glucose at a clamp level (target level) and to avoid a drop of plasma glucose caused by the given insulin is used to quantify the glucose lowering effect of a drug (e.g. insulin).*

To date glucose clamps are either performed manually or using automated glucose clamp devices. Manual glucose clamp studies are technically challenging, non-continuous, labour intensive and thus expensive. In contrast to that, automated clamp devices allow to clamp patients continuously in a fully automated manner reducing labour costs and increase the clamp quality.

State of the art automated clamp devices, which sample blood from a peripheral double lumen catheter were introduced in 1960 by Weller et al. [11]. These are: Nikkiso STG-22, Glucostator or Biostator. The Nikkiso STG-22 is not licensed for use in Europe or the USA, whereas the Glucostator and Biostator have a certification for these markets but suffer from the following disadvantages:

- considerably high blood loss of 2.5 ml/h
- unreliable glucose measurement due to variability $> 5\%$
- recalibration intervals ≤ 30 minutes
- insufficient algorithm
- experienced study staff needed
- non-availability of spare parts

To overcome these limitations the industry is interested in a new automated clamp device, which uses innovative and modern technologies. Profil (Institute for Metabolic Research GmbH) coordinates the EU funded project called EU-CLAMP (EUglycaemic- CLinical Application for Metabolic Profiling) with the target to develop such a device.

The prerequisite for a glucose clamp device is to quantify plasma glucose in a reproducible manner, which was the main focus of this thesis. Glucose monitoring systems known from the treatment of type 1 diabetic patients measure glucose in the interstitial fluid (ISF) of the subcutaneous adipose tissue. Due to a physiological time lag between glucose concentration of plasma and ISF, which was reported in various studies, these techniques are not suitable for an automated glucose clamp device [12] [16] [17].

Another method to determine the plasma glucose concentration without blood loss is the intravenous microdialysis (ivMD) technique which was introduced by Ungerstedt [13]. Recent studies by Hage et al. and Rooyackers et al. have shown promising results in this research area [14] [15].

Thereby a microdialysis probe is inserted into a peripheral vein through a standard catheter (e.g. Venflon). Figure 2 shows the components of an intravenous microdialysis probe. Such a probe has a semipermeable membrane at the tip and consists of two lumens, which are connected to an inlet- and outlet- tubing. When a perfusate is pumped through this probe, the outcoming perfusate (dialysate) is enriched with glucose molecules, which have passed over the membrane and thus reflect the plasma glucose (for detailed information refer to 2.1 Intravenous Microdialysis).

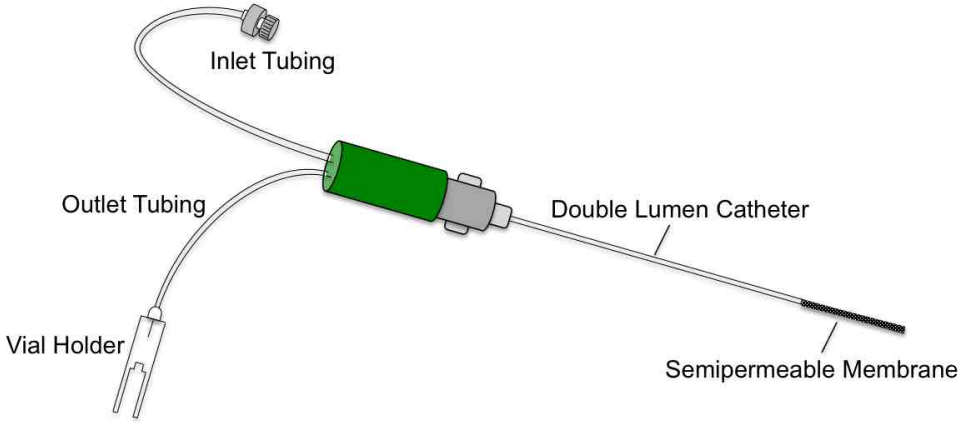


Figure 2: *Components of a microdialysis probe*

When a venous microdialysis probe is inserted into the human body it is recognised by the immune system, triggering some reactions, like deposition of cells and proteins. These effects decrease the permeability of the membrane for glucose and thus change its diffusion characteristics and therefore the degree of equilibration (recovery) between blood and the dialysate. A stable recovery is a prerequisite for reliable glucose monitoring, but since this is not feasible in reality, corrections have to be performed, applying special techniques (2.2 Ionic Reference Technique and 2.7 Calibration of In Vivo Data).

1.2 Objectives

The primary objective of this thesis was to assess if blood glucose can be sampled by means of intravenous microdialysis combined with the ionic reference technique (IRT) in a reproducible manner in humans. In other words the relationship between concentrations of glucose in venous blood (plasma) and glucose sampled with the ivMD technique and corrected with the IRT should be determined.

To achieve this primary objective the following subtasks were performed:

- Determine the quality of the manual glucose clamp.
- Determine the quality of the pumps (stability of the flow rate).
- Determine the glucose- and ion recovery of the ivMD technique.
- Determine changes of the glucose- and ion recovery.
- Determine the relationship between glucose- and ion-recovery.
- Apply two different correction models (Linear & MassBalance) for the IRT regarding the relationship between glucose and ion-recovery.
- Proof whether the IRT improves the correlation between concentrations of glucose in venous blood (plasma) and glucose sampled with the ivMD.
- Validate quality of clinical treatment with Error Grid Analysis (EGA) and Bland & Altman (B&A).
- Proof whether the IRT reduces the amount of required calibration points.
- Find the best setup (microdialysis probe, pump type, flow rate, anticoagulation-dose and -type)

2 Research Design and Methods

2.1 Intravenous Microdialysis

Microdialysis (MD) is based on the technique of conventional dialysis but in a miniaturized manner. MD was introduced by Ungerstedt and Pycock [28] in 1974 primarily for brain research. In 1991 Ungerstedt used the MD-technique to extract analytes via a semi-permeable membrane out of the interstitial fluid of the subcutaneous adipose tissue in humans [13]. Later the development of secure and certified intravascular MD probes opened a new field of research and allowed to extract analytes out of the blood stream. These MD probes, like the CMA 64 IView MD cathether by CMA Microdialysis AB or the MicroEye by Probe Scientific, allow continuous sampling of glucose.

The MD probe is inserted into a vein through a standard peripheral venous catheter (Venflon). The semi-permeable membrane at the tip of the MD-probe (refer to Figure 2) sticks out of the catheter to be fully in contact with the venous bloodstream. Molecules smaller than the membrane´s molecular weight cut-off (PME011 ≈ 10 kDalton, CMA64 ≈ 20 kDalton) diffuse from the blood over the membrane to an analyte-free solution (perfusate) located inside the probe. This fluid is continuously pumped through the MD probe enriched with the analyte (in our case glucose), collected at the extracorporeal outlet and called dialysate (Figure 3).

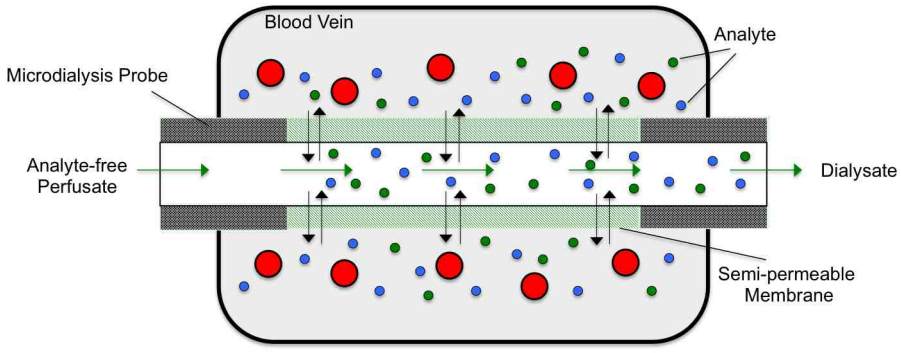


Figure 3: *Principle of a MD catheter: Molecules smaller than the membrane´s molecular weight cut-off (e.g. glucose, lactate) are able to pass the membrane and diffuse into the perfusate.*

The analyte concentration in the dialysate reflects the concentration in the venous blood and depends on parameters like the membrane´s surface area, analyte´s molecular weight and charge, temperature and the flow rate of the perfusate. Full equilibration of an analyte between dialysate and venous blood is only possible at very low flow rates (<1 μl/min) [18], which are inapplicable for practical use due to long delay times. Thus at higher flow rates the analyte concentration found in the dialysate (C_{Dia}) is always lower than in venous blood (C_{Blood}), which can be expressed through the relative recovery of a substance.

$$relative\ Recovery = \frac{C_{\mathrm{Dia}}}{C_{\mathrm{Blood}}} \tag{1}$$

As a consequence at least a 1-point calibration is mandatory for MD-systems. Furthermore the relative recovery might change over time as a result of a varying flow rate, changing membrane permeability due to movement, swelling or clot formation (deposition of proteins or thrombocytes around the membrane). Several publications exist describing this behaviour which occurs shortly after probe implantation [19] [20] [21]. As a consequence frequent calibrations are mandatory for MD-systems. To compensate this changing permeability, the ionic reference technique (IRT) was introduced by Schaupp et al. and shown to be beneficial [26] [27].

2.2 Ionic Reference Technique

As mentioned above the main problem of measuring blood glucose by ivMD is that the glucose concentration in the dialysate ($Gluc_{Dia}$) does not directly reflect the glucose concentration in blood ($Gluc_{Blood}$).

$$Rec_{\mathrm{Gluc}} = \frac{Gluc_{\mathrm{Dia}}}{Gluc_{\mathrm{Blood}}} \tag{2}$$

The relative recovery of glucose (Rec_{Gluc}) can only be determined if both the dialysate glucose concentration ($Gluc_{Dia}$) and blood glucose concentration ($Gluc_{Blood}$) are known. As the blood glucose concentration is the parameter to be determined and unknown, the

relative recovery of glucose can not be calculated. In contrast the relative recovery of ions ($\mathrm{Rec_{Ions}}$) can be determined assuming a constant ion concentration in blood ($\mathrm{Ions_{Blood}}$) by measuring the ion concentration in the dialysate ($\mathrm{Ions_{Dia}}$).

$$Rec_{\mathrm{Ions}} = \frac{Ions_{\mathrm{Dia}}}{Ions_{\mathrm{Blood}}} \tag{3}$$

Due to the fact that for small concentrations the overall ion concentration of a fluid is proportional to its electrical conductivity which is very simple to measure, the determination of the ion concentration is substituted by the measurement of the electrical conductivity. If the electrical conductivity of the dialysate ($\mathrm{Ions_{Dia}}$) is measured and the conductivity of blood plasma ($\mathrm{Ions_{Blood}}$) is assumed to be close to that of NaCl saline solution (0.9%), the relative recovery of ions can be determined as follows:

$$Rec_{\mathrm{Ions}} = \frac{Conductivity\ of\ Dialysate\ [\%\ of\ NaCl\ 0.9\%]}{Conductivity\ of\ NaCl\ 0.9\%} \tag{4}$$

To determine the real glucose concentration in blood ($\mathrm{Gluc_{Blood}}$) a functional relationship between $\mathrm{Rec_{Gluc}}$ and $\mathrm{Rec_{Ions}}$ must be found. Additionally a good correlation between these two recoveries is manadory to achieve a good outcome of the corrected glucose data if the IRT is applied.

$$Rec_{\mathrm{Gluc}} = f(Rec_{\mathrm{Ions}}) \qquad\qquad Gluc_{\mathrm{Blood}} = \frac{Gluc_{\mathrm{Dia}}}{f(Rec_{\mathrm{Ions}})} \tag{5}$$

Thus a change of the relative recovery of glucose can be determined by frequently or continuously measuring the conductivity of the dialysate. As a consequence the changing permeability of the MD membrane can be compensated with the ionic reference technique (IRT), which is based on using an ion-free perfusate and the simultaneous measurement of the electrical conductivity and glucose concentration in the dialysate.

There are two different approaches which were investigated during the work of this thesis:

- Linear Model
- Mass Balance Model

2.2.1 Linear Model

The simplified linear approach assumes that the relative recoveries of glucose and ions can be described with a linear relationship of the form:

$$Rec_{\text{Gluc}} = Rec_{\text{Ions}} * k \tag{6}$$

The concentration of the unknown blood glucose is then calculated as:

$$Gluc_{\text{Blood}} = \frac{Gluc_{\text{Dia}}}{Rec_{\text{Ions}} * k} = Gluc_{\text{Dia}} * \frac{Ions_{\text{Blood}}}{Ions_{\text{Dia}}} * \frac{1}{k} \tag{7}$$

Figure 4 shows the linear relationship between both recoveries with different slopes k. k = 1.00 (line of identity) implies that both recoveries are the same, i.e. the diffusion coefficients of glucose and ions are the same. k < 1.00 implies that the recovery of glucose (Rec_{Gluc}) is smaller than the recovery of ions (Rec_{Ions}).

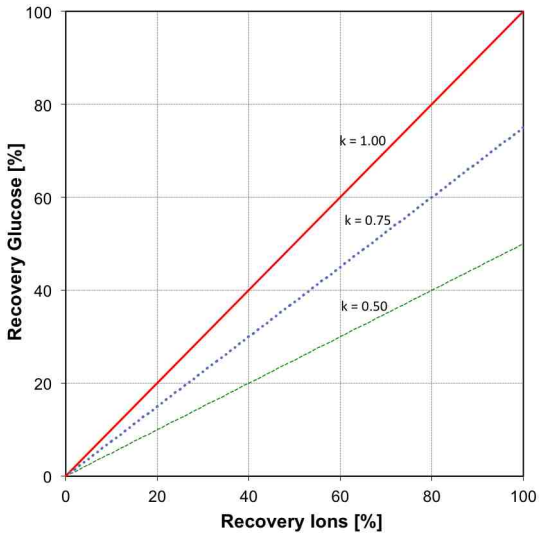


Figure 4: *Schematically linear relationship between relative recoveries of glucose and ions.*

2.2.2 Mass Balance Model

Due to different molecular weights and/or charge of ions and glucose molecules the relative recovery of ions is assumed to be higher than the relative recovery of glucose and the relationship between them is nonlinear.

Assuming that the relative recovery of glucose and ions depend on a flow rate Q and a mass transfer resistance R they can be described as follows [29]:

$$Rec_{\text{Ions}} = 1 - e^{\frac{-1}{R_{\text{Ions}} * Q}} \tag{8}$$

$$Rec_{\text{Gluc}} = 1 - e^{\frac{-1}{R_{\text{Gluc}} * Q}} \tag{9}$$

If the flow rate Q is determined out of equation (8)

$$Q = \frac{-1}{R_{\text{Ions}} * ln(1 - Rec_{\text{Ions}})} \tag{10}$$

and substituted in equation (9) the recovery of glucose can be expressed as a function of the recovery of ions and both mass transfer resistances R_{Ions} and R_{Gluc}.

$$Rec_{\text{Gluc}} = 1 - e^{\frac{R_{\text{Ions}}}{R_{\text{Gluc}}} * ln(1 - Rec_{\text{Ions}})} \tag{11}$$

The quotient k of the mass transfer resistances was determined by fitting (LSM) the function using in-vitro data.

$$k = \frac{R_{\text{Ions}}}{R_{\text{Gluc}}} \tag{12}$$

This is described as “Mass Balance” model.

$$Rec_{\text{Gluc}} = 1 - e^{k * ln(1 - Rec_{\text{Ions}})} \tag{13}$$

The concentration of the unknown blood glucose is then calculated as:

$$Gluc_{\text{Blood}} = Gluc_{\text{Dia}} * \frac{1}{1 - e^{k * ln(1 - \frac{Ions_{\text{Blood}}}{Ions_{\text{Dia}}})}} \tag{14}$$

Figure 5 and 6 depict the Least Squares Fit (green dashed line) of recovery data (orange squares) recorded during an in-vitro investigation for a PME011- and CMA64-MD probe, respectively. Both MD probes were investigated using anticoagulated whole blood at a temperature of 37°C. The flow rate of the perfusate (5% Mannitol) was increased stepwise from 1 to 250 μl/min obtaining recoveries over the whole range and thus increasing the quality of the fit. The calculated value for k was 0.61 for the PME011- and 0.76 for the CM64-MD probe. These values were used for correcting the in vivo data with the Mass Balance model.

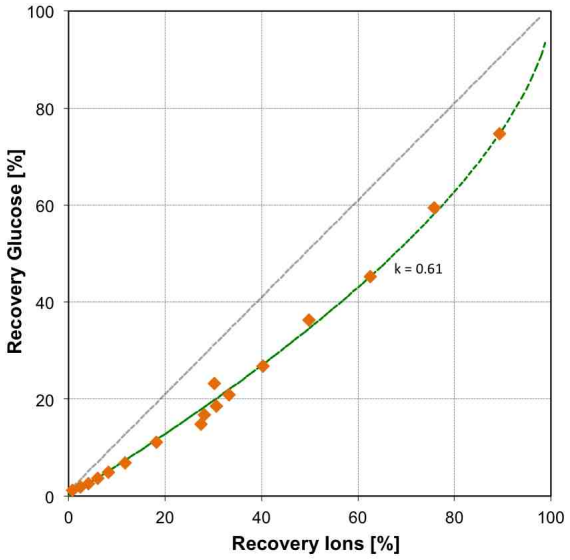


Figure 5: *Least Squares Fit of PME011 in-vitro data with the Mass Balance model (k=0.61)*

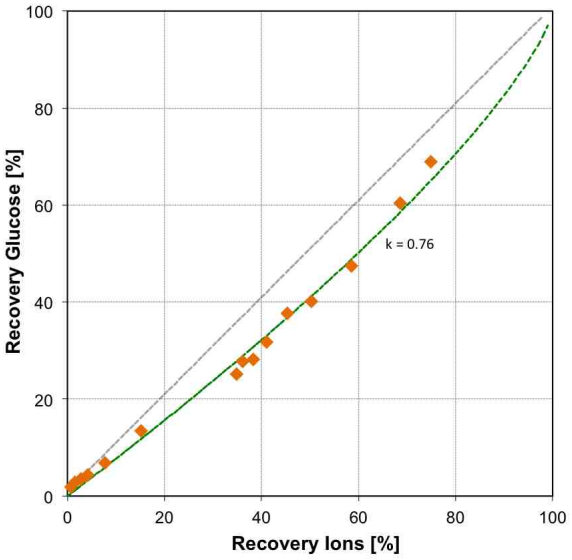


Figure 6: *Least Squares Fit of CMA64 in-vitro data with the Mass Balance model (k=0.76).*

2.3 In Vivo Investigations

The overall goal of the clinical study was to assess, if blood glucose can be sampled in a reproducible manner by means of ivMD in human volunteers. This was done by inserting two CE-certified ivMD probes into veins of the lower arm, wrist or hand of each subject through a standard peripheral venous catheter (Venflon). The probes were perfused with a sterile, isotonic and ion-free fluid (Mannitol 5%). The dialysate was sampled and fractionised for offline analysis (flow rate, glucose, conductivity measurement). Furthermore two hand- or ante cubital veins were cannulated for sampling blood as reference and for the glucose infusion which was used during the clamp. The overall study duration for each subject was 24 hours. After the study occasionally an ultrasound investigation of the implanted MD probes was performed.

2.3.1 Protocol

After optimal operating conditions had been found during the preclinical experiments a technical and clinical evaluation of the EU-CLAMP sampling system was performed in a 24h open mono-centre clinical feasibility trial in 20 healthy, non-diabetic subjects. The study was performed according to Good Clinical Practice (GCP) [22] guidelines at the Clinical Research Centre located at the Medical University of Graz (MUG). Approval was obtained from the local ethical committee of the MUG and the Austrian Agency for Health and Food Safety (AGES). The study was conducted in accordance with the Declaration of Helsinki [23]. Signed informed consent was obtained from each subject before any trial related activities. The protocol for the in vivo investigations was designed to evaluate the sampling process of MD peripheral venous catheter, tubing, pump and perfusate as part of the EU-CLAMP device. Furthermore the influence of different application sites (subcutaneous, perfusate or both) and concentrations for the applied anticoagulation drugs on the system performance was investigated. The subjects were manually clamped to four different glucose levels for six hours each, using manually withdrawn reference blood samples (Figure 7).

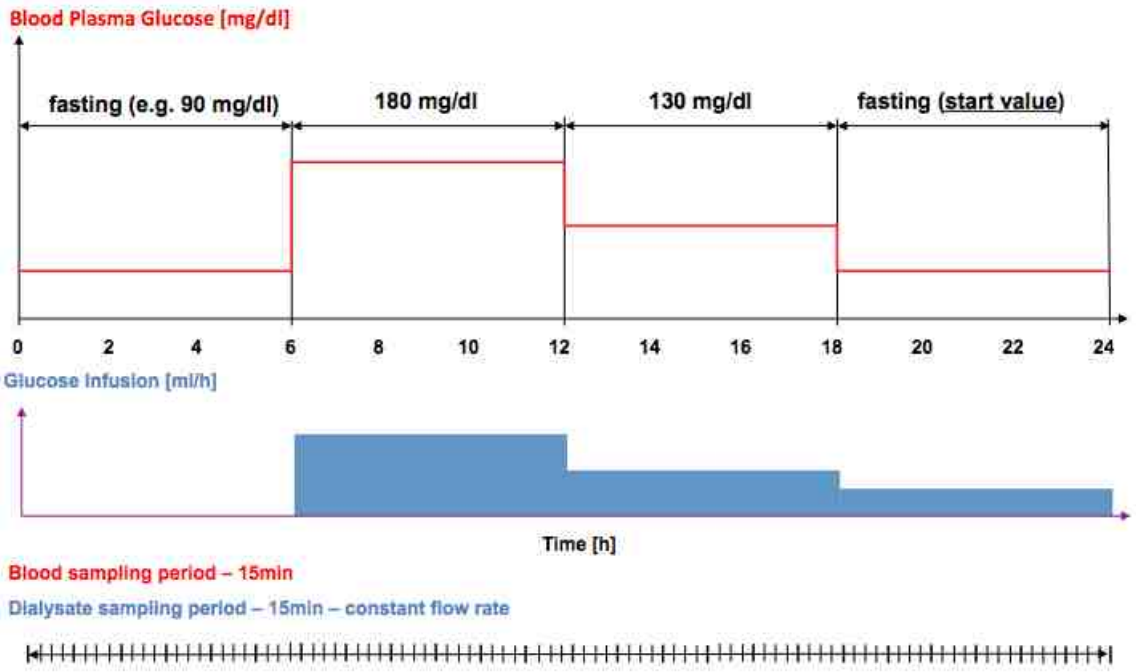


Figure 7: *Study protocol for evaluating the performance of the MD sampling system.*

Glucose Clamp Procedure

Subjects arrived in the morning of the trial in a fasting condition. Per subject four peripheral venous catheters were applied. Two were applied on the right and two on the left arm of the subjects for reference blood sampling, glucose infusion and to attach two MD systems, respectively. Throughout the first 6 hours of the experiment no glucose infusion was given, so an euglycaemic level of about 90 mg/dl could be maintained by the subjects themselves. After 6 hours of fasting an intravenous glucose bolus (Glucosteril 20%) was given to reach the 180 mg/dl clamp level within minutes, which was calculated taken into consideration the body surface area according to the Dubois-method [30]. After the infusion of the bolus the blood glucose was clamped to the level of 180 mg/dl for 6 hours followed by a glucose level of 130 mg/dl for another 6 hours. Throughout the last 6 hours the subjects were fixed to the same euglycaemic level as at the start of the investigation (about 90 mg/dl). Occasionally subjects got a breakfast during the last hour of the study.

Blood Sampling Procedure

Every 15 minutes reference blood samples (approx. 200 μl) were taken from the reference catheter, centrifuged and supernatant plasma was collected for offline glucose analysis. In addition further blood samples were taken (e.g. every 5 minutes) to be able to clamp the glucose levels with a better quality. This was done especially during the phases with target glucose levels of 180 and 130 mg/dl, respectively. Furthermore four blood samples for ion determination were taken at the beginning, after 9, 15 and 24 hours, respectively.

Dialysate Sampling Procedure

Two MD systems were evaluated for each of the 20 subjects (one on the left and one on the right arm). The flow rate of the perfusate, which was pumped through the MD probe, was tried to be held constant throughout the whole experiment at flow rates of 5, 10 or 20 μl/min, respectively. The gathered dialysate was collected either in Eppendorf-vials at flow rates of 10 and 20 μl/min or in PCR-vials at a flow rate of 5 μl/min for a period of 15 minutes. Thus, dialysate samples reflect the average glucose and ion concentrations over the sampling period. In total 96 dialysate samples per system were collected throughout 24 hours and were analysed offline for weight, ion- and glucose- concentration.

2.3.2 Setup Overview

An overview of the whole in vivo setup including the position and function of the catheters applied to each subject is shown in Figure 8.

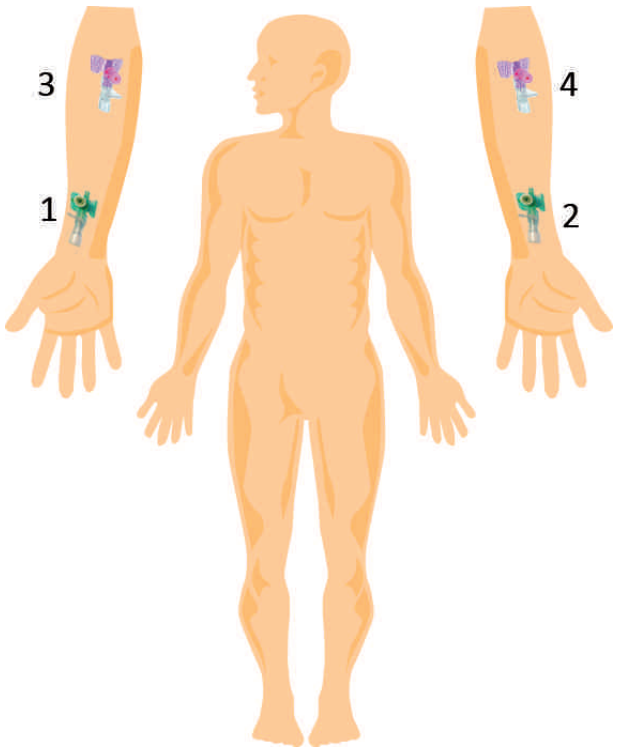


Figure 8: *Microdialysis probes (PME012, PME011, CMA64) were attached to catheter 1 and 2 at the distal position of the arm. The proximal catheter 3 was used for taking reference blood samples whereas the proximal catheter 4 was used for glucose infusion to clamp the subject's to the target blood glucose levels. As catheter 3 and 4 were placed proximal a dilution of the collected dialysate samples through infused flushing fluid could be excluded.*

Figure 9 describes the function of the two MD-systems attached to the left and the right arm. The MD-probes were attached to the subject´s vein using a peripheral venous catheter at the distal position of the arm. A perfusate was pumped through the tubing into the MD-probe. In most cases of the experiments additionally an anticoagulation drug was added to the perfusate to avoid coagulation (blood clotting) around the probe´s membrane. To transport the perfusate through the MD probe either a bedside syringe pump or a portable peristaltic pump were used. The glucose and ion enriched dialysate was collected at the outlet tubing in probe containers.

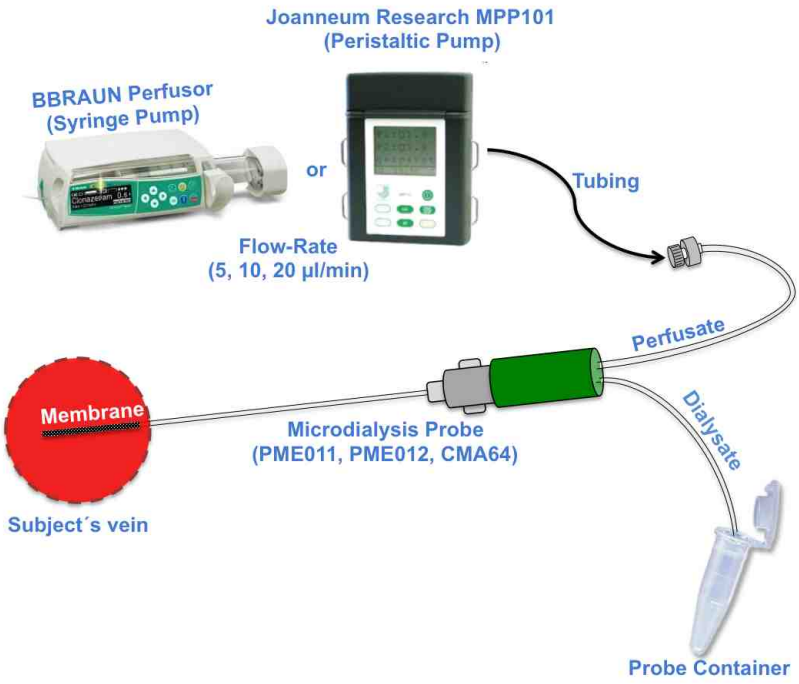


Figure 9: *Setup overview of the in vivo investigations*

To find the best setup and thus the best correlation between blood- and dialysate glucose, the following parameters were varied:

- Type of MD-probe (PME011, PME012, CMA64)
- Type of pump and operation mode (Push vs. Push-Pull)
- Flow rate (5, 10 or 20 μl/min)
- Concentration of anticoagulation drug in perfusate (0, 25, 50 and 100 IU/ml Heparin or 2.5 mg/20.5 ml and 2.5 mg/10.5 ml Arixtra®)
- Systemic anticoagulation (None or subcutaneous injection of 2.5 mg Arixtra®)

All of these parameters are described more precisely in the following sections.

2.3.3 Microdialysis Probes

Three different MD-probes were investigated throughout the in vivo studies.

- PME012 (MicroEye, Probe Scientific) - 10mm membrane length
- PME011 (MicroEye, Probe Scientific) - 20mm membrane length
- CMA64 (CMA Microdialysis AB) - 20mm membrane length

All probes were inserted into a vein through a standard peripheral venous catheter (Venflon 18G). To reduce the risk of a membrane breakage the probe and the venflon were withdrawn together at the end of the study. Due to different shaft lengths of the MD probes two different types of Venflons were used to attach them to the subject's vein. The Introcan Safety 18G x 1 1/4" was used together with the CMA64 whereas the Vasofix Safety 18G x 1 3/4" was used together with the two MicroEye probes (PME011, PME012).

Figure 10: *MicroEye MD probe by Probe Scientific*

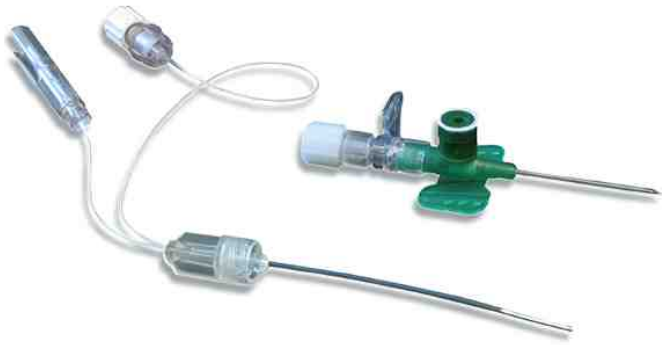


Figure 11: *CMA64 MD probe by CMA Microdialysis AB*

The MicroEye probes PME012 and PME011 (Figure 10) use two adjacent capillaries for the fluidic transportation. In contrast the CMA64 (Figure 11) is built up with two concentric capillaries. The molecular weight cut-off of the MicroEye membrane is about 10 kDalton compared to the 20 kDalton of the CMA64 membrane.

2.3.4 Pumps

Two different pump types were used throughout the investigations:

- Portable peristaltic pump (Joanneum Research MPP101)
- Bedside syringe pump (BBRAUN Perfusor Space)

Compared to the syringe pump the peristaltic pump supports push-pull mode, which was expected to yield a better performance of the sampling process. On the other hand the syringe pump is widely used in clinical practice and thus well-known and even handier than the peristaltic pump.

From the results gained throughout prior in vitro experiments, it was expected to operate the systems at flow rates up to 50 μl/min in the clinical study. As in vivo glucose and ion recovery rates were significantly lower than those obtained during the in vitro experiments in heparinised whole blood and thus the glucose levels were below the lower limit of quantification (LLOQ) of the glucose- and ion analysers, the maximum flow rate was set to 20 μl/min. Therefore 3 different flow rates were investigated for the BBRAUN pump: 5, 10 and 20 μl/min, respectively. As the peristaltic pump MPP101 supports only flow rates up to 10 μl/min, the push-pull investigations were only done with 5 and 10 μl/min.

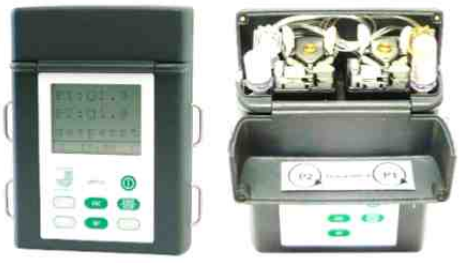


Figure 12: *MPP101 portable peristaltic pump (Joanneum Research)*

Figure 13: *Perfusor Space syringe pump (BBRAUN)*

2.3.5 Perfusate & Anticoagulant

5% Mannitol was the main component of the used perfusate, which is isoosmotic and ion-free. To reduce blood clotting on the probe´s membrane different concentrations of an anticoagulant (heparin, fondaparinux) were added to the perfusate. The influence of the different heparin- and fondaparinux (Arixtra®) concentrations on glucose- and conductivity measurement was analysed prior to the in-vitro investigations. For the glucose signals no significant influence was observed. In contrast the electrical conductivity of the perfusate increased from 0% to 12.5% of the conductivity of physiological saline solution (NaCl 0.9%) when adding 150 IU/ml heparin and from 0% to 5.9% when adding a concentration of 2.5 mg/10.5 ml Arixtra® to the perfusate. The anticoagulant concentrations for the in vivo investigations were chosen to keep the influence of the perfusate conductivity in an acceptable range.

The following concentrations of either heparin (Ebewe Pharma, Heparin Immuno, 1000 IU/ml) or Arixtra® (Glaxo Smith Kline, fondaparinux-sodium, 5 mg/ml) were added to the perfusate:

- None
- 25 IU/ml of Heparin Immuno
- 50 IU/ml of Heparin Immuno
- 100 IU/ml of Heparin Immuno
- Arixtra®:
    - 2.5 mg/20.5 ml at flowrate 10 μl/min
    - 2.5 mg/10.5 ml at flowrate 5 μl/min

Additionally a subcutaneous injection of Arixtra® (2.5 mg/0.5 ml) was given to subjects 11, 12, 13 and 14, respectively, prior to the study.

2.3.6 Tubing & Perfusate Container

When using the bedside syringe pump a BD Plastipak syringe was used as perfusate container with a volume of either 50 ml (flow rate 20 μl/min) or 20 ml (for flow rates 5 and 10 μl/min). The perfusate container was connected to the MD probe with a rigid tubing (CODAN, E-87P). This extension line offers the advantage that a pinching of the tubing causes less flow rate-fluctuations than those observed in conventional, soft tubing.

In case the portable peristaltic pump was integrated in the setup, the sterile perfusate was injected into an appropriate sterile reservoir bag (JR, BEB001) with a capacity of 10 ml, which was refilled via a septum throughout the experiment. Furthermore this pump was used with its appropriate tubing set (SCS001), whereby just 1 out of 3 channels was used.

2.4 Analytical Methods

2.4.1 Conductivity Measurement

Conductivity was determined using the TraceDec® capacitively coupled contactless conductivity measuring device [31]. Normally from less than 5 μl an offline measurement can be guaranteed. To measure a sample it has to be withdrawn from its container with a peristaltic pump (Gilson Minipuls) using a tubing system and a fused silica capillary that is placed inside the sensor. The sensor consists of two electrodes, the pick-up and the actuator electrode between which a high AC voltage is applied. It drives a current which has to flow through the capillary wall, the detection gap inside the capillary and back to the pick-up electrode.

Since the device measures no absolute values (e.g. S/m) it has to be calibrated to a fluid of known conductivity e.g. physiological saline solution (0.9 % sodium chloride solution). To determine the calibration curve a dilution series with a conductivity between 0 and 100% was prepared diluting 0.9% sodium chloride solution with distilled water. All investigated concentrations were measured five-fold. From these values the calibration curve (Figure 14, equals the fit function between the instrument response and the conductivity) was calculated which was later applied on the data obtained during the in vivo study. Coefficient of variation (CV) was found to be less than 2%.

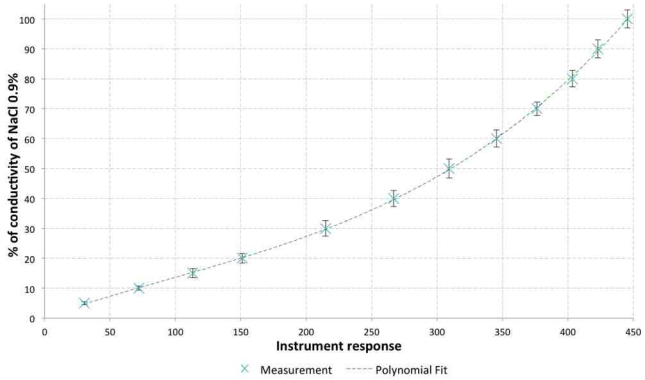


Figure 14: *Nonlinear calibration curve of the TraceDec® conductivity measuring device (error bars indicate SD). Polynomial fit function: $y = 0.3824 + 0.1503x - 0.0003x^2 + 10^{-6}x^3$, $R^2 = 0.9998$*

2.4.2 Glucose Measurement

Supernatant derived from the reference blood samples and dialysate samples were analysed with respect to their glucose concentration using the bench top glucose analyser SUPER GL2 (Dr. Müller Gerätebau GmbH). The glucose analyser was initially 2-point calibrated. During the study fully automated 1-point calibrations were performed. The coefficient of variation (CV) of the device is smaller than 1.5% for a glucose concentration of 216 mg/dl.

Figure 15: *Bench top glucose analyser SUPER GL2*

To quantify samples in the standard working range from 11 to 910 mg/dl, 20 μl of the sample were pipetted into caps with 1000 μl buffer solution (Glucocapil). Due to low recovery rates the glucose concentration in the dialysate could have been below the lower limit of quantification (LLOQ = 11 mg/dl) of the glucose analyser. To quantify dialysate samples in a lower measurement range, up to 400 μl were pipetted into the Glucocapil caps instead of the requested 20 μl. Results were than corrected by volume and glucose concentration.

As usually V_1 (20 μl) are pipetted into a Glucocapil cap the internal volume correction of the SUPER GL2 is

$$C_{\text{Sample}} = C_{\text{Glucocapil}} * \frac{V_0 + V_1}{V_1} \tag{15}$$

with V_0 (1000 μl) - the volume of the fluid in the glucocapil cap and V_1 (20 μl) - the added sample volume.

If the pipetted volume V_1 gets changed to a volume $V_2 = V_1 * x$, the SUPER GL2 measures the following glucose concentration C_{Sample}

$$C_{\text{Sample}} = C_{\text{Glucocapil}} * \frac{V_0 + V_1 * x}{V_1 * x} \tag{16}$$

To return to the real glucose concentration $C_{corr\_spiking}$ a further correction term has to be applied to the corrected sample concentration C_{Sample}:

$$C_{\text{corr\_spiking}} = C_{\text{Sample}} * \frac{V_1}{V_2} * \frac{V_0 + V_2}{V_0 + V_1} \tag{17}$$

This correction term (17) was validated by an in-vitro investigation, pipetting different volumes (20 - 400 μl) of prepared glucose concentrations into glucocapils and correcting them after the measurement. CV values of this investigation were found to be smaller than 1.3%.

2.4.3 Flow Rate

As the recovery rate of glucose changes with the flow rate of the perfusate, an almost constant flow rate had to be assured during the experiment. Due to a lack of existing in vivo, CE-certified, sterile online flow sensors, the flow had to be assessed taking into account the sample's mass, assuming a density of 1 g/ml, and the duration of the sampling process (min). Therefore the mass of the sample containers was measured using a laboratory scale when they were empty and full (containing the dialysate sample). The flow was calculated according to the following equation:

$$Flow[\mu l/min] = \frac{Mass_{\text{Full}}[g] - Mass_{\text{Empty}}[g]}{Time_{\text{Sampling}}[min]} * 10^3 \tag{18}$$

2.5 Data Acquisition

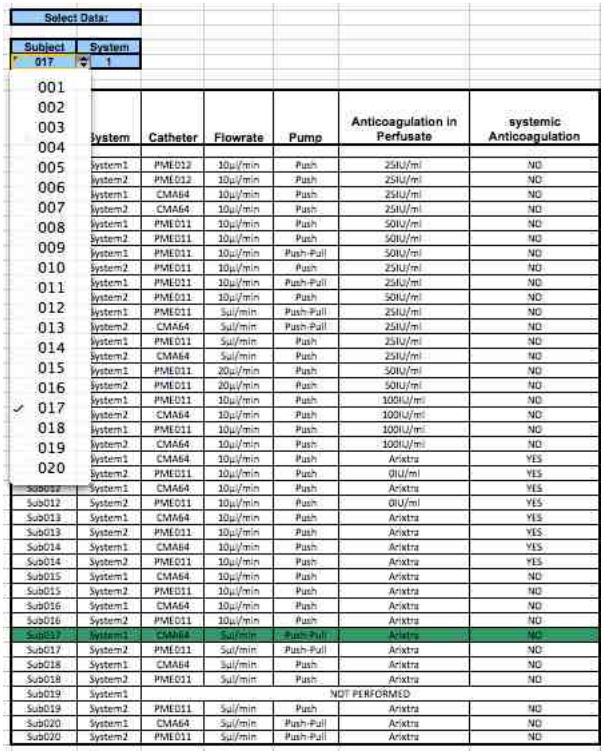
Data measured during the study were stored twofold in handwritten case report forms (CRF) and in Microsoft Excel worksheet files (Microsoft Corporation, Redmond, WA). For each MD system and per sampling interval (15 min) the following measurement values had to be stored:

- Start- and end-time of sampling interval [hh:mm:ss]
- Mass of probe container with dialysate [g]
- Plasma glucose concentration [mg/dl]
- Dialysate glucose concentration [mg/dl]
- Pipetted volume of dialysate for glucose measurement [μl]
- Dialysate conductivity value [% of NaCl 0.9%]
- Calibration standard conductivity value [% of NaCl 0.9%]

Per study day four MD systems (2 subjects) were tested simultaneously. Probe containers of each system were labelled with different colours to decrease the possibility of failures due to swapping. Additionally to decrease the workload and thus failures, two different input masks were created in Visual Basic. The input mask used for entering the mass- and conductivity-measurements to the particular Excel files is depicted in Figure 16.

Figure 16: *Input Mask for entering mass- and conductivity measurement values to the particular Excel files.*

For further analysis the entered data was rechecked with the CRF´s and copied to one single Excel file which is called „Master File - EUClamp“. In this template file the desired subject and system is selectable via drop-down menu (Figure 17). The file allows an investigation and comparison of raw (uncorrected) data and data corrected with the IRT. Statistical values like System Error, Mean Absolute Relative Difference (MARD), Median Absolute Relative Difference (M2ARD), correlation coefficient (R) etc. can be investigated. Furthermore the glucose- and recovery profiles over time, the Error Grid Analysis (EGA) and the Bland & Altman (B&A) analysis are shown. Additionally it is possible to calibrate data automatically by choosing an interval or manually by setting calibration points.



Subject	System	Catheter	Flowrate	Pump	Anticoagulation in Perfusate	systemic Anticoagulation
	ystem1	PME012	10µl/min	Push	25IU/ml	NO
	ystem2	PME012	10µl/min	Push	25IU/ml	NO
	ystem1	CMA64	10µl/min	Push	25IU/ml	NO
	ystem2	CMA64	10µl/min	Push	25IU/ml	NO
	ystem1	PME011	10µl/min	Push	50IU/ml	NO
	ystem2	PME011	10µl/min	Push	50IU/ml	NO
	ystem1	PME011	10µl/min	Push-Pull	50IU/ml	NO
	ystem2	PME011	10µl/min	Push	25IU/ml	NO
	ystem1	PME011	10µl/min	Push-Pull	25IU/ml	NO
	ystem2	PME011	10µl/min	Push	50IU/ml	NO
	ystem1	PME011	5µl/min	Push-Pull	25IU/ml	NO
	ystem2	CMA64	5µl/min	Push-Pull	25IU/ml	NO
	ystem1	PME011	5µl/min	Push	25IU/ml	NO
	ystem2	CMA64	5µl/min	Push	25IU/ml	NO
	ystem1	PME011	20µl/min	Push	50IU/ml	NO
	ystem2	PME011	20µl/min	Push	50IU/ml	NO
	ystem1	PME011	10µl/min	Push	100IU/ml	NO
	ystem2	CMA64	10µl/min	Push	100IU/ml	NO
	ystem1	PME011	10µl/min	Push	100IU/ml	NO
	ystem2	CMA64	10µl/min	Push	100IU/ml	NO
	ystem1	CMA64	10µl/min	Push	Arixtra	YES
	ystem2	PME011	10µl/min	Push	0IU/ml	YES
Sub012	System1	CMA64	10µl/min	Push	Arixtra	YES
Sub012	System2	PME011	10µl/min	Push	0IU/ml	YES
Sub013	System1	CMA64	10µl/min	Push	Arixtra	YES
Sub013	System2	PME011	10µl/min	Push	Arixtra	YES
Sub014	System1	CMA64	10µl/min	Push	Arixtra	YES
Sub014	System2	PME011	10µl/min	Push	Arixtra	YES
Sub015	System1	CMA64	10µl/min	Push	Arixtra	NO
Sub015	System2	PME011	10µl/min	Push	Arixtra	NO
Sub016	System1	CMA64	10µl/min	Push	Arixtra	NO
Sub016	System2	PME011	10µl/min	Push	Arixtra	NO
Sub017	System1	CMA64	5µl/min	Push-Pull	Arixtra	NO
Sub017	System2	PME011	5µl/min	Push-Pull	Arixtra	NO
Sub018	System1	CMA64	5µl/min	Push	Arixtra	NO
Sub018	System2	PME011	5µl/min	Push	Arixtra	NO
Sub019	System1	NOT PERFORMED				
Sub019	System2	PME011	5µl/min	Push	Arixtra	NO
Sub020	System1	CMA64	5µl/min	Push-Pull	Arixtra	NO
Sub020	System2	PME011	5µl/min	Push-Pull	Arixtra	NO

Figure 17: *Drop Down menu for the selection of the desired system.*

In another Excel file called „Overview File - EUClamp“ different data sets (flow rate, recoveries, EGA, B&A, R etc.) of all 39 systems can be investigated. Every system can be added or removed manually from the analysis. Furthermore systems can be selected with predefined filters choosing the parameters probe type, flow rate, pump type and used anticoagulant.

2.6 Statistical Methods

All statistic methods described where used to quantify the accuracy of the ivMD sampling system, comparing the glucose concentration determined by ivMD (estimate) with blood plasma glucose concentration (reference). Furthermore these methods were used to evaluate the use of the ionic reference technique (IRT) and different calibration methods.

2.6.1 Correlation Coefficient (R)

The correlation coefficient (R) is a measure for the strength of a linear relation between two variables, in our case estimate- and reference values. It is calculated as follows [32]:

$$R = \frac{\sum_{n=1}^{N}(Estimate_{\mathrm{n}} - \overline{Estimate})(Reference_{\mathrm{n}} - \overline{Reference})}{\sqrt{\sum_{n=1}^{N}(Estimate_{\mathrm{n}} - \overline{Estimate})^2}\sqrt{\sum_{n=1}^{N}(Reference_{\mathrm{n}} - \overline{Reference})^2}} \tag{19}$$

Results are always between -1 and 1, indicating whether the linear correlation between estimate and reference is positive (1 = fully correlated), negative (-1 = fully anticorrelated) or zero (0 = uncorrelated).

2.6.2 Mean Absolute Relative Difference (MARD)

The mean absolute relative difference (MARD) is the mean of all absolute relative differences between estimate and reference. It is calculated as follows [33]:

$$MARD[\%] = 100 * \frac{1}{N} * \sum_{n=1}^{N}\left|\frac{Estimate_{\mathrm{n}} - Reference_{\mathrm{n}}}{Reference_{\mathrm{n}}}\right| \tag{20}$$

The lower the result, the better the agreement between estimate and reference.

2.6.3 Median Absolute Relative Difference (M2ARD)

The median absolute relative difference (M2ARD) is the median of all absolute relative differences between estimate and reference. It is calculated as follows [33]:

$$M2ARD[\%] = 100 * Median \sum_{n=1}^{N} \left| \frac{Estimate_{\mathrm{n}} - Reference_{\mathrm{n}}}{Reference_{\mathrm{n}}} \right| \tag{21}$$

The lower the result the better the agreement between estimate and reference. The median causes a smaller impact of outliers on the result.

2.6.4 Predicted Error Sum of Squares in % (%PRESS)

The %PRESS method is used to compare a reference and an estimate in chemistry (e.g. concentrations). Hence this method was recommended for comparing the glucose concentration determined by ivMD and the blood plasma glucose concentration. It is calculated as follows [34]:

$$\%PRESS[\%] = 100 * \sqrt{\frac{\sum_{n=1}^{N} (Estimate_{\mathrm{n}} - Reference_{\mathrm{n}})^2}{\sum_{n=1}^{N} (Reference_{\mathrm{n}})^2}} \tag{22}$$

The %PRESS method is more sensitive to outliers. The lower the result, the better the agreement between reference and estimate. A disadvantage of the %PRESS is the loss of the sign (reference smaller than estimate or vice versa) by use of the squared difference.

2.6.5 Error Grid Analysis (EGA)

The Error Grid Analysis (EGA), developed by William L. Clarke in 1987, is the gold standard for quantifying the clinical accuracy of glucose concentration estimates obtained by blood glucose meters compared to the "true" blood glucose concentration obtained by a newly introduced method. Additionally the EGA describes the clinical significance of the degree of accuracy of a specific glucose measurement system [24].

As shown in Figure 18, the glucose measurement estimate of the newly introduced method (y-axis) is plotted against the reference method (x-axis). Furthermore the EGA is divided into five zones reflecting the possible clinical consequences caused by the inaccuracy of the measured values. Zones A and B contain all values leading to an appropriate treatment. Zone D indicates a possible non-detection of hypo- or hyperglycaemia, whereas measurements lying in Zone C and E would lead to a treatment which would even enhance hypo- or hyperglycaemia. Thus data in A (accurate) and B are clinically acceptable whereas data in C, D and E are unacceptable [24].

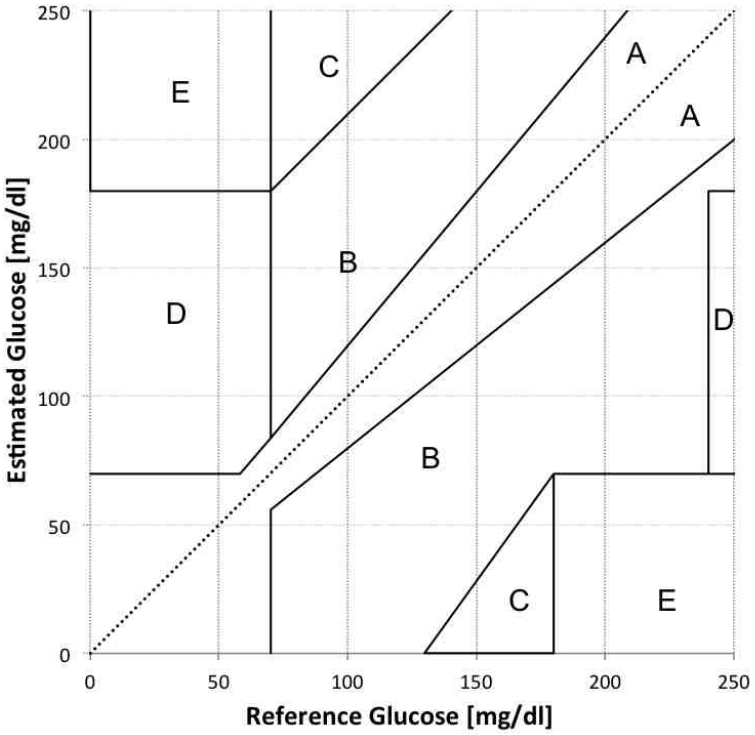


Figure 18: *Clarke´s Error Grid Analysis (EGA)*

2.6.6 Bland & Altman (B&A)

The Bland & Altman (B&A) analysis, named by their inventors J. Martin Bland and Douglas G. Altman, uses a scatter diagram plotting the difference of two methods (absolute error on y-axis) against the mean of them (x-axis). This plot allows an investigation of any possible relationship between the absolute error and the true value. As the true value is often unknown the mean is the best estimate. Additionally a horizontal line visualizes the mean of all differences and two separate lines represent the mean ± 1.96 SD, often called the "limits of agreement". In case of normally distributed (Gaussian) differences 95% of all values lie in this confidence interval. Consequently fluctuation range, possible systematic errors, a bias and outliers can be investigated easily [25].

As in our case the true value is assumed to be the blood plasma glucose (reference glucose) the x-axis of the B&A graph was modified (Figure 19). Thus a possible relationship between the differences and the reference can be shown.

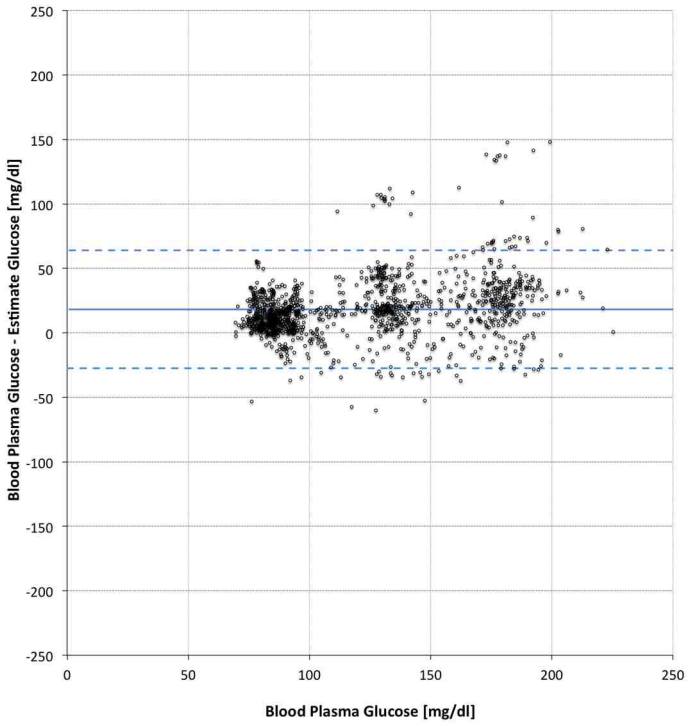


Figure 19: *Example of the Bland & Altman Analysis (B&A). Solid blue line indicates the mean of all differences and the two dashed blue lines represent mean ± 1.96 SD.*

2.7 Calibration of In Vivo Data

The dialysate glucose concentration (Gluc_{Dia}) obtained by ivMD is always lower than the blood plasma glucose concentration ($\text{Gluc}_{\text{Blood}}$), which is a result of the relative recovery of substances when passing a semi-permeable membrane (see 2.1 Intravenous Microdialysis). The IRT compensates only changes (mainly decrease) of this relative recovery over time but is not able to compensate the absolute difference between blood plasma glucose concentration and dialysate glucose concentration. Thus calibration is mandatory for MD-systems.

The calibration of the dialysate glucose concentration against blood plasma glucose concentration is performed by linear regression with the equation:

$$Gluc_{\text{Blood}} = Gluc_{\text{Dia}} * k + d \tag{23}$$

where k is the slope and d the intercept of the regression line. A calibration with more than one point (e.g. 2-point calibration) is only advantageous if different blood plasma glucose concentrations are used. As the clamp device provides more or less a constant blood plasma glucose level only 1-point calibrations with no intercept (d=0) were performed. The slope k at each calibration point was determined as follows:

$$k = \frac{Gluc_{\text{Blood}}}{Gluc_{\text{Dia}}} \tag{24}$$

All subsequent points are also corrected using this slope until the next calibration point. In case of insufficient data, e.g. lack of blood plasma glucose- or dialysate glucose value, the calibration is performed as soon as both values are available.

With the described procedures there are limitations which have to be taken into account. All dialysate glucose values are time-integrated (smeared) concentrations derived from sampling the dialysate over 15 minutes into a probe container. Additional blood plasma glucose values are time-corrected for 7.5 minutes taking into consideration the blood plasma glucose values at the beginning and end of the 15 min sampling interval of the dialysate. These single values are used to one-point calibrate the dialysate glucose values.

2.7.1 Calibration based on a Limit of the System Error (|SE|<10%)

A calibration algorithm based on a limit of the system error was applied to uncorrected (raw) as well as IRT-corrected dialysate data of all analysed 38 systems. A calibration point was generated if the relative error (system error) between the reference- and dialysate-glucose (uncorrected and IRT-corrected) exceeded a limit of ±10%.

The average calibration interval was used for evaluation and calculated as follows:

$$Avg\ Calibration\ Interval\ [min] = \frac{DataPoints}{CalibrationPoints} * Sampling\ Interval \qquad (25)$$

3 Results

3.1 Overview

20 healthy, non-diabetic subjects (17 males, 3 females; age: 26.4±3.8 years; BMI: 24.2±2.3 kg/m^2) were investigated during the study. All subjects successfully completed the study and no adverse events were reported during and after the study. No MD probe had to be removed or renewed before the end of the study and none of them broke during use.

The investigation of subject 019 system 1 could not be performed due to problems with the peripheral venous catheter. At subject 020 system 2 large fluctuations of the dialysate glucose and recoveries above 100% were detected. This system is excluded from further analysis as a pre-contamination of some probe containers with 20% glucose is assumed. Subject 014 received 2 meals instead of the glucose clamp due to problems with the glucose infusion line. At subject 006 System 2 the battery of the peristaltic pump was discharged after 17.5 hours.

The following parameters were varied during the study:

- **MD probes:** 2 x PME012, 22 x PME011 and 15 x CMA64
- **Pumps:** 8 x push-pull- and 31 x push-systems
- **Flow rates:** 11 x 5 μl/min, 26 x 10 μl/min and 2 x 20 μl/min
- **Anticoagulation (Perfusate):** 2 x 0 IU/ml, 10 x 25 IU/ml, 6 x 50 IU/ml, 4 x 100 IU/ml, 10 x Arixtra® 2.5 mg/20.5 ml and 7 x Arixtra® 2.5 mg/10.5 ml
- **Anticoagulation (systemic):** 8 x 0.5 ml Arixtra® 5 mg/ml (4 subjects)

In total 3132 dialysate samples were analysed for their glucose concentration. These values were compared to the time-corrected (7.5 minutes) blood plasma glucose values taking into account an average of the values at the beginning and end of the 15 min sampling interval of the dialysate.

An overview of the parameters under which the 39 systems (20 subjects) were tested, is given in Table 1.

Sub	**Sys**	**Probe Type**	**Flowrate (μl/min)**	**Pump Type**	**Anticoagulant in Perfusate**	**Systemic Anticoagulation**
001	1	PME012	10	Push	Hep 25 IU/ml	None
001	2	PME012	10	Push	Hep 25 IU/ml	None
002	1	CMA64	10	Push	Hep 25 IU/ml	None
002	2	CMA64	10	Push	Hep 25 IU/ml	None
003	1	PME011	10	Push	Hep 50 IU/ml	None
003	2	PME011	10	Push	Hep 50 IU/ml	None
004	1	PME011	10	Push-Pull	Hep 50 IU/ml	None
004	2	PME011	10	Push	Hep 25 IU/ml	None
005	1	PME011	10	Push-Pull	Hep 25 IU/ml	None
005	2	PME011	10	Push	Hep 50 IU/ml	None
006	1	PME011	5	Push-Pull	Hep 25 IU/ml	None
006	2	CMA64	5	Push-Pull	Hep 25 IU/ml	None
007	1	PME011	5	Push	Hep 25 IU/ml	None
007	2	CMA64	5	Push	Hep 25 IU/ml	None
008	1	PME011	20	Push	Hep 50 IU/ml	None
008	2	PME011	20	Push	Hep 50 IU/ml	None
009	1	PME011	10	Push	Hep 100 IU/ml	None
009	2	CMA64	10	Push	Hep 100 IU/ml	None
010	1	PME011	10	Push	Hep 100 IU/ml	None
010	2	CMA64	10	Push	Hep 100 IU/ml	None
011	1	CMA64	10	Push	Arixtra 2.5 mg/20.5 ml	Arixtra 5 mg/ml
011	2	PME011	10	Push	None	Arixtra 5 mg/ml
012	1	CMA64	10	Push	Arixtra 2.5 mg/20.5 ml	Arixtra 5 mg/ml
012	2	PME011	10	Push	None	Arixtra 5 mg/ml
013	1	CMA64	10	Push	Arixtra 2.5 mg/20.5 ml	Arixtra 5 mg/ml
013	2	PME011	10	Push	Arixtra2.5 mg/20.5 ml	Arixtra 5 mg/ml
014	1	CMA64	10	Push	Arixtra 2.5 mg/20.5 ml	Arixtra 5 mg/ml
014	2	PME011	10	Push	Arixtra 2.5 mg/20.5 ml	Arixtra 5 mg/ml
015	1	CMA64	10	Push	Arixtra 2.5 mg/20.5 ml	None
015	2	PME011	10	Push	Arixtra 2.5 mg/20.5 ml	None
016	1	CMA64	10	Push	Arixtra 2.5 mg/20.5 ml	None
016	2	PME011	10	Push	Arixtra 2.5 mg/20.5 ml	None
017	1	CMA64	5	Push-Pull	Arixtra 2.5 mg/10.5 ml	None
017	2	PME011	5	Push-Pull	Arixtra 2.5 mg/10.5 ml	None
018	1	CMA64	5	Push	Arixtra 2.5 mg/10.5 ml	None
018	2	PME011	5	Push	Arixtra 2.5 mg/10.5 ml	None
019	1	NOT PERFORMED				
019	2	PME011	5	Push	Arixtra 2.5 mg/10.5 ml	None
020	1	CMA64	5	Push-Pull	Arixtra 2.5 mg/10.5 ml	None
020	2	PME011	5	Push-Pull	Arixtra 2.5 mg/10.5 ml	None

Table 1: *Overview of parameters of all 39 tested systems (20 subjects). Additionally subject 011, 012, 013 and 014 were systemically anticoagulated with 2.5 mg Arixtra®. At subject 020 system 2 large fluctuations of the dialysate glucose and recoveries above 100% were detected. This system is excluded from further analysis as a pre-contamination of some probe containers with 20% glucose is assumed. Due to problems with the venous access only one MD-system could be applied at subject 019. Subject 014 received 2 meals instead of the glucose clamp due to problems with the glucose infusion line.*

3.2 Glucose Clamp

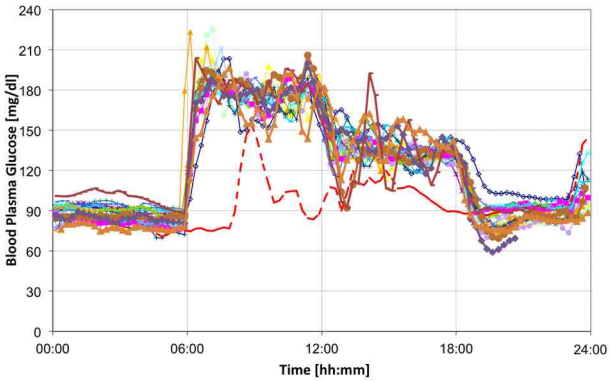


Figure 20: *Individual blood plasma glucose profiles for all 20 subjects*

Figure 20 depicts the individual manually clamped blood plasma glucose profiles for all 20 subjects. The outlying red dashed curve shows the glucose profile of subject 014, which could not be clamped due to a malfunction of the glucose infusion catheter. No glucose infusion could be performed and thus only meals were given (e.g. peak at 09:00). The increased glucose levels at the end of the experiment were caused by the breakfast, which was given to all subjects after 23.5 hours.

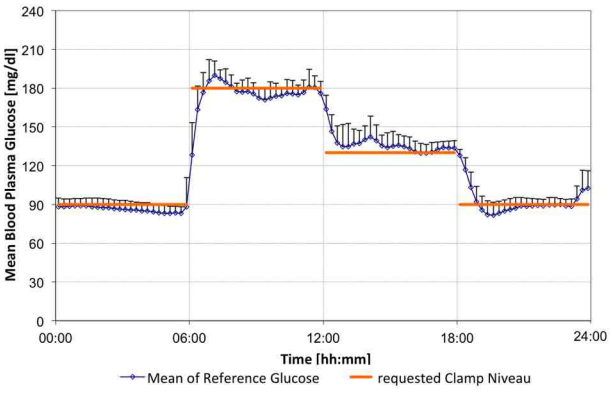


Figure 21: *Mean values of the individual blood glucose profiles and the standard deviation (black bars) of 19 subjects.*

Figure 21 depicts the mean values of the individual blood glucose profiles and the standard deviation (black bars) of 19 subjects (subject 014 was excluded from the analysis). The horizontal, solid, orange lines indicate the target glucose levels (180 mg/dl from 06:00 - 12:00 and 130 mg/dl from 12:00 - 18:00). The increased glucose levels at the end of the experiment were caused by the breakfast, which was given to all subjects after 23.5 hours.

3.3 Pumping

3.3.1 Push Mode

Figure 22 depicts the flow rate of all 31 tested push systems (BBRAUN Perfusor Space). At system 2 of subject 003 (red squares) the flow rate was changed from 20 to 10 μl/min after 2 hours, because the glucose values were below the LLOQ of the glucose analyser. Most of the flow rate deviations were caused by toilet breaks, spillage of the dialysate samples or were artefacts caused by movements.

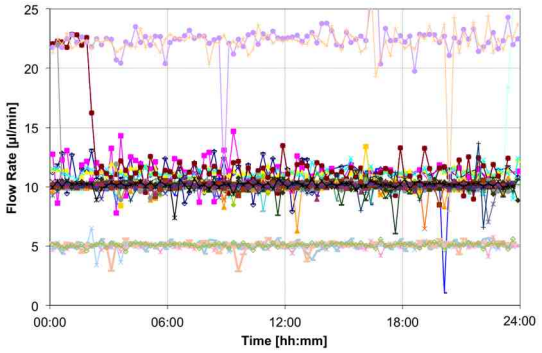


Figure 22: *Flow rate of all 31 tested push systems (BBRAUN Perfusor Space)*

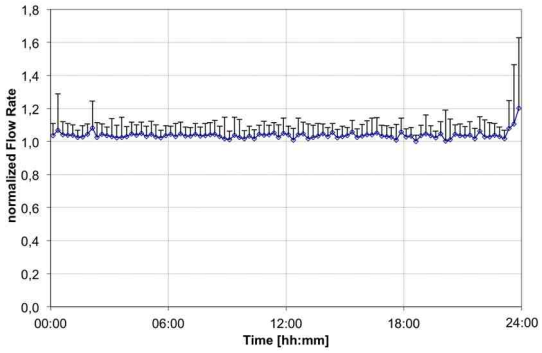


Figure 23: *Mean values of normalized flow rates of all 31 tested push systems (BBRAUN Perfusor Space)*

Figure 23 depicts the mean values of the normalized flow rate of the BBRAUN system (measured flow rate/nominal flow rate) and the standard deviation (black bars) of all 31 tested push systems. Flow rates and the flow variations remained stable throughout the whole experiment (slope of trendline = -0.0097 $\frac{\mu l/min}{24h}$), but were generally higher (+4%) than the expected nominal flow.

3.3.2 Push-Pull Mode

Figure 24 depicts the flow rate of the 8 tested push-pull systems (Joanneum Research MPP101). As the tubing systems were not designed to be operated at flow rates of 10 μl/min a decrease of the flow rate (blue squares), caused by wearing of one tubing system, can be observed. After changing the tubing system twice, after 8.5 and 14 hours, the flow rate returned to its initial level. For flow rates of 5 μl/min only a small decrease of the flow rate can be observed. Within one system the battery of the peristaltic pump was discarged after 17.5 hours and was not replaced.

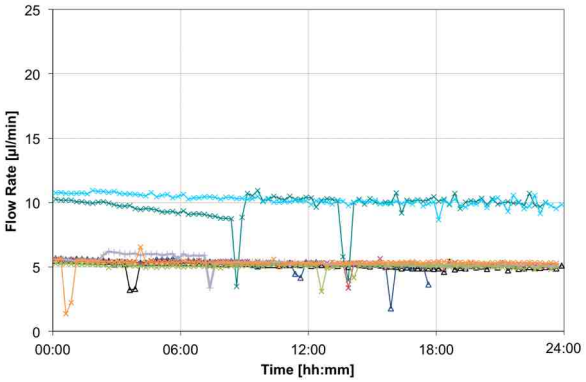


Figure 24: *Flow rate of all 8 tested push-pull systems (Joanneum Research MPP101)*

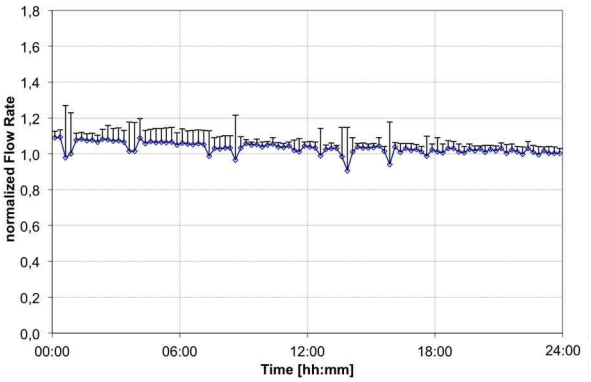


Figure 25: *Mean values of normalized flow rates of all 8 tested push-pull systems (Joanneum Research MPP101)*

Figure 25 depicts the mean values of the normalized flow rate of the JR system (measured flow rate/nominal flow rate) and the standard deviation (black bars) of all 8 tested push-pull systems. A decreasing flow rate (slope of trendline = -0.0625 $\frac{\mu l/min}{24h}$) can be observed from the beginning to the end of the experiment.

3.4 Recovery

Figure 26 depicts the mean relative recovery of glucose (red columns) and ions (blue columns) for all 39 tested systems, subdivided by the used MD probes PME012, PME011 and CMA64. Mean recoveries gained by the two tested PME012 probes with the membrane length of 10 mm were 2.1% for glucose and 2.2% for ions. Thus glucose concentrations in the dialysate were below the LLOQ of the glucose analyzer even when using the whole 15 min sampling volume for determination. In this case the sampling interval was increased to 30 minutes to double the sampling volume and thus the glucose amount for determination. To achieve higher recoveries all further investigations were done either with the PME011 or CMA64, the membrane of which is twice as long (20 mm). The mean glucose recovery of all tested systems using the PME011 or CMA64 with a flow rate of 5 μl/min was 19.2% and 29.4%, respectively. Achieved mean ion recoveries were 26.4% and 34.4%. In contrast the mean recoveries of all systems using a flow rate of 10 μl/min were 6.4% and 19.0% for glucose and 9.8% and 23.9% for ions, respectively. In one PME012- (Sub 001 Sys 2) and two PME011-systems (Sub 008 Sys 1, Sub 020 Sys 2) the mean ion recovery was lower than the mean glucose recovery. The highest mean recovery was achieved using a CMA64 probe (Sub 018 Sys 1) with 48.1% for glucose and 48.7% for ions at a flow rate of 5 μl/min with the push pump.

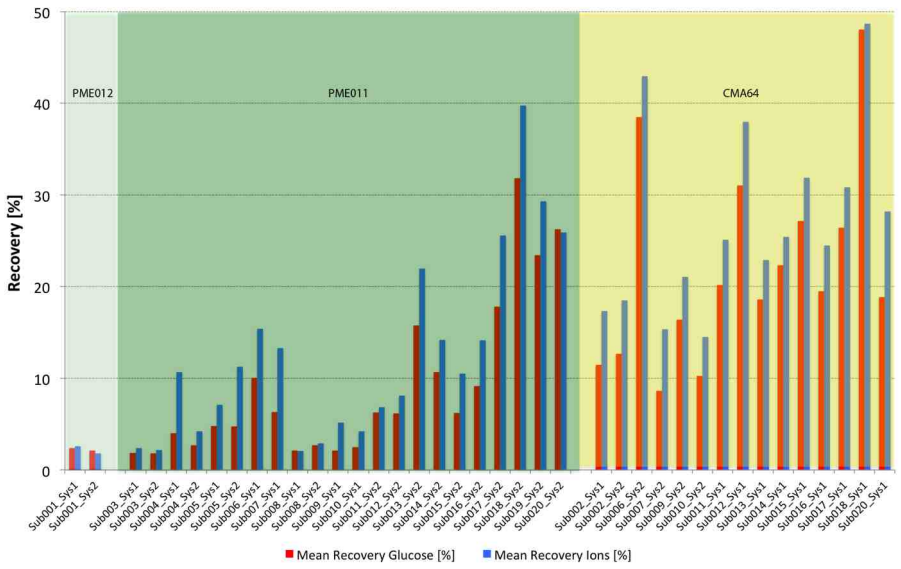


Figure 26: *Mean relative recovery of glucose (red columns) and ions (blue columns) for all 39 tested systems, subdivided by the MD probe types.*

Figure 27 depicts the mean relative recovery of glucose (red) and ions (blue) over time of all tested PME011- and CMA64-systems subdivided by the used flow rates 5 and 10 μl/min, respectively. Black bars indicate the SD for each point. An initial decrease of both recoveries can be shown at both probes. PME011 probes showed a mean decrease of -6.1 % for glucose and -8.5% for ion-recovery in the first 2 hours. The mean duration of the initial phase of all CMA64-probes was 1.5 hours with a decrease of -8.0% for glucose and -4.6% for ion-recovery.

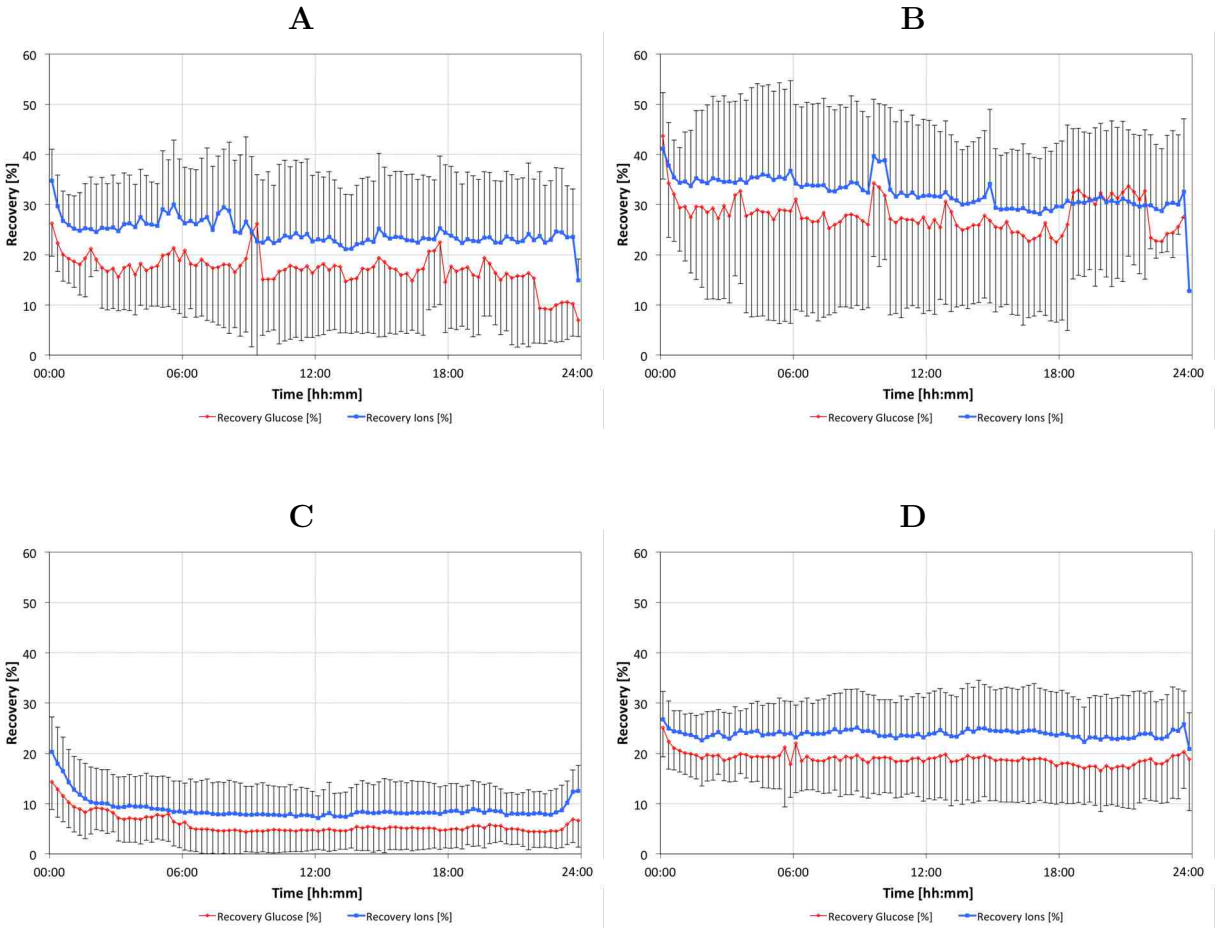


Figure 27: *Mean relative recovery and SD´s of glucose (red) and ions (blue) over time of* ***(A)*** *all 5 PME011 probes using a flow rate of 5 µl/min,* ***(B)*** *all 5 CMA64 probes using a flow rate of 5 µl/min,* ***(C)*** *all 14 PME011 probes using a flow rate of 10 µl/min and* ***(D)*** *all 10 CMA64 probes using a flow rate of 10 µl/min.*

Figure 28 and 29 depict the mean ratio between glucose- and ion recovery of all tested PME011- and CMA64-systems. The mean recovery ratio of PME011- and CMA64-probes was 0.69±0.30 and 0.78±0.14, respectively. These ratio values confirm the assumption that the relative recovery for ions is higher than the glucose recovery. Compared to the PME011 probes, the ratio between glucose- and ion-recovery of the CMA64 probes is more stable and the SD´s are smaller.

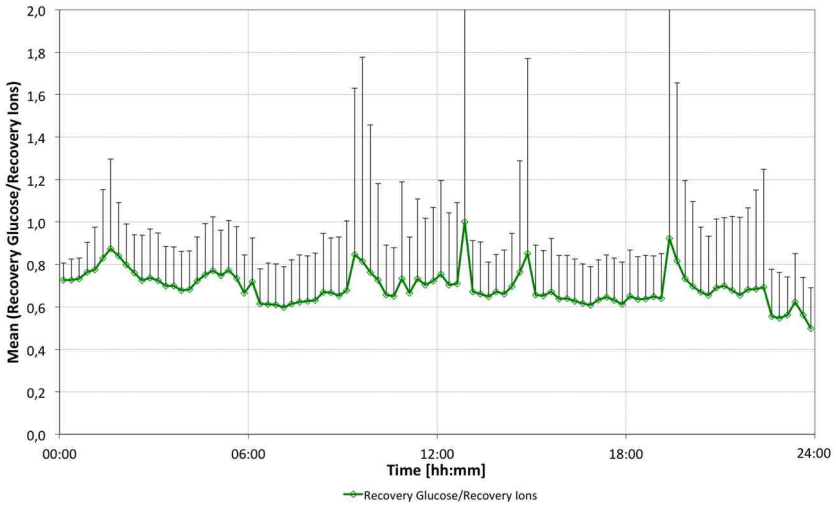


Figure 28: *Mean ratio between glucose- and ion-recovery over time of all tested PME011-systems.*

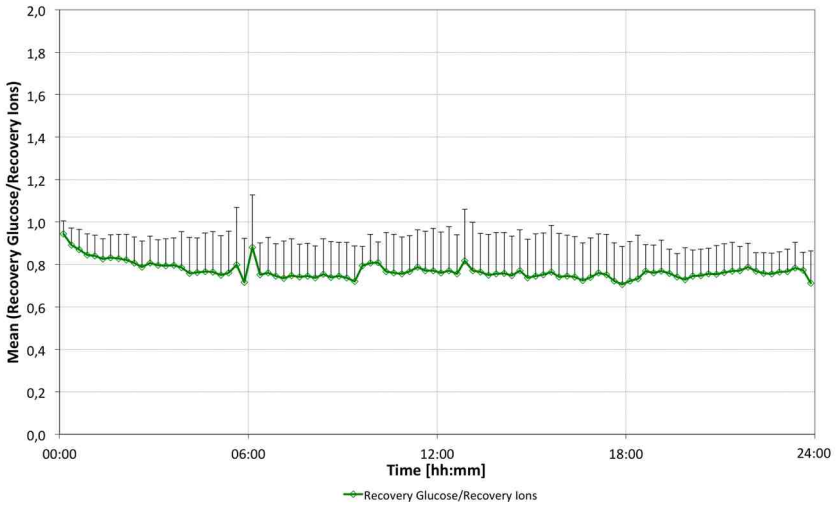


Figure 29: *Mean ratio between glucose- and ion-recovery over time of all tested CMA64-systems.*

Figure 30 and 31 depict the glucose recovery plotted against the ion recovery of all tested PME011- and CMA64-probes, respectively.

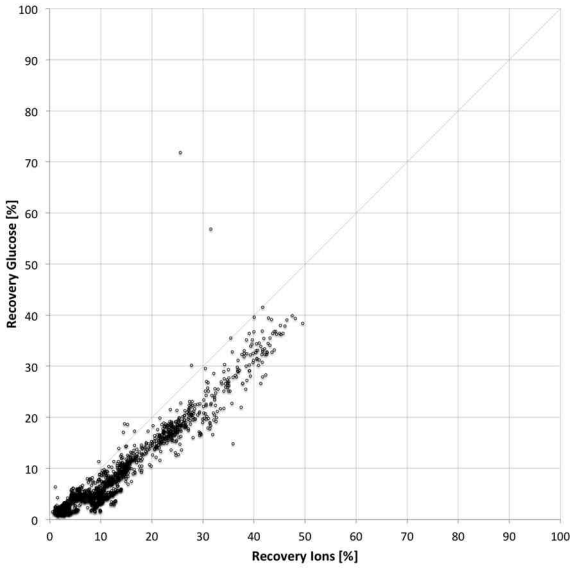


Figure 30: *Glucose recovery plotted against ion recovery of all PME011 probes.*

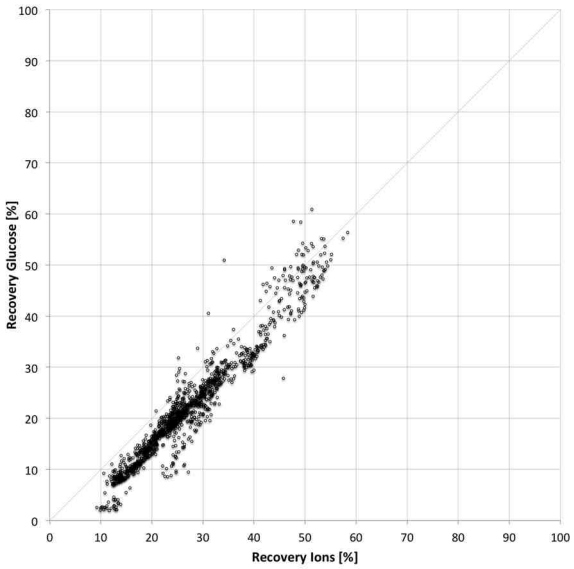


Figure 31: *Glucose recovery plotted against ion recovery of all CMA64 probes.*

3.5 Glucose Profiles

In this section four different glucose profiles over 24 hours of three exemplarily systems are depicted. A green triangle on the x-axis in the graphs indicates the calibration point.

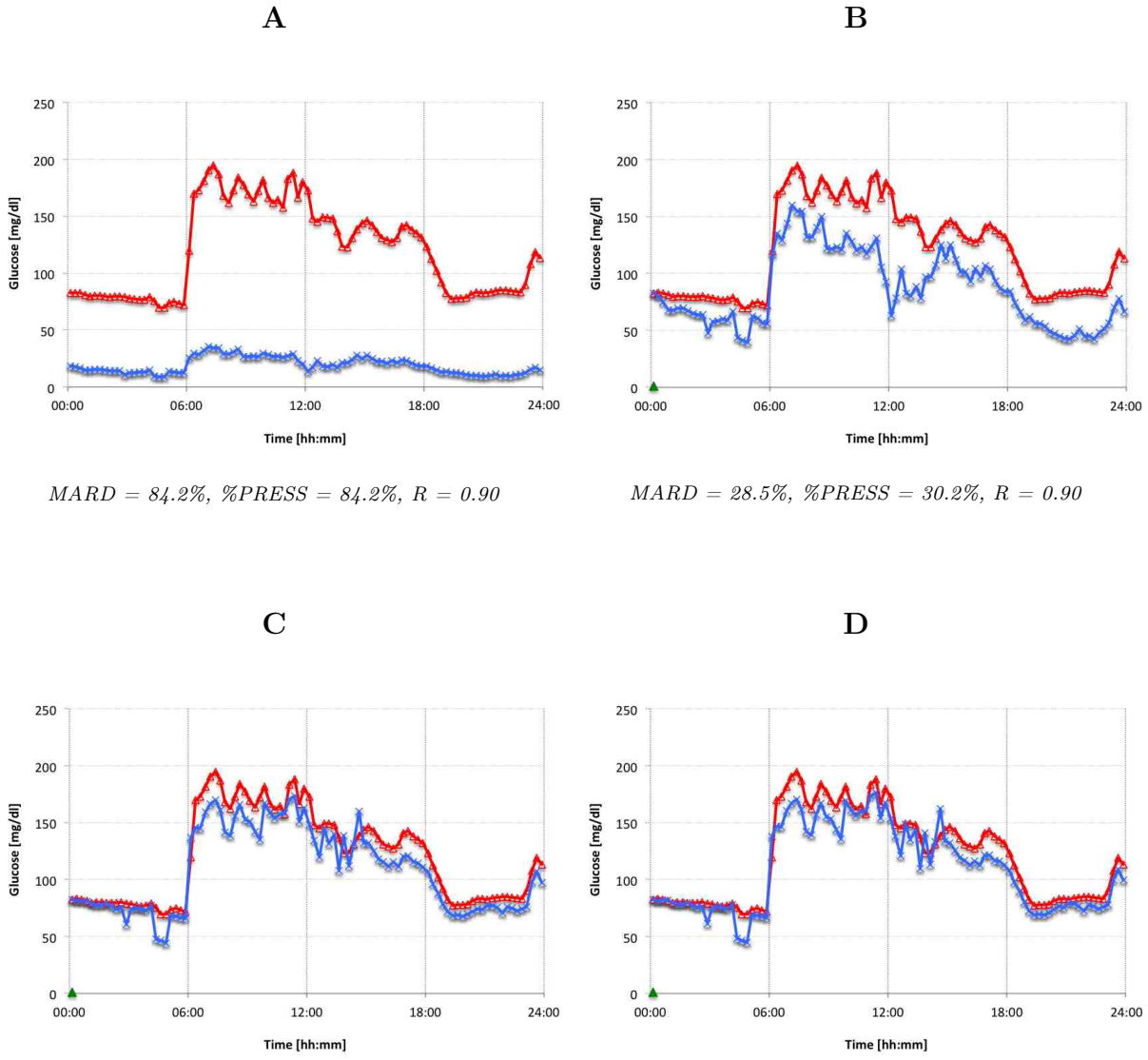
The following data are shown:

- Uncorrected & uncalibrated (Figure 32A, Figure 33A, Figure 34A)
- Uncorrected & one-point calibrated on the first available plasma glucose concentration (Figure 32B, Figure 33B, Figure 34B)
- IRT-Linear corrected & one-point calibrated on the first available plasma glucose concentration (Figure 32C, Figure 33C, Figure 34C)
- IRT-Mass Balance corrected & one-point calibrated on the first available plasma glucose concentration (Figure 32D, Figure 33D, Figure 34D)

The following three systems are shown:

- Subject 013 System 2 (PME011, 10μl/min, Push, Arixtra 2.5 mg/ 20.5 ml)
- Subject 013 System 1 (CMA64, 10μl/min, Push, Arixtra 2.5 mg/20.5 ml)
- Subject 012 System 1 (CMA64, 10μl/min, Push, Arixtra 2.5 mg/20.5 ml)

Table 2 shows an overview of MARD, M2ARD, %PRESS and the correlation coefficient R of all 38 evaluated systems (subject 020 system 2 was excluded). Green labeled cells show the best result of MARD, M2ARD, %PRESS and R for each system, respectively.



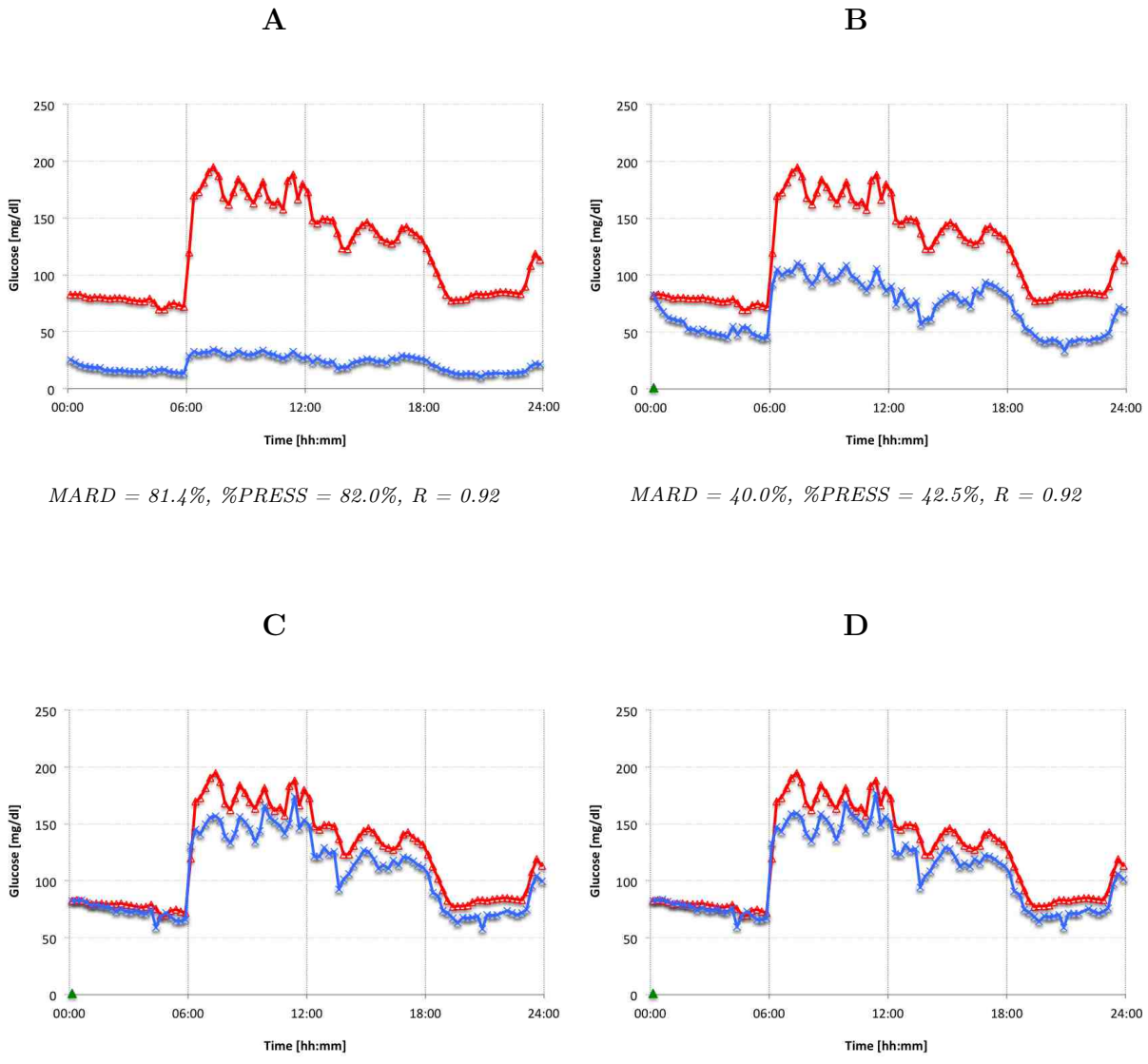
MARD = 84.2%, %PRESS = 84.2%, R = 0.90

MARD = 28.5%, %PRESS = 30.2%, R = 0.90

MARD = 10.9%, %PRESS = 12.4%, R = 0.97

MARD = 9.9%, %PRESS = 11.5%, R = 0.97

Figure 32: *Glucose profiles of subject 013 system 2 (PME011, 10µl/min, Push, Arixtra®). The red curves indicate the blood plasma glucose concentrations, respectively. Green triangles on the x-axis indicate calibration points. Blue curves indicate* ***(A)*** *uncorrected and uncalibrated dialysate glucose,* ***(B)*** *uncorrected dialysate glucose calibrated on the first available plasma glucose concentration,* ***(C)*** *IRT-Linear corrected dialysate glucose calibrated on the first available plasma glucose concentration,* ***(D)*** *IRT-Mass Balance corrected dialysate glucose calibrated on the first available plasma glucose concentration. The IRT, whether with the Linear or the Mass Balance method, increases the overall performance of the system and the correlation coefficient R between blood plasma glucose and dialysate glucose.*



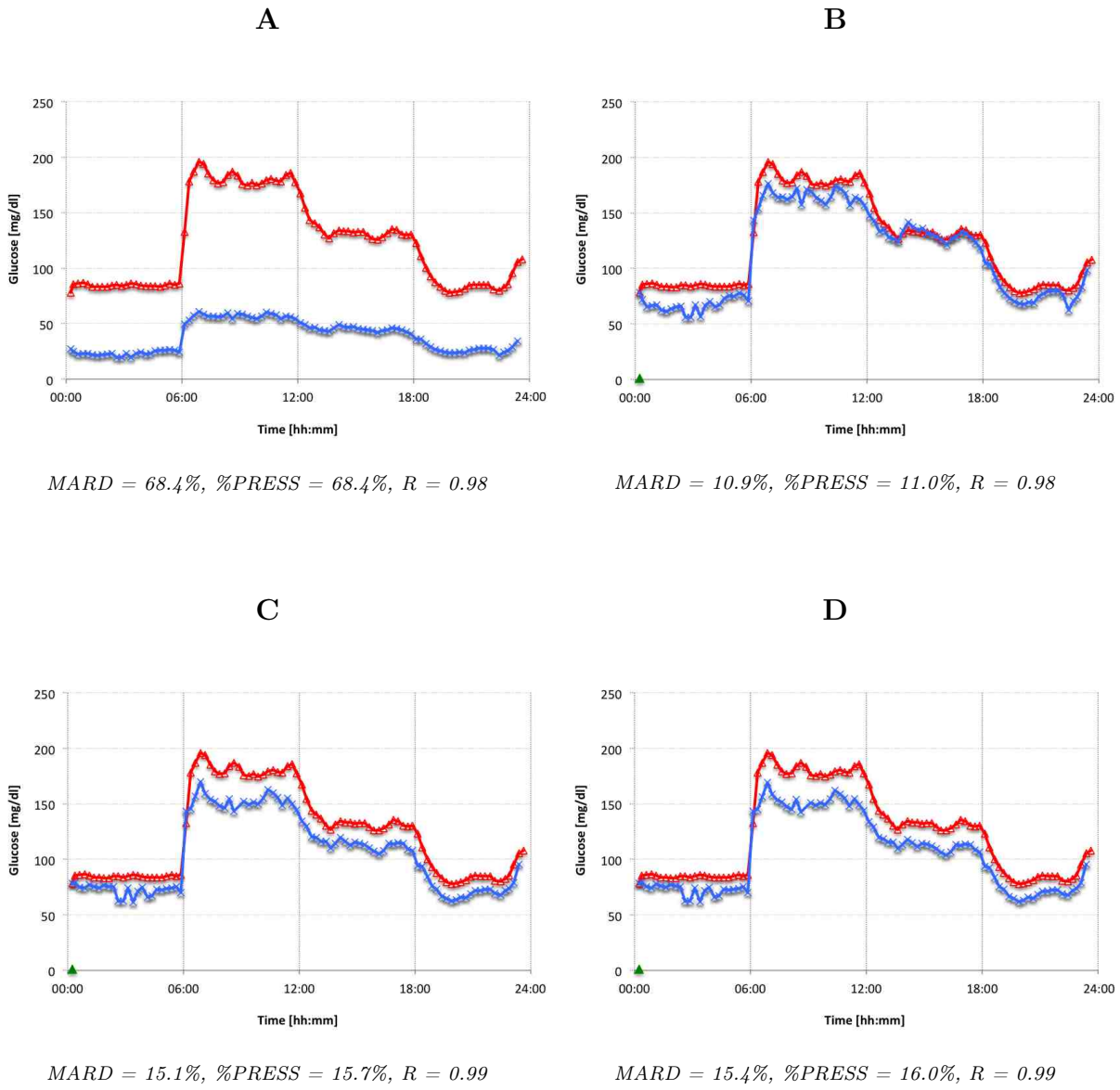
MARD = 81.4%, %PRESS = 82.0%, R = 0.92

MARD = 40.0%, %PRESS = 42.5%, R = 0.92

MARD = 12.7%, %PRESS = 14.9%, R = 0.98

MARD = 11.4%, %PRESS = 13.6%, R = 0.98

Figure 33: *Glucose profiles of subject 013 system 1 (CMA64, 10µl/min, Push, Arixtra®). The red curves indicate the blood plasma glucose concentrations, respectively. Green triangles on the x-axis indicate calibration points. Blue curves indicate* ***(A)*** *uncorrected and uncalibrated dialysate glucose,* ***(B)*** *uncorrected dialysate glucose calibrated on the first available plasma glucose concentration,* ***(C)*** *IRT-Linear corrected dialysate glucose calibrated on the first available plasma glucose concentration,* ***(D)*** *IRT-Mass Balance corrected dialysate glucose calibrated on the first available plasma glucose concentration. The IRT, whether with the Linear or the Mass Balance method, increases the overall performance of the system and the correlation coefficient R between blood plasma glucose and dialysate glucose..*



MARD = 68.4%, %PRESS = 68.4%, R = 0.98

MARD = 10.9%, %PRESS = 11.0%, R = 0.98

MARD = 15.1%, %PRESS = 15.7%, R = 0.99

MARD = 15.4%, %PRESS = 16.0%, R = 0.99

Figure 34: *Glucose profiles of subject 012 system 1 (CMA64, 10µl/min, Push, Arixtra®). The red curves indicate the blood plasma glucose concentrations, respectively. Green triangles on the x-axis indicate calibration points. Blue curves indicate* ***(A)*** *uncorrected and uncalibrated dialysate glucose,* ***(B)*** *uncorrected dialysate glucose calibrated on the first available plasma glucose concentration,* ***(C)*** *IRT-Linear corrected dialysate glucose calibrated on the first available plasma glucose concentration,* ***(D)*** *IRT-Mass Balance corrected dialysate glucose calibrated on the first available plasma glucose concentration. The IRT, whether with the Linear or the Mass Balance method, worsens the overall performance of the system. The correlation coefficient R between blood plasma glucose and dialysate glucose was increased by 0.01.*

			Uncorrected				IRT Linear Model				IRT Mass Balance Model			
Sub	**Sys**	**Probe Type**	**MARD** [%]	**M2ARD** [%]	**%PRESS** [%]	**R**	**MARD** [%]	**M2ARD** [%]	**%PRESS** [%]	**R**	**MARD** [%]	**M2ARD** [%]	**%PRESS** [%]	**R**
001	1	PME012	56.0	61.8	65.3	0.32	30.7	18.1	30.3	0.20	31.2	19.2	30.8	0.20
001	1	PME012	41.4	49.9	54.2	0.30	29.3	21.1	30.0	0.01	29.4	20.9	30.0	0.00
002	1	CMA64	28.8	27.2	30.6	0.88	23.9	24.0	23.9	0.96	23.8	24.0	23.8	0.96
002	2	CMA64	53.4	58.5	58.1	0.72	35.4	37.8	37.6	0.96	34.8	37.0	36.8	0.96
003	1	PME011	75.2	82.4	80.3	0.01	27.9	22.5	30.3	0.54	27.6	21.2	30.3	0.54
003	2	PME011	68.3	71.1	70.8	0.49	23.9	13.9	35.1	0.67	24.2	13.5	35.9	0.66
004	1	PME011	64.2	70.6	72.4	0.25	32.8	36.5	42.2	0.50	31.4	34.9	40.8	0.53
004	2	PME011	69.0	77.3	76.4	0.36	16.1	11.8	17.8	0.76	16.3	10.4	18.1	0.76
005	1	PME011	49.5	53.6	54.3	0.75	20.8	7.1	22.8	0.72	22.1	8.5	24.2	0.71
005	2	PME011	74.5	79.2	79.3	0.08	32.9	39.9	40.2	0.62	30.5	36.9	37.6	0.63
006	1	PME011	72.3	85.3	79.5	0.02	26.6	32.2	31.1	0.88	21.5	25.9	25.6	0.91
006	2	CMA64	15.5	11.1	19.9	0.88	7.7	7.1	8.5	0.98	7.3	6.6	8.2	0.98
007	1	PME011	66.7	71.9	72.5	0.20	27.8	32.6	33.2	0.85	25.6	29.7	30.5	0.86
007	2	CMA64	84.4	93.9	91.7	0.38	59.7	74.1	72.3	0.25	57.5	72.2	70.7	0.22
008	1	PME011	68.1	71.4	71.9	0.46	33.5	26.4	51.4	0.77	34.8	28.1	53.0	0.77
008	2	PME011	65.1	67.4	67.3	0.62	32.0	12.9	51.3	0.43	33.1	13.1	52.7	0.42
009	1	PME011	81.8	86.7	86.1	0.14	44.2	48.3	47.6	0.74	42.7	46.7	46.2	0.75
009	2	CMA64	45.5	46.6	46.1	0.85	20.8	20.9	21.7	0.97	19.7	19.6	20.7	0.97
010	1	PME011	83.6	92.5	90.3	0.41	43.5	48.6	48.2	0.61	41.8	46.7	46.4	0.64
010	2	CMA64	45.5	49.8	48.0	0.74	25.9	28.2	27.5	0.93	25.4	27.7	27.0	0.93
011	1	CMA64	10.9	10.0	14.9	0.92	11.5	11.8	15.6	0.94	11.6	11.8	15.7	0.94
011	2	PME011	70.1	76.9	75.4	0.19	25.3	23.4	26.6	0.91	30.8	29.5	32.1	0.91
012	1	CMA64	10.9	9.8	11.0	0.98	15.1	14.9	15.7	0.99	15.4	15.5	16.0	0.99
012	2	PME011	39.2	47.8	43.8	0.59	14.7	14.5	19.8	0.95	14.3	14.3	19.4	0.95
013	1	CMA64	40.0	41.4	42.5	0.92	12.7	14.0	14.9	0.98	11.4	12.3	13.6	0.98
013	2	PME011	28.5	25.8	30.3	0.90	10.9	11.0	12.4	0.97	9.9	9.3	11.5	0.97
014	1	CMA64	14.7	14.2	17.0	0.80	14.3	14.6	16.4	0.82	14.3	14.6	16.4	0.81
014	2	PME011	37.7	38.9	41.2	0.45	10.7	9.5	13.9	0.71	10.2	8.2	13.5	0.71
015	1	CMA64	11.8	9.2	14.9	0.94	6.1	4.2	8.9	0.96	6.5	4.5	9.1	0.96
015	2	PME011	63.7	70.1	70.4	0.14	23.8	27.3	28.5	0.87	22.0	25.0	26.4	0.88
016	1	CMA64	11.8	10.5	13.2	0.92	9.3	8.7	11.1	0.98	9.4	8.7	11.2	0.98
016	2	PME011	52.7	53.7	56.8	0.69	17.2	17.7	20.4	0.94	15.2	15.3	18.4	0.94
017	1	CMA64	25.0	26.3	29.2	0.94	14.2	15.5	17.7	0.98	13.7	14.9	17.1	0.97
017	2	PME011	19.7	19.3	21.7	0.94	8.4	6.6	9.5	0.97	8.4	5.8	9.4	0.96
018	1	CMA64	21.6	19.9	23.0	0.95	12.6	11.5	14.3	0.97	12.0	10.5	13.8	0.96
018	2	PME011	21.3	21.0	27.4	0.93	8.0	6.5	10.3	0.96	7.4	5.2	9.5	0.95
019	2	PME011	22.4	19.8	34.3	0.75	13.5	7.7	41.3	0.68	13.1	6.9	42.2	0.68
020	1	CMA64	27.8	26.8	30.7	0.86	15.4	11.4	17.1	0.92	15.2	11.0	16.9	0.92

Table 2: *Overview of the results (MARD, M2ARD, %PRESS, R) of all 38 evaluated systems (subject 020 system 2 was excluded). Green labeled cells show the best result of MARD, M2ARD, %PRESS and correlation coefficient R for each system, respectively.*

3.6 Error Grid Analysis

A comparison of all EGA results between uncalibrated (raw), calibrated on first available plasma glucose concentration, IRT-Linear corrected and IRT-Mass Balance corrected MD data of all probes can be found in Figure 35.

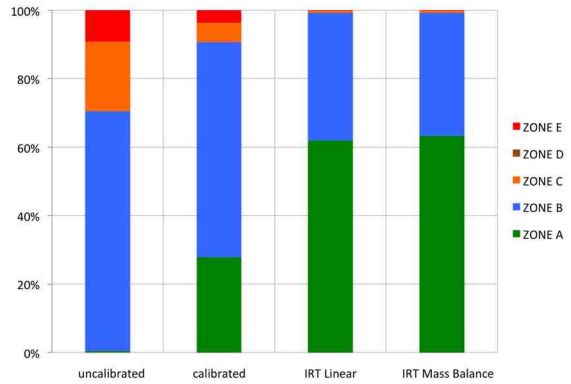


Figure 35: *Comparison of EGA results of all probes.*

Figure 36A depicts the EGA graph for all measured raw dialysate glucose concentrations. All of these uncorrected glucose concentrations are smaller than the blood plasma glucose concentration. 13 values (0.4%) can be found in zone A-, 2195 (70.1%) in zone B-, 635 (20.3%) in zone C- and 289 (9.2%) in zone E-.

Figure 36B depicts the EGA graph for all dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration. 872 values (27.8%) can be found in zone A, 1970 (62.9%) in zone B, 174 (5.6%) in zone C and 116 (3.7%) in zone E.

Figure 36C depicts the EGA graph for all IRT-corrected (Linear) dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration. 1936 values (61.9%) can be found in zone A, 1165 (37.3%) in zone B, 21 (0.7%) in zone C and 4 (0.1%) in zone E.

Figure 36D depicts the EGA graph for all IRT-corrected (Mass Balance) dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration. 1976 values (63.2%) can be found in zone A, 1124 (36.0%) in zone B, 22 (0.7%) in zone C and 4 (0.1%) in zone E.

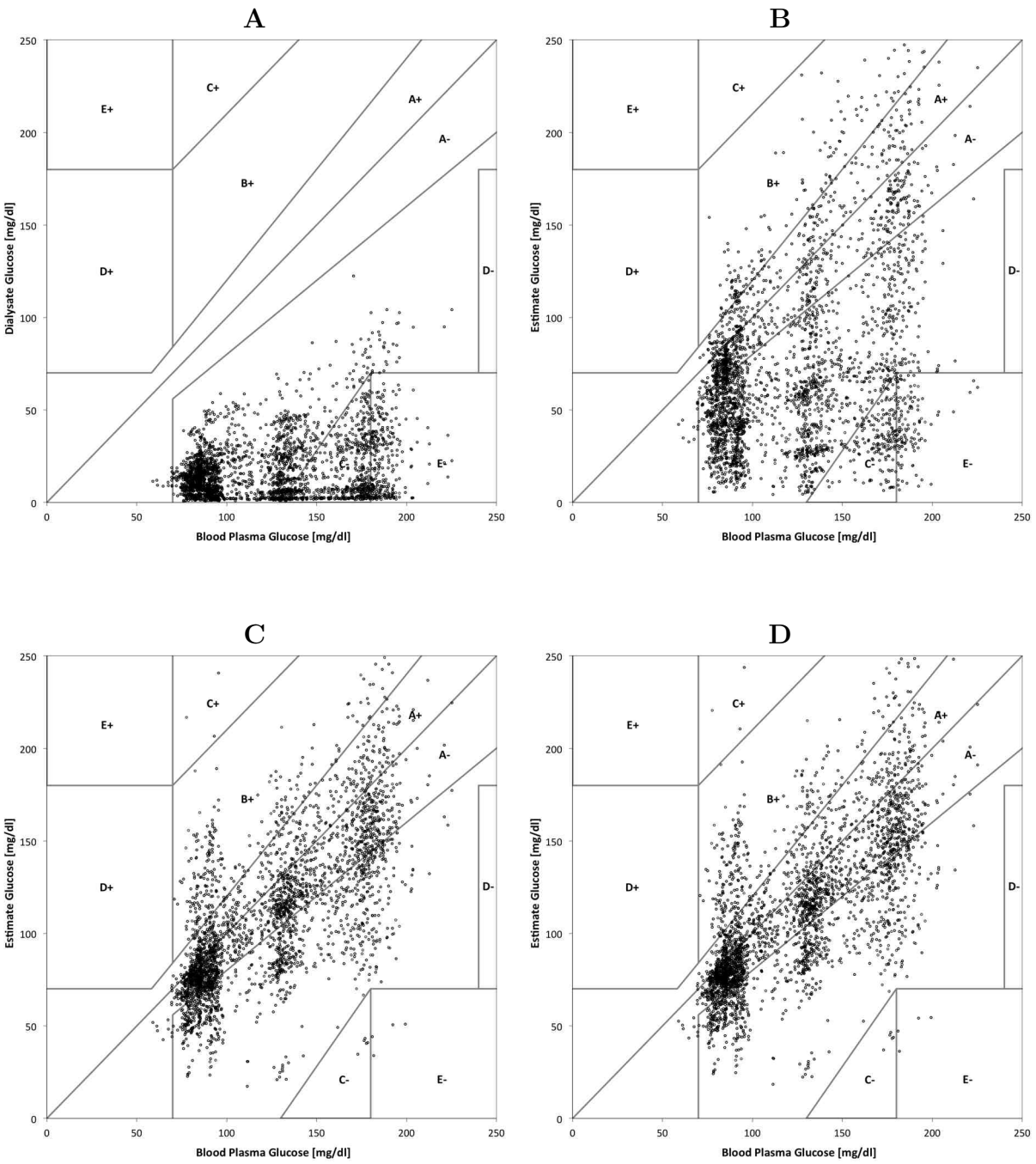


Figure 36: *EGA graphs of all evaluated systems (subject 020 system 2 was excluded).* ***(A)*** *depicts uncorrected and uncalibrated dialysate glucose,* ***(B)*** *depicts uncorrected and calibrated (first available plasma glucose concentration) dialysate glucose,* ***(C)*** *depicts IRT-Linear corrected and calibrated (first available plasma glucose concentration) dialysate glucose,* ***(D)*** *depicts IRT-Mass Balance corrected and calibrated (first available plasma glucose concentration) dialysate glucose.*

3.6.1 PME011

A comparison of EGA results between uncalibrated (raw), calibrated on first available plasma glucose concentration, IRT-Linear corrected and IRT-Mass Balance corrected MD data of all PME011 probes can be found in Figure 37.

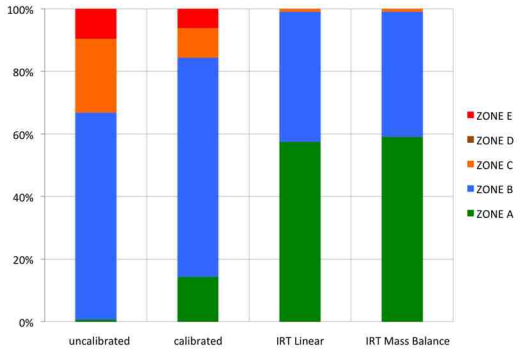


Figure 37: *Comparison of EGA results for all PME011 probes.*

Figure 38A depicts the EGA graph for all measured raw dialysate glucose concentrations of all 21 evaluated (subject 020 system 2 was excluded) PME011 probes. 10 values (0.6%) can be found in zone A-, 1085 (66.8%) in zone B-, 395 (23.9%) in zone C- and 160 (9.7%) in zone E-.

Figure 38B depicts the EGA graph for all dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration, of all 21 evaluated (subject 020 system 2 was excluded) PME011 probes. 236 values (14.3%) can be found in zone A, 1156 (70.1%) in zone B, 156 (9.5%) in zone C and 102 (6.2%) in zone E.

Figure 38C depicts the EGA graph for all IRT-corrected (Linear) dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration, of all 21 evaluated (subject 020 system 2 was excluded) PME011 probes. 946 values (57.4%) can be found in zone A, 686 (41.6%) in zone B and 16 (1.0%) in zone C.

Figure 38D depicts the EGA graph for all IRT-corrected (Mass Balance) dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration, of all 21 evaluated (subject 020 system 2 was excluded) PME011 probes. 973 values (59.0%) can be found in zone A, 658 (39.9%) in zone B and 17 (1.0%) in zone C.

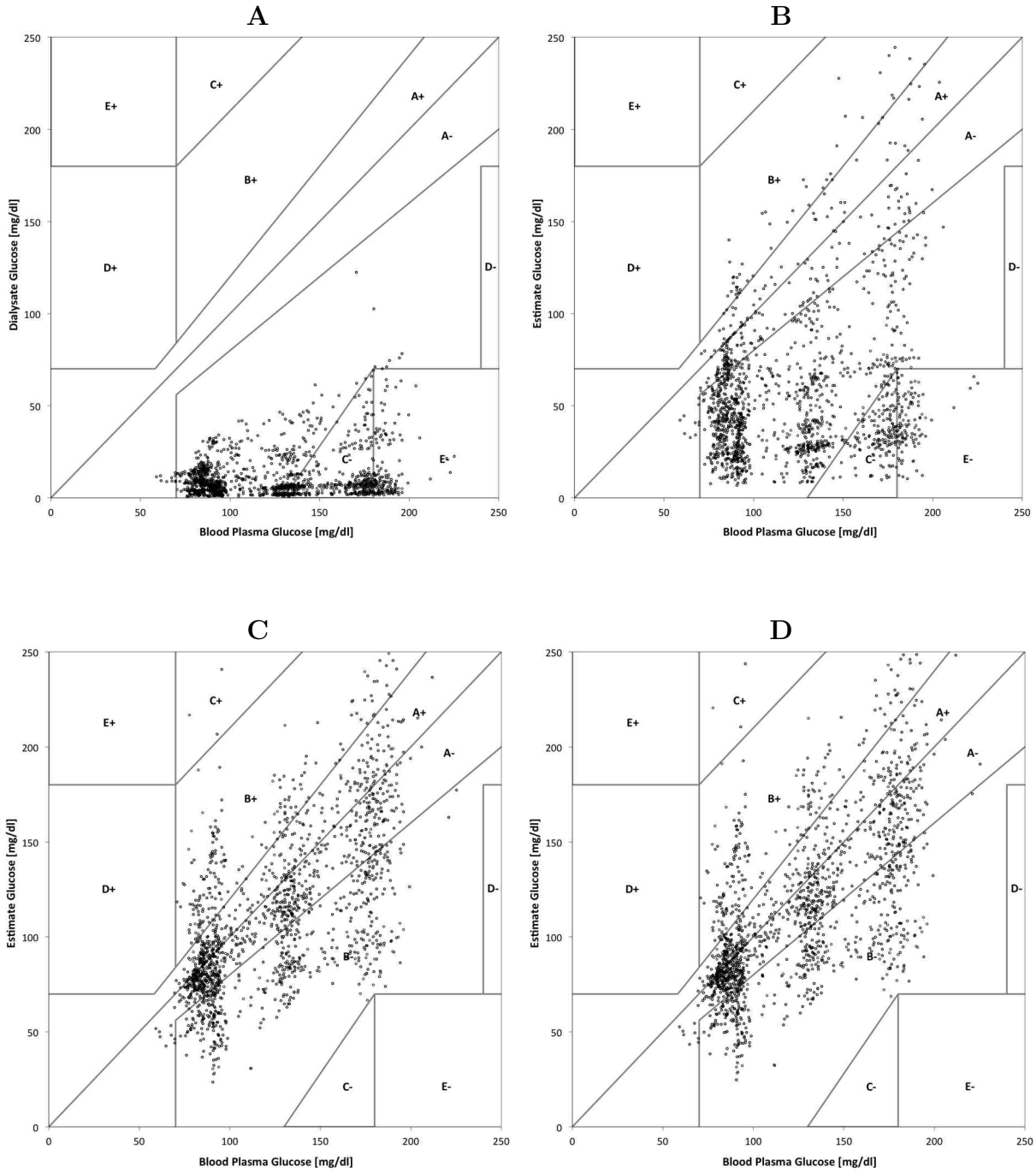


Figure 38: *EGA graphs of all systems using PME011 probes (subject 020 system 2 was excluded).* ***(A)*** *depicts uncorrected and uncalibrated dialysate glucose,* ***(B)*** *depicts uncorrected and calibrated (first available plasma glucose concentration) dialysate glucose,* ***(C)*** *depicts IRT-Linear corrected and calibrated (first available plasma glucose concentration) dialysate glucose,* ***(D)*** *depicts IRT-Mass Balance corrected and calibrated (first available plasma glucose concentration) dialysate glucose.*

3.6.2 CMA64

A comparison of EGA results between uncalibrated (raw), calibrated on first available plasma glucose concentration, IRT-Linear corrected and IRT-Mass Balance corrected MD data of all CMA64 probes can be found in Figure 39.

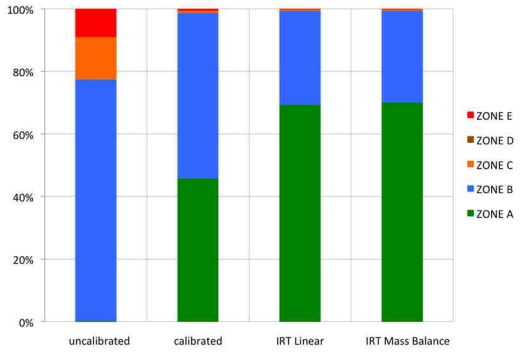


Figure 39: *Comparison of EGA results for all CMA64 probes.*

Figure 40A depicts the EGA graph for all measured raw dialysate glucose concentrations of all 15 evaluated CMA64 probes. 3 values (0.2%) can be found in zone A-, 1031 (77.2%) in zone B-, 180 (13.5%) in zone C- and 121 (9.1%) in zone E-.

Figure 40B depicts the EGA graph for all dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration, of all 15 evaluated CMA64 probes. 612 values (45.8%) can be found in zone A, 705 (52.8%) in zone B, 8 (0.6%) in zone C and 10 (0.7%) in zone E.

Figure 40C depicts the EGA graph for all IRT-corrected (Linear) dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration, of all 15 evaluated CMA64 probes. 923 values (69.2%) can be found in zone A, 401 (30.1%) in zone B, 5 (0.4%) in zone C and 4 (0.3%) in zone E.

Figure 40D depicts the EGA graph for all IRT-corrected (Mass Balance) dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration, of all 15 evaluated CMA64 probes. 934 values (70.1%) can be found in zone A, 390 (29.3%) in zone B, 5 (0.4%) in zone C and 4 (0.3%) in zone E.

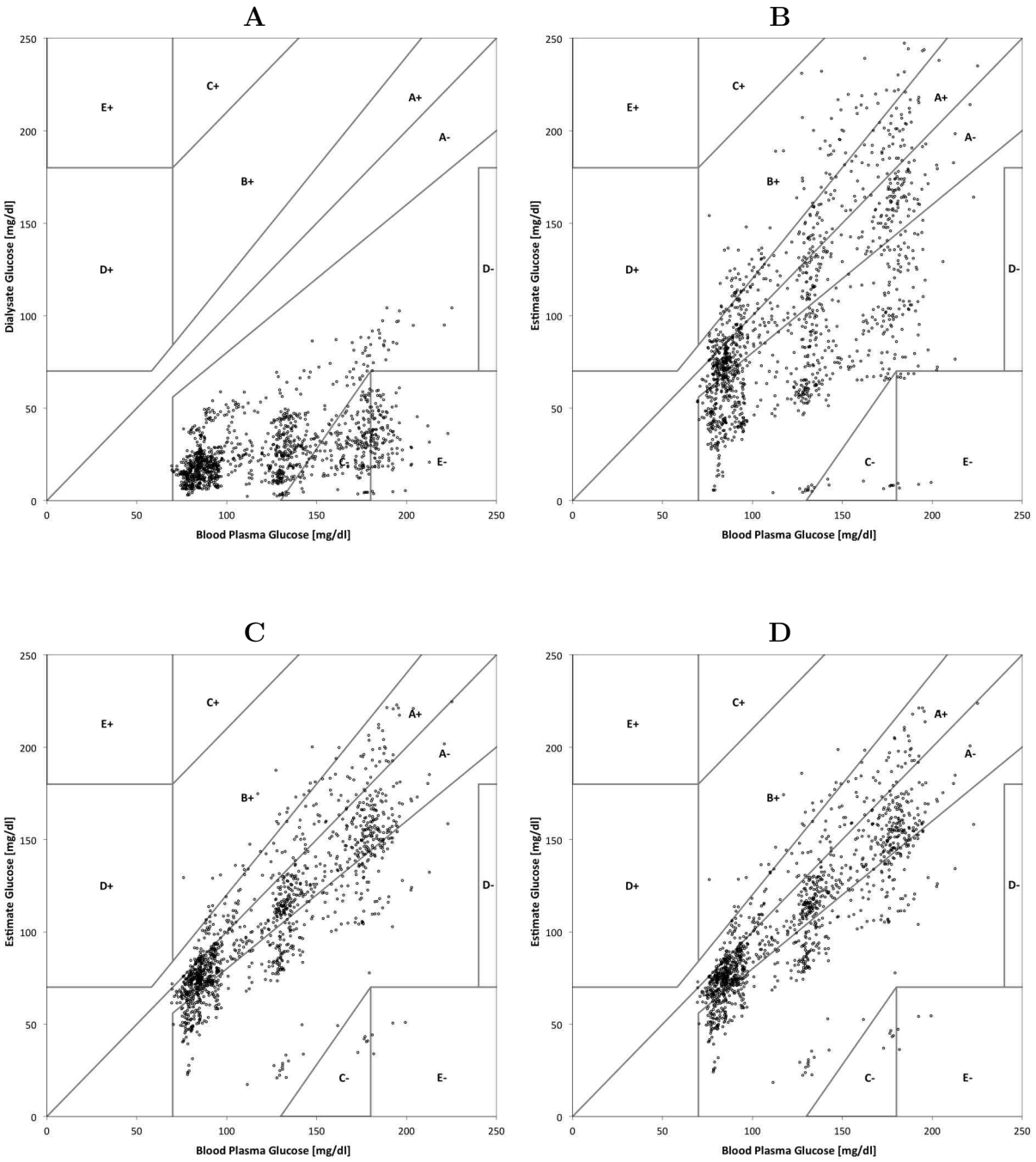


Figure 40: *EGA graphs of all systems using CMA64 probes.* ***(A)*** *depicts uncorrected and uncalibrated dialysate glucose,* ***(B)*** *depicts uncorrected and calibrated (first available plasma glucose concentration) dialysate glucose,* ***(C)*** *depicts IRT-Linear corrected and calibrated (first available plasma glucose concentration) dialysate glucose,* ***(D)*** *depicts IRT-Mass Balance corrected and calibrated (first available plasma glucose concentration) dialysate glucose.*

3.7 Bland & Altman

A comparison of all B&A results between uncalibrated (raw), calibrated on first available plasma glucose concentration, IRT-Linear corrected and IRT-Mass Balance corrected MD data of all probes can be found in Figure 41.

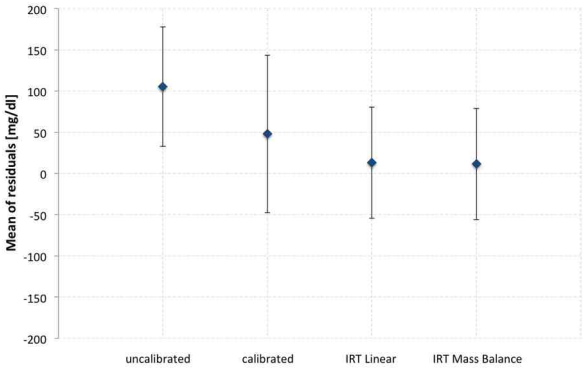


Figure 41: *Comparison of B&A results (Mean±1.96SD) between uncalibrated (raw), calibrated on first available plasma glucose concentration, IRT-Linear corrected and IRT-Mass Balance corrected MD data of all probes.*

Figure 42A depicts the B&A graph for all measured raw dialysate glucose concentrations. The Mean±1.96SD (105.3±72.4 mg/dl) is indicated by the solid and the dashed blue lines, respectively. A trend of the absolute error (blood plasma glucose - dialysate glucose) can be seen with increasing plasma glucose concentrations.

Figure 42B depicts the B&A graph for all dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration. An increase of SD of the absolute error (plasma glucose concentration - dialysate glucose concentration) with increasing blood plasma glucose concentrations can be seen (48.0±95.5 mg/dl).

Figure 42C depicts the B&A graph for all IRT-corrected (Linear) dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration. (13.0±67.3 mg/dl).

Figure 42D depicts the B&A graph for all IRT-corrected (Mass Balance) dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration. (11.6±67.5 mg/dl).

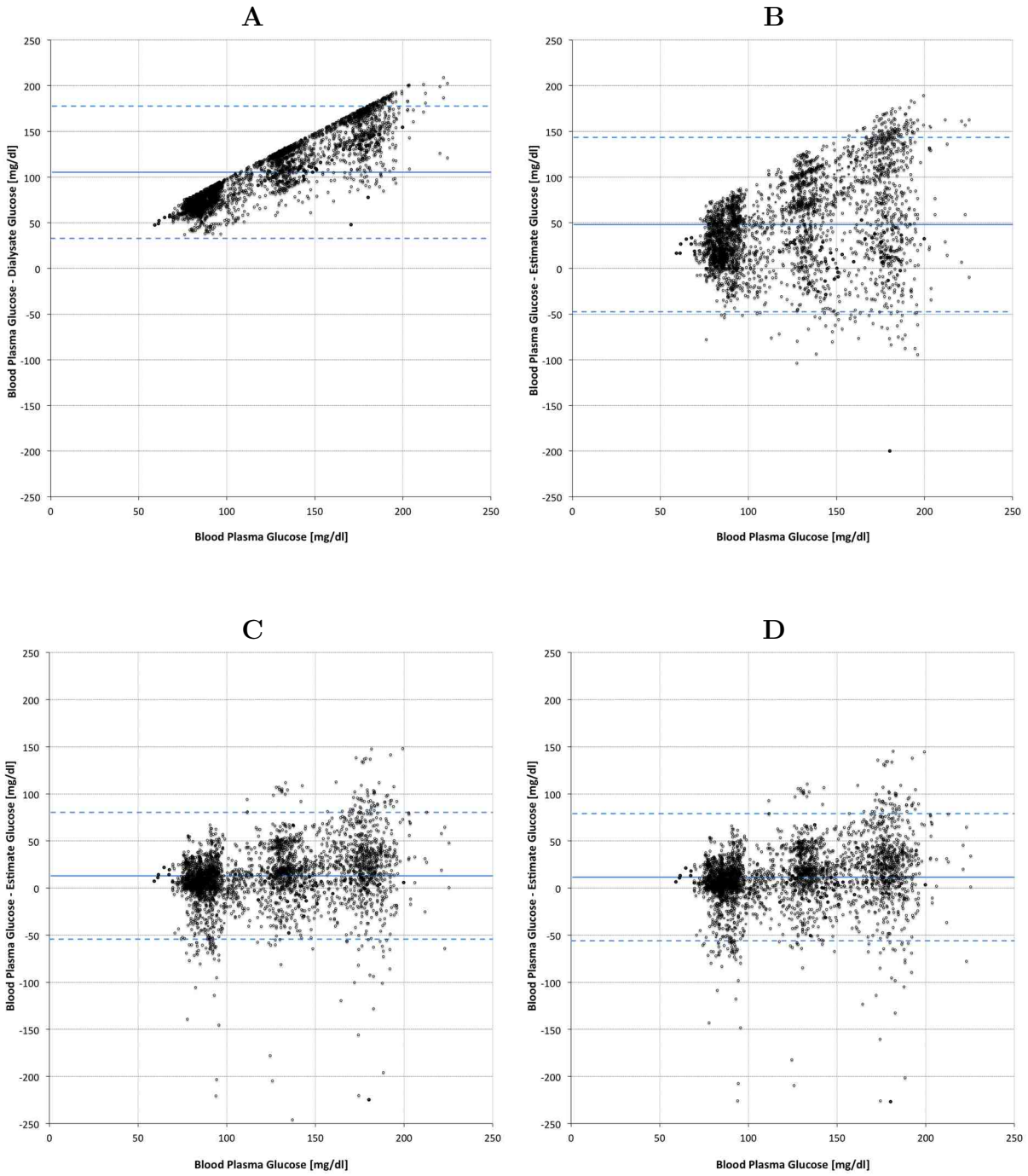


Figure 42: *B&A graphs of all evaluated systems (subject 020 system 2 was excluded).* ***(A)*** *depicts uncorrected and uncalibrated dialysate glucose,* ***(B)*** *depicts uncorrected and calibrated (first available plasma glucose concentration) dialysate glucose,* ***(C)*** *depicts IRT-Linear corrected and calibrated (first available plasma glucose concentration) dialysate glucose,* ***(D)*** *depicts IRT-Mass Balance corrected and calibrated (first available plasma glucose concentration) dialysate glucose.*

3.7.1 PME011

A comparison of all B&A results between uncalibrated (raw), calibrated on first available plasma glucose concentration, IRT-Linear corrected and IRT-Mass Balance corrected MD data of all PME011 probes can be found in Figure 43.

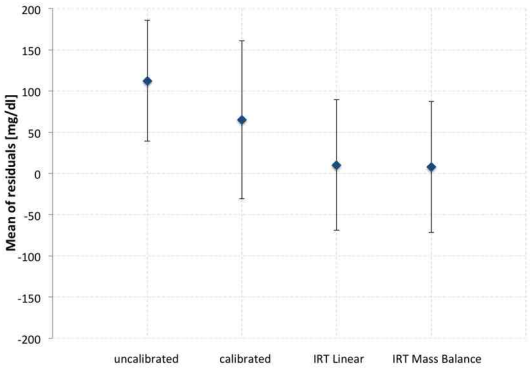


Figure 43: *Comparison of B&A results (Mean±1.96SD) between uncalibrated (raw), calibrated on first available plasma glucose concentration, IRT-Linear corrected and IRT-Mass Balance corrected MD data of all PME011 probes.*

Figure 44A depicts the B&A graph for all measured raw dialysate glucose concentrations of all 21 evaluated PME011 probes. (112.3±73.4 mg/dl).

Figure 44B depicts the B&A graph for all dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration, of all 21 evaluated PME011 probes. (65.2±95.9 mg/dl).

Figure 44C depicts the B&A graph for all IRT-corrected (Linear) dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration, of all 21 evaluated PME011 probes. (10.1±79.1 mg/dl).

Figure 44D depicts the B&A graph for all IRT-corrected (Mass Balance) dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration, of all 21 evaluated PME011 probes. (7.7±79.4 mg/dl).

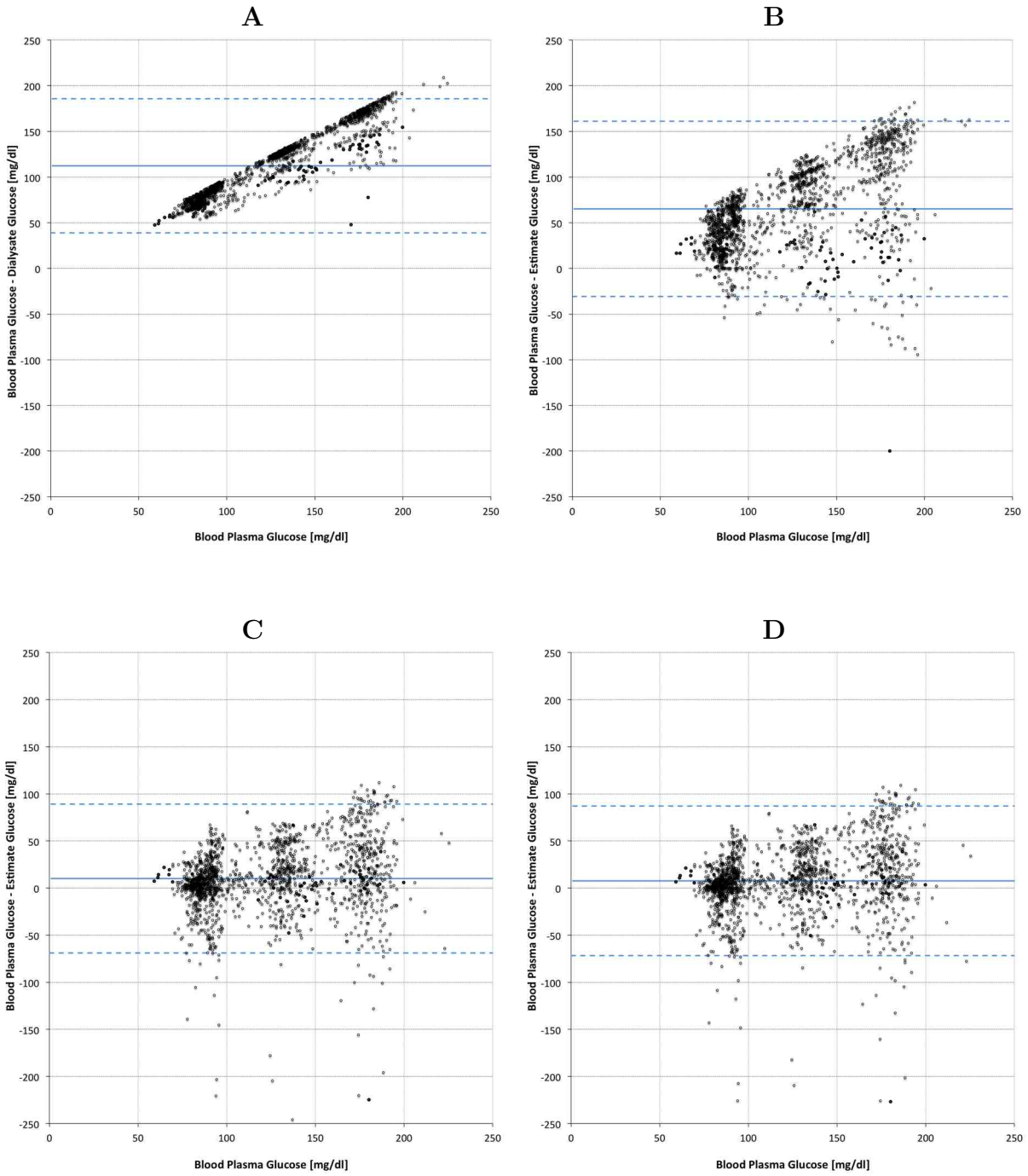


Figure 44: *B&A graphs of all systems using PME011 probes (subject 020 system 2 was excluded).* ***(A)*** *depicts uncorrected and uncalibrated dialysate glucose,* ***(B)*** *depicts uncorrected and calibrated (first available plasma glucose concentration) dialysate glucose,* ***(C)*** *depicts IRT-Linear corrected and calibrated (first available plasma glucose concentration) dialysate glucose,* ***(D)*** *depicts IRT-Mass Balance corrected and calibrated (first available plasma glucose concentration) dialysate glucose.*

3.7.2 CMA64

A comparison of all B&A results between uncalibrated (raw), calibrated on first available plasma glucose concentration, IRT-Linear corrected and IRT-Mass Balance corrected MD data of all CMA64 probes can be found in Figure 45.

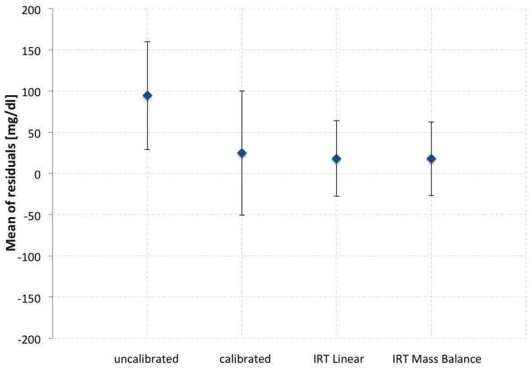


Figure 45: *Comparison of B&A results (Mean±1.96SD) between uncalibrated (raw), calibrated on first available plasma glucose concentration, IRT-Linear corrected and IRT-Mass Balance corrected MD data of all CMA64 probes.*

Figure 46A depicts the B&A graph for all measured raw dialysate glucose concentrations of all 15 evaluated CMA64 probes. (94.5±65.4 mg/dl).

Figure 46B depicts the B&A graph for all dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration, of all 15 evaluated CMA64 probes. (24.7±75.3 mg/dl).

Figure 46C depicts the B&A graph for all IRT-corrected (Linear) dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration, of all 15 evaluated CMA64 probes. (18.2±45.7 mg/dl).

Figure 46D depicts the B&A graph for all IRT-corrected (Mass Balance) dialysate glucose concentrations, one-point calibrated to the first available plasma glucose concentration, of all 15 evaluated CMA64 probes. (17.9±44.7 mg/dl).

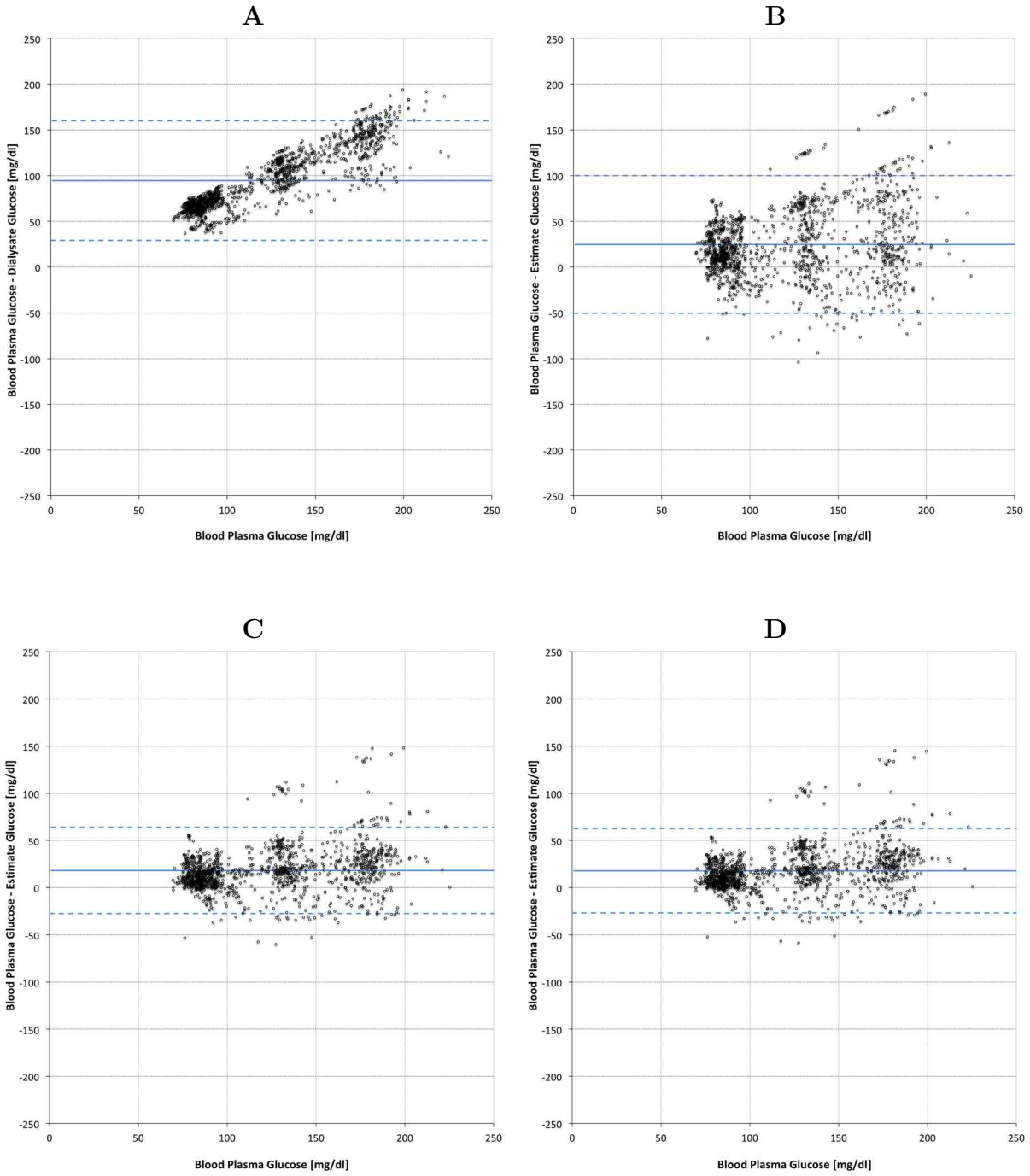


Figure 46: *B&A graphs of all systems using CMA64 probes.* ***(A)*** *depicts uncorrected and uncalibrated dialysate glucose,* ***(B)*** *depicts uncorrected and calibrated (first available plasma glucose concentration) dialysate glucose,* ***(C)*** *depicts IRT-Linear corrected and calibrated (first available plasma glucose concentration) dialysate glucose,* ***(D)*** *depicts IRT-Mass Balance corrected and calibrated (first available plasma glucose concentration) dialysate glucose.*

3.8 Correlation Coefficient

Figure 47 depicts the relationship of the correlation coefficient R between uncorrected (raw) and IRT-corrected (IRT-Linear) data for all analysed 38 systems (subject 020 system 2 was excluded). The correlation coefficient is determined by the Least Mean Squares method with blood plasma glucose and dialysate glucose. In 32 of total 38 (84.2%) systems the IRT-Linear method improves the correlation coefficient (green lines). In 6 systems (2 PME012, 3 PME011, 1 CMA64) the IRT-Linear decreases the correlation coefficient indicated by a negative slope (red lines).

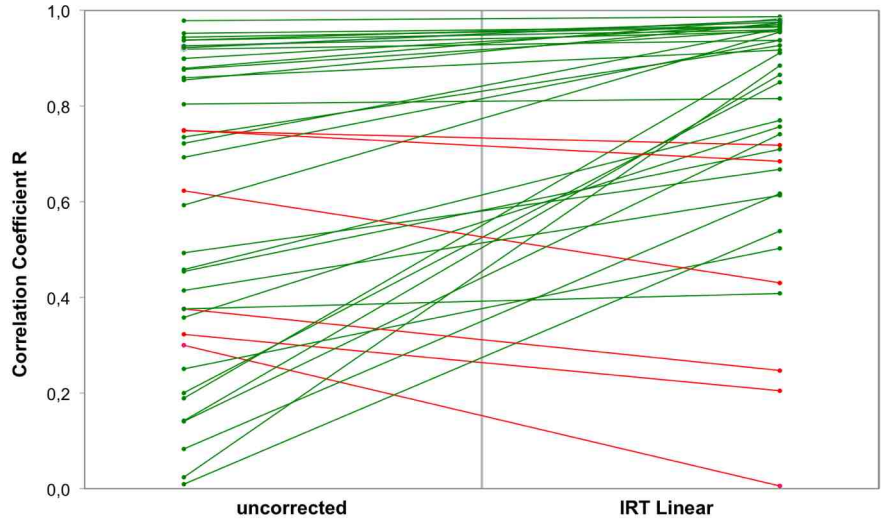


Figure 47: *Relationship of correlation coefficient R between uncorrected and IRT corrected (IRT-Linear) data.*

The relationship of the correlation coefficient R between uncorrected and IRT-data, using the mass balance model, showed results comparable to those of the linear model. The same six systems decrease the correlation coefficient when using IRT-Mass Balance.

Figure 48 depicts the relationship between correlation coefficient and mean glucose recovery for all analysed 38 systems (subject 020 system 2 was excluded). The correlation coefficient was determined by the Least Squares Method (LSM) with blood plasma glucose and dialysate glucose for the whole duration of the experiment. Blue squares represent data derived from the CMA64 probes, whereas the orange and green circles show data of the MicroEye probes (PME012, PME011). The 2 systems with the red circle represent subject 014, which was not clamped. The system with the blue triangle represents subject 019 system 2, where two outlying dialysate values worsen the correlation coefficient to 0.68. When these two outlying points are neglected the correlation coefficient increases to 0.90. Except for these two systems and one outlying system all systems are either located in the yellow or the green area. The yellow area represents data with a poor correlation, smaller than 0.80, whereas the green area shows data with a good correlation, greater than 0.80. All systems, apart from three, with a mean glucose recovery greater than 5% showed good correlation. 37.5% of MicroEye probes and 93.3% of CMA64 probes are located in the green area and show good correlation. Comparable results were achieved using the IRT-Mass Balance model.

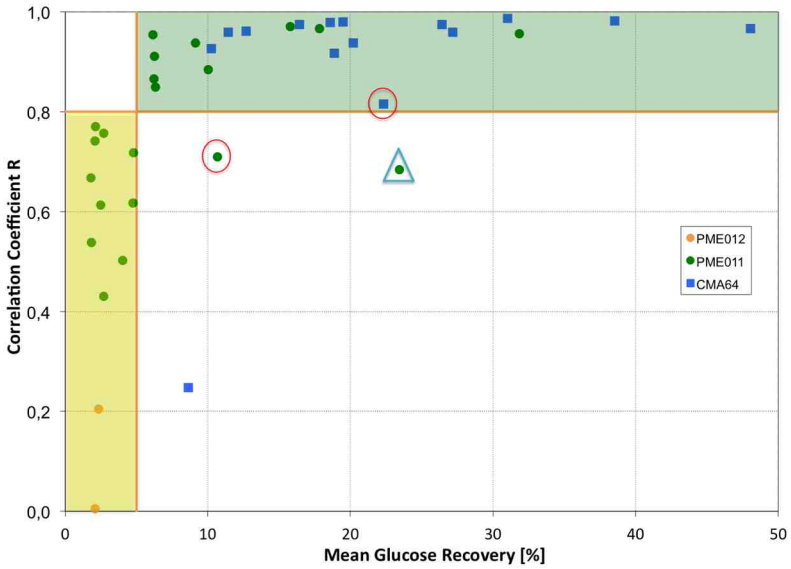


Figure 48: *Relationship between correlation coefficient and mean glucose recovery for all analysed 38 systems after applying IRT (without calibration).*

3.9 Ultrasound Investigations

In 25 systems (9 CMA64, 2 PME012, 14 PME011) an ultrasound investigation was performed. In 6 CMA64- and 8 PME011-probes no thrombus formation was found whereas in 3 CMA64- and 6 PME011-probes thrombus formations were found.

Figure 49 depicts the ultrasound image of the right arm vein of subject 003 system 1 (PME011, 10μl/min, Push, Heparin 50 IU/ml). The bright point in the lower right corner of the red circle is the tip of the PME011 microdialysis probe. Around the catheter a thrombus formation can be observed. Figure 50 depicts the glucose- and ion-recovery over time of the same system. A strong initial decrease of both recoveries during the first 2 hours can be observed. The mean value of glucose recovery was very low (1.8%). An explanation might be the thrombus formation which was confirmed by the ultrasound image. The strong decrease could only be partially compensated with the IRT.

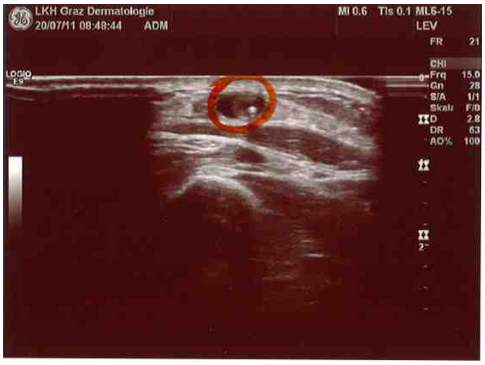


Figure 49: *Ultrasound image of subject 003 system 1.*

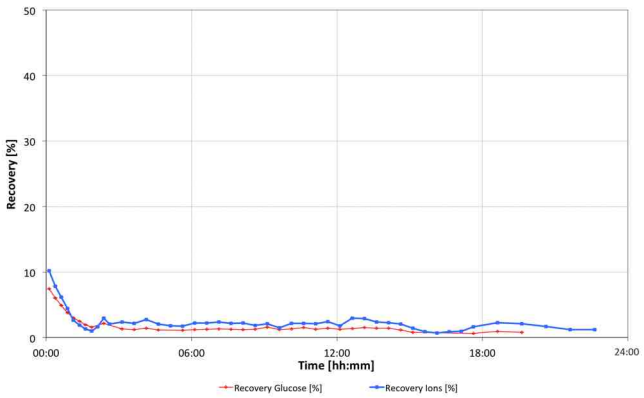


Figure 50: *Recovery profiles of glucose and ions of subject 003 system 1.*

Figure 51 depicts the ultrasound image of the right arm vein of subject 011 system 2 (PME011, 10μl/min, Push, no anticoagulation). Around the probe a low blood flow due to a thrombus formation was observed. Figure 52 depicts the glucose- and ion-recovery over time of the same system. A strong initial decrease of both recoveries during the first 6 hours can be observed. The mean value of glucose recovery was low (6.3%). An explanation might be the low flow due to a thrombus formation around the MD probe which was confirmed by the ultrasound image. The strong decrease could be compensated with the IRT.

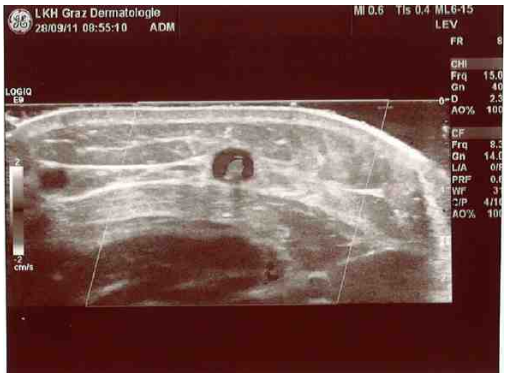


Figure 51: *Ultrasound image of subject 011 system 2.*

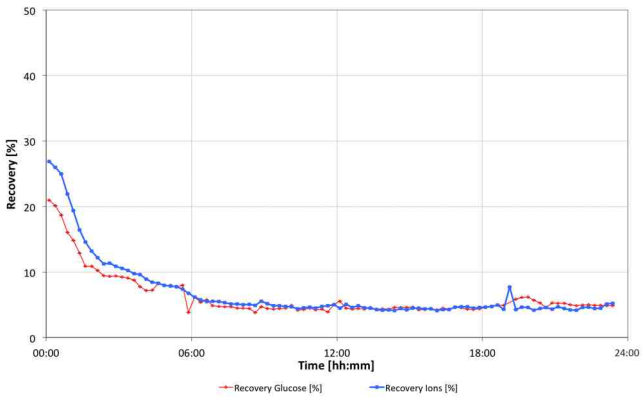


Figure 52: *Recovery profiles of glucose and ions of subject 011 system 2.*

Figure 53 depicts the ultrasound image of the right arm vein of subject 012 system 1 (CMA64, 10μl/min, Push, Arixtra® 2.5 mg/ 20.5 ml). No thrombus formation and a good blood flow around the probe was observed. Figure 54 depicts the glucose- and ion-recovery over time of the same system. The mean value of glucose recovery was very good (31.0%). This might be explained by the good flow around the MD probe which was confirmed by the ultrasound image. The IRT-corrected dialysate glucose showed good results.

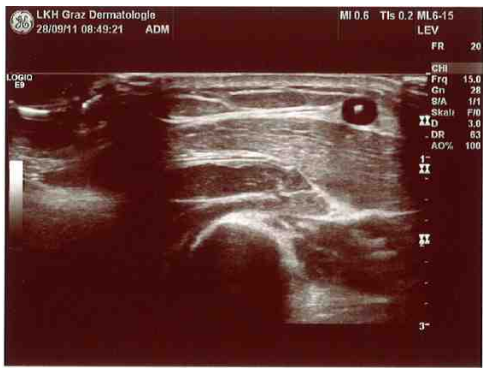


Figure 53: *Ultrasound image of subject 012 system 1.*

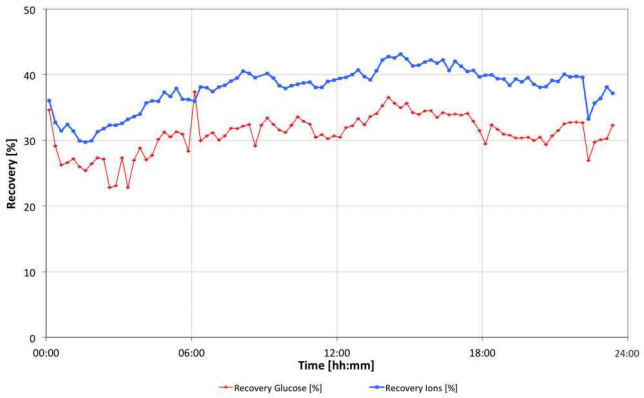


Figure 54: *Recovery profiles of glucose and ions of subject 012 system 1.*

3.10 Anticoagulation

Figure 55 depicts the mean relative recovery of glucose (red columns) and ions (blue columns) for all 39 tested systems, subdivided by the used type and concentration of anticoagulation drug in the perfusate. Despite also other parameters like the flow rate or the used MD probe exert an influence on the recovery, no significant increase of the recoveries was observed by increasing the concentration of heparin immuno in the perfusate. Mean recoveries achieved by the two tested systems without anticoagulation were 6.1% for glucose and 7.4% for ions. The mean glucose- and ion recovery for the systems using heparin immuno were 9.5% and 13.4% for a concentration of 25 IU/ml, 3.1% and 6.0% for 50 IU/ml (2 out of 4 systems performed with flow rate 20 μl) and 10.5% and 13.9% for 100 IU/ml, respectively. In contrast the mean glucose- and ion-recovery for the systems using Arixtra® were 18.1% and 22.8% for the concentration of 2.5 mg/20.5 ml (flow rate 10 μl/min) and 26.9% and 32.3% for the concentration of 2.5 mg/10.5 ml (flow rate 5 μl/min), respectively.

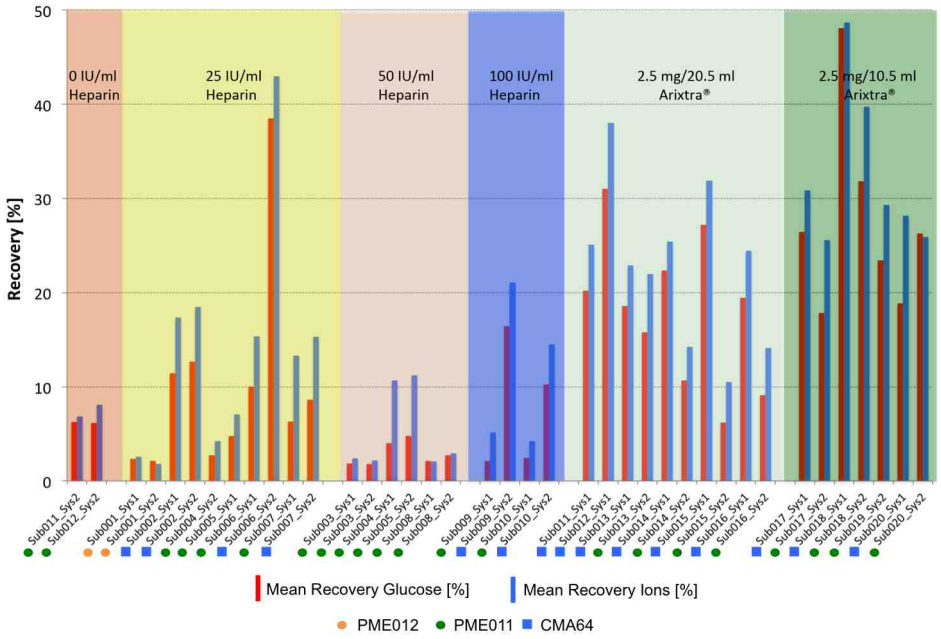


Figure 55: *Mean relative recovery of glucose (red columns) and ions (blue columns) for all 39 tested systems, subdivided by the used type and concentration of anticoagulation drug given to the perfusate.*

3.11 Calibration based on a Limit of the System Error (|SE|<10%)

Figure 56 and 57 depict the calibrated data of subject 013 system 2 (PME011, 10μl/min, Push, Arixtra® 2.5 mg/ 20.5 ml) for uncorrected and IRT-corrected data, respectively. In case of the uncorrected data 27 calibration points are required to assure a system error lower than ±10%. In contrast 15 calibration points are required when using IRT corrected data.

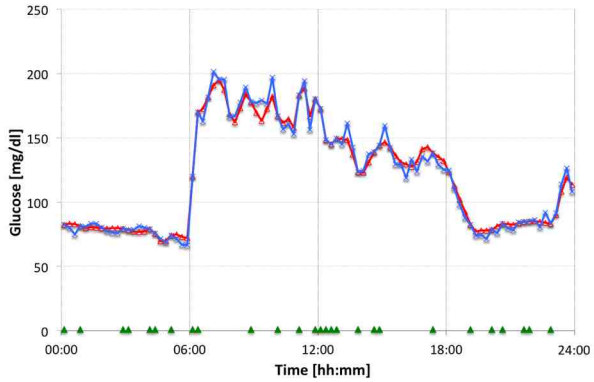


Figure 56: *Calibrated glucose profiles of blood plasma (red) and uncorrected dialysate (blue) over 24 hours of subject 013 system 2. Green triangles indicate the 27 calibration points.*

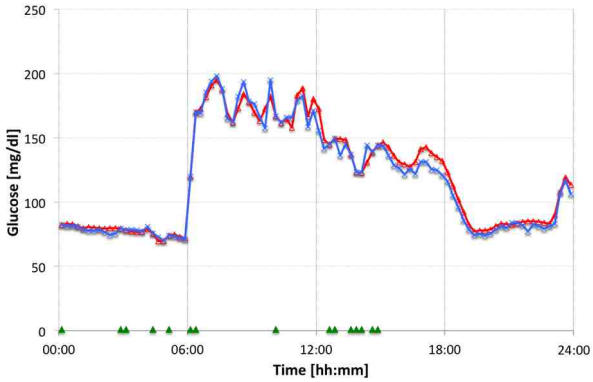


Figure 57: *Calibrated glucose profiles of blood plasma (red) and IRT-corrected dialysate (blue) over 24 hours of subject 013 system 2. Green triangles indicate the 15 calibration points.*

Figure 58 and 59 depict the calibrated data of subject 013 system 1 (CMA64 10μl/min Push Arixtra® 2.5 mg/20.5 ml) for uncorrected and IRT-corrected data, respectively. In case of the uncorrected data 24 calibration points are required to assure a system error lower than ±10%. In contrast 12 calibration points are required when using IRT corrected data.

Figure 60 and 61 depict the calibrated data of subject 012 system 1 (CMA64 10μl/min Push Arixtra® 2.5 mg/20.5 ml) for uncorrected and IRT-corrected data, respectively. In case of the uncorrected data 15 calibration points are required to assure a system error lower than ±10%. In contrast 12 calibration points are required when using IRT corrected data.

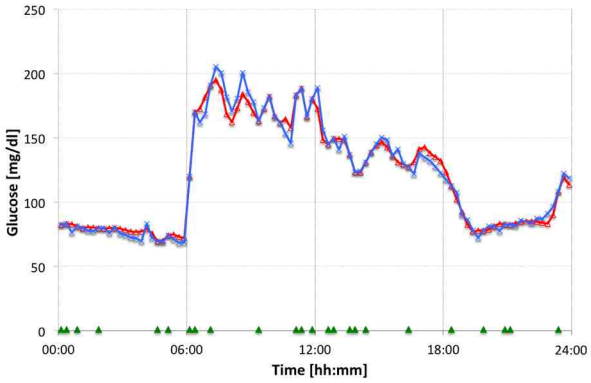


Figure 58: *Calibrated glucose profiles of blood plasma (red) and uncorrected dialysate (blue) over 24 hours of subject 013 system 1. Green triangles indicate the 24 calibration points.*

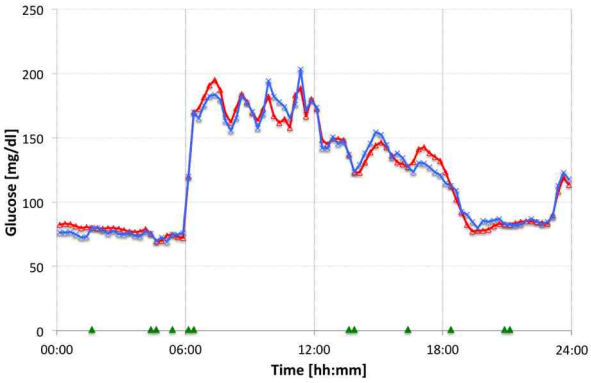


Figure 59: *Calibrated glucose profiles of blood plasma (red) and IRT-corrected dialysate (blue) over 24 hours of subject 013 system 1. Green triangles indicate the 12 calibration points.*

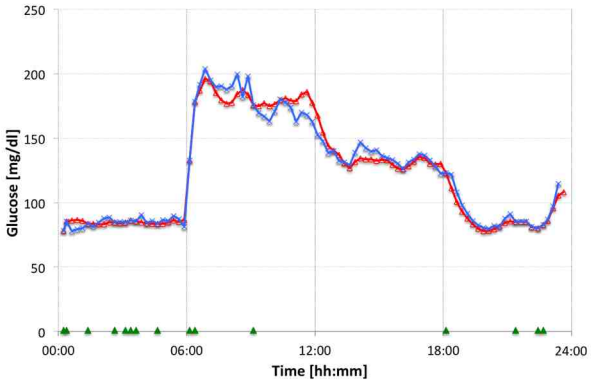


Figure 60: *Calibrated glucose profiles of blood plasma (red) and uncorrected dialysate (blue) over 24 hours of subject 012 system 1. Green triangles indicate the 15 calibration points.*

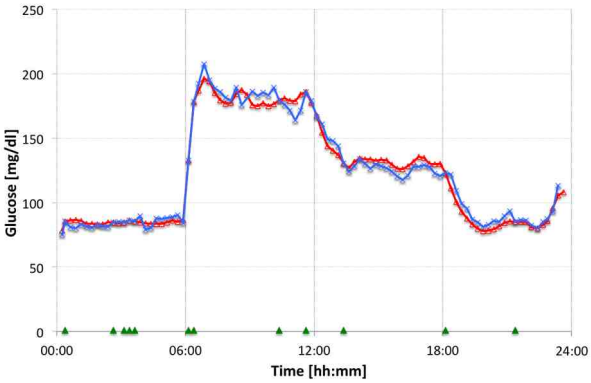


Figure 61: *Calibrated glucose profiles of blood plasma (red) and IRT-corrected dialysate (blue) over 24 hours of subject 012 system 1. Green triangles indicate the 12 calibration points.*

Figure 62 depicts either the increase or the decrease of the number of needed calibration points to meet the limit of the allowed system error of ±10% after applying the Ionic Reference Technique (IRT) onto data of all 38 systems. Blue squares represent data derived from the CMA64 probes, whereas orange and green circles show data of the MicroEye probes (PME012, PME011). In 31 out of 38 systems (81.6%) a decrease of the required number of calibration points, to meet the limit of the system error, can be observed when applying IRT. All 15 tested CMA64 systems show a decrease of the number of calibration points when applying IRT (mean decrease = 12.1 calibration points). At 2 PME012 and 6 PME011 catheters (27.3% of all PME011) an increase of the number of required calibration points can be observed when using IRT. All 17 systems with Arixtra® added to the perfusate show a decrease of the number of calibration points.

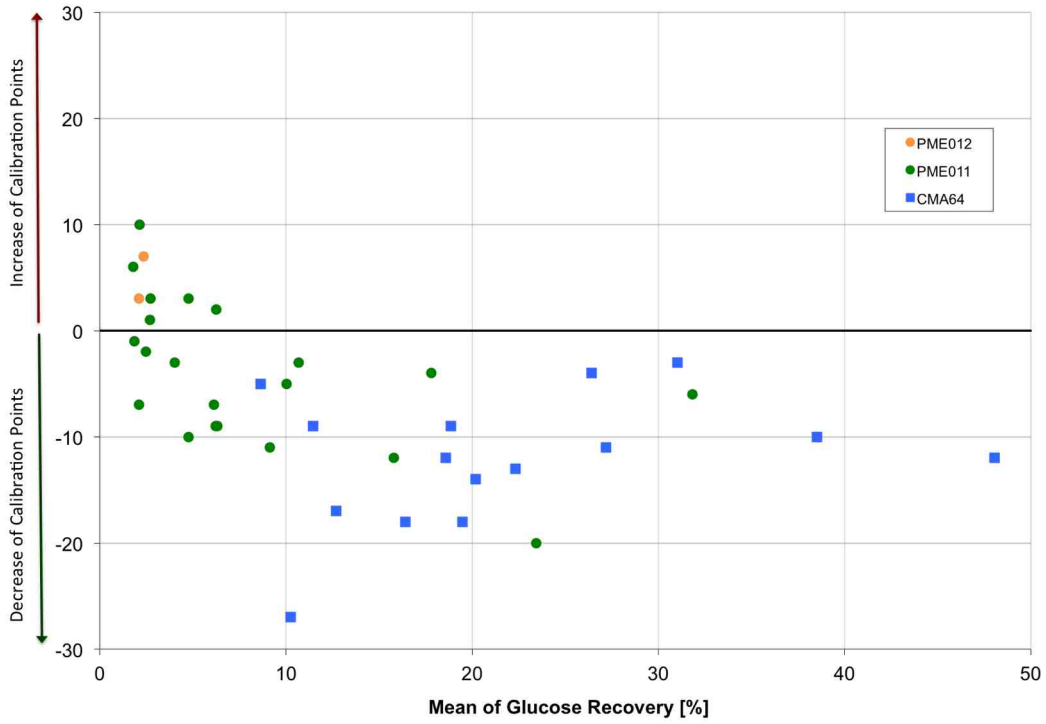


Figure 62: *Increase/Decrease of used calibration points when using IRT (Linear) over mean glucose recovery.*

4 Discussion

In 2010 Hage et al. published the results of a clinical study in which they monitored the blood glucose of 14 patients applying the ivMD and compared them to conventional venous plasma glucose concentrations. They could show an acceptable overall congruence between the two measurements in 10 patients. *„In conclusion, the microdialysis technique appears promising as a future option for continuous glucose monitoring. However, further development of the technology is needed to improve the accuracy.“* [14] Another clinical study by Rooyackers et al. showed the technical feasibility of the ivMD but also concluded that some kind of calibration procedure is required to increase the overall accuracy of the system [15].

It seems that the problems which raised in these studies resulted from changing membrane characteristics of the ivMD probe over time. This behaviour can also be seen in the results gained in the in vivo investigations shown in this thesis. The uncorrected (raw) one-point calibrated dialysate glucose is not adequate for practical use as 9.3% of all glucose estimates are located in the clinically unacceptable zones C, D and E of the EGA. As the IRT should compensate these changing membrane characteristics a significant benefit was expected using this technique. Thus the primary objective of this thesis was to assess if blood glucose can be sampled by means of ivMD combined with the IRT in a reproducible manner in humans. To proof this hypothesis several subtasks were performed, the results of which are discussed below.

The quality of the manual glucose clamp was adequate for the investigation of the MD sampling system. As all 20 subjects are healthy and do not suffer from diabetes, the hyperglycaemic clamp level of 180 mg/dl was the hardest to maintain and thus showed the largest deviations (SD_{Max} = 29.5 mg/dl). Although the plasma glucose concentration was measured in very short intervals (5 min) during this clamp level, fluctuations of the plasma glucose could not be avoided.

The use of the push-pull pump (Joanneum Research MPP101) does not improve the flow stability and its variations. Contrariwise the setup with the push syringe pump (BBRAUN Perfusor) shows a constant flow and fewer variations at flow rates up to 20 μl/min. Furthermore the use of the syringe pump is the more convenient setup as the pump is not attached to the subject´s arm and thus allows unrestricted movements of the subjects. Additionally a built-in overpressure detection decreases the risk of a membrane breakage. Further advantages are, that the system is well known to medical health personal, easier to handle and no battery changes have to be performed due to an external power supply. Thus the push syringe pump is recommended, except if a push-pull system is mandatory to operate an online glucose sensor at the ivMD outlet where an occurring back pressure might influence the dialysis process at the membrane.

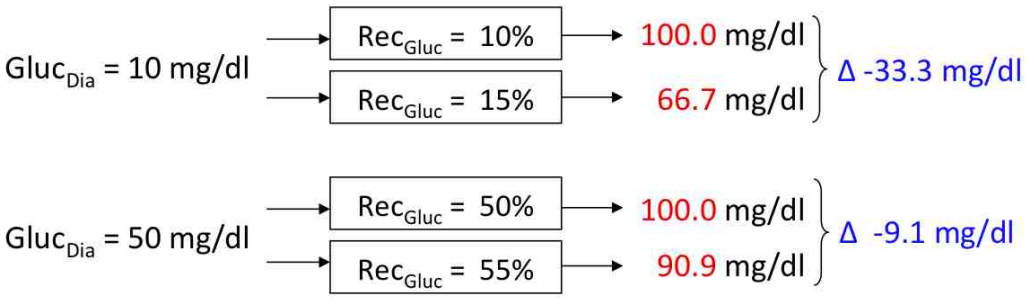
As hypothesized, the mean relative recovery of ions is higher than the mean relative recovery of glucose in most (37 out of 39) systems. In general CMA64 probes yield higher relative recoveries than PME011 probes. Basically it can be assumed that the PME011´s membrane surface is more prone to thrombus formation than the CMA64 membrane and thus the relative recovery for glucose and ions in the dialysate is lower, too. Both probes show a mean initial decrease of recovery which may be caused by swelling (biofouling) of the membrane and deposition of cells causing e.g. thrombus formations around the membrane in the blood vessel.

The flow rate of 20 μl/min results in very low relative recoveries ($< 3.0\%$) which are lower than the limit of quantification of the glucose analyser. For this reason all further investigations were performed with flow rates of 5 and 10 μl/min. The two tested systems with the PME012 probes also show very low relative recoveries (< 2.6 %) caused by the short membrane length of 10 mm. Therefore all further investigations were done with the PME011 and CMA64, the membranes of which have a length of 20 mm.

In order to achieve good results using the IRT, whether with the linear approach or the mass balance model, the correlation between glucose- and ion-recovery should be high. A change of the unknown glucose recovery should be compensated by the ion recovery which is determined by measuring the conductivity of the perfusate. As the mean ratio over time between glucose- and ion-recovery is more stable when using CMA64 probes,

the IRT shows better results using this probes. Only few values show a glucose- and ion-recovery below 10%.

One of the mayor findings in this thesis was, that the correlation between glucose- and ion-recovery and thus the overall performance of the IRT-corrected system increases with increasing relative recoveries of glucose and ions. An explanation might be that the absolute error caused by an uncorrected change of recovery or a wrong determined recovery is smaller, if this change appears in a higher recovery region, as shown in the following example:



As shown in Figure 48 in most cases a good correlation (> 0.8) between blood plasma- and dialysate-glucose is associated with a mean relative recovery higher than 5%. Consequently in 14 out of 15 (93.3%) CMA64 probes the overall performance (correlation) can be improved using the IRT, both with the linear or with the mass balance model. Thus these 14 systems show a good correlation (> 0.8) after applying IRT. As the mean relative recovery for PME011 probes was generally lower, in only 9 out of 22 (40.9%) systems the correlation was higher than 0.8. Furthermore both PME012 probes and 3 PME011 probes show a decrease of correlation after applying the IRT, whether linear or with the mass balance model. In 32 out of all 38 analysed systems (84.2%) the overall performance (correlation) could be increased using the IRT.

EGA results indicate an overall improvement using the IRT, as estimate points move closer to the line of identity. In case of the PME011 probes the amount of values located in zone A of the EGA can be increased from 14.3% to 57.4% (Δ +43.1%) and the amount of values located in zone C, D and E decreased from 15.7% to 1.0% (Δ -14.7%) after applying IRT Linear. In case of the CMA64 probes the amount of values located in zone

A of the EGA can be increased from 45.9% to 69.2% (Δ +23.3%) and the amount of values located in zone C, D and E decreased from 1.3% to 0.7% (Δ -0.6%) after applying IRT Linear.

The analysis using the method of B&A shows that the absolute error and the SD decreases when applying the IRT. The higher the clamp level the bigger is the absolute error and the SD of the estimated values. In case of the PME011 probes the mean absolute error decreases from 65.2 to 10.1 mg/dl (Δ -55.1 mg/dl) and the 1.96SD decreases from 95.9 to 79.1 mg/dl (Δ -16.8) after applying IRT-Linear. In case of the CMA64 probes the mean absolute error decreases from 24.7 to 18.2 mg/dl (Δ -6.5 mg/dl) and the 1.96SD decreases from 75.3 to 45.7 mg/dl (Δ -29.6) after applying IRT-Linear.

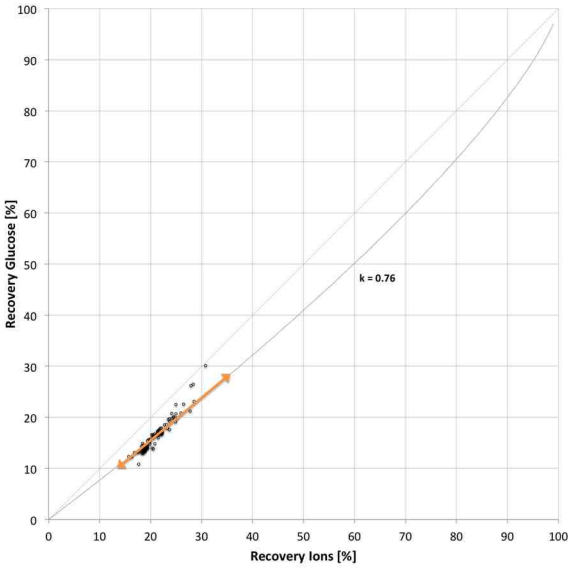


Figure 63: *Linear working range of the mass balance model in Subject 009 System 2 reaching from 15.7 to 30.8% of relative recovery of ions.*

The mass balance and the linear model show comparable results. The percentage of corrected dialysate glucose values in zone A of the EGA can only be increased from 61.9% to 63.2% (Δ +1.3%) when applying the mass balance model instead of the linear method. The same 6 systems (Figure 47) show a decrease of the correlation coefficient, regardless of the used correction model. The mass balance model would only show a significant benefit if the relative recovery of glucose and ions decreases significantly from the beginning to the end of the glucose clamp. As the dynamic range of the relative recoveries between the beginning and end was rather small, the mass balance correction model worked in an almost linear range (Figure 63).

Another issue to increase the relative recoveries and thus the performance of the system was the addition of an anticoagulant to the perfusate. In the first 20 systems (10 subjects) the relative recoveries could not be improved by increasing the concentration of heparin immuno added to the perfusate. The molecular weight of heparin immuno is indicated with 8 - 25 kDalton, the CMA64- and PME011-membrane´s weight cut off is 20 and 10 kDalton, respectively. Thus it may be speculated that only a part of the heparin molecules could diffuse over the membrane to avoid clotting at the outside of the membrane. In contrast Arixtra® another widely used anticoagulant, has a molecular weight of 1.7 kDalton, which is far below the membrane's cut-off of both probes. As a consequence Arixtra® was added to the perfusate instead of heparin immuno to reduce blood clotting around the membrane of the MD probe. The mean relative recoveries for glucose and ions and thus the overall performance was increased using Arixtra®. In 16 out of 17 (94.1%) systems using Arixtra® as anticoagulant in the perfusate the correlation between blood plasma- and dialysate glucose was improved when applying the IRT. In 15 out of these 17 systems (88.2%) a correlation > 0.8 was observed.

Additionally to the anticoagulant in the perfusate subject 011, 012, 013 and 014 received a subcutaneous injection of Arixtra® (2.5 mg/0.5 ml) prior the study. To prove a possible benefit of the systemic anticoagulation no Arixtra® was added to the perfusate at system 2 of subject 011 and subject 012, respectively. In these systems no improvement due to systemic anticoagulation can be observed. Hence to avoid clotting on the membrane it is sufficient to add Arixtra® to the perfusate.

One mayor advantage of the IRT is the decrease of time- and personal-consuming recalibration points, associated with the loss of blood. The IRT decreases the amount of required recalibration points to meet the limit of the allowed system error of $\pm 10\%$ in 30 out of 38 (78.9%) systems. In total all 15 CMA64-systems were improved using the IRT. In total 2 PME012- and 15 PME011-systems were improved using the IRT whereas data sets of 7 PME011 were worsened. In these systems the mean recalibration interval can be increased from 54 minutes to 86 minutes (Δ +32 minutes). This interval could be further increased by introducing calibration criteria, e.g. allowing calibrations only if the slope of dialysate glucose is lower than a predefined limit.

The following limitation should be kept in mind when looking at the data in this thesis. All dialysate glucose values are time-integrated (smeared) concentrations derived from sampling the dialysate over 15 minutes into a probe container. Additional blood plasma glucose concentrations are time-corrected for 7.5 minutes taking into consideration the blood plasma glucose values at the beginning and end of the 15 min sampling interval of the dialysate. Thus the mean of two single blood plasma glucose concentrations is compared to the time-integrated value of dialysate glucose concentration. This drawback will be circumvented by using a continuous glucose sensor as planned for the future development of the clamp device.

5 Conclusion and Outlook

In this thesis the relationship between concentrations of glucose in venous blood plasma ($Gluc_{Blood}$) and glucose sampled with the ivMD technique ($Gluc_{Dia}$) was determined during an in vivo study. A clinically inacceptable low correlation between $Gluc_{Blood}$ and uncorrected but one-point calibrated $Gluc_{Dia}$ was observed caused by changing membrane characteristics of the ivMD probe. The use of the IRT improves this correlation in 32 out of 38 (84.2%) systems. Thus the amount of estimated glucose concentrations in the clinical critically EGA zones C, D and E decreases from 9.3% to 0.8% for all systems.

A relationship between the mean glucose recovery and the performance of the system expressed by the correlation coefficient can be observed. All systems, apart from three, with a mean glucose recovery greater than 5% show a good correlation ($R > 0.8$). Hence one of the mayor findings of this work was that the recovery should be $> 5\%$ in order to achieve proper results when applying the IRT. As a consequence the IRT decreases the amount of required recalibration points to meet the limit of the allowed system error of $\pm 10\%$ in 30 out of 38 (78.9%) systems.

A flow rate of 20 μl/min results in a too low recovery ($< 2.5\%$) and is not recommended. Highest recoveries were achieved using a flow rate of 5 μl/min. Hence 5 μl/min is recommended for the final setup.

Using the push-pull pump (Joanneum Research MPP101) did not improve the flow stability and its variations compared to the push syringe pump (BBRAUN Perfusor Space). Therefore further investigations should be done with the push pump, except if a push-pull system is mandatory to operate an online glucose sensor and its flow cell.

The use of Arixtra® as an anticoagulant locally given to the perfusate to avoid clotting on the MD membrane is recommended as it increases the mean recovery and thus the performance.

The systemic anticoagulation was not shown to be beneficial and thus it is not mandatory for the final setup.

Three different MD probe types were tested throughout the study. The use of the PME012 is not recommended because of the low recoveries (< 2.6 %) caused by the short membrane length of 10 mm. Better results were achieved using the CMA64- and PME011-probes, the membranes of which have a length of 20 mm. In general CMA64 probes yield higher recoveries than the PME011 probes. The IRT improves the correlation of 93.3% of all CMA64 probes to a value > 0.8 and decreases the required amount of calibration points for all probes. In contrast in 86.4% of all PME011 probes the correlation improves by the use of the IRT but only 42.9% of them show a correlation > 0.8. In conclusion glucose estimates achieved with the CMA64 probes show a better correlation with the blood plasma glucose than the PME011 probes.

In general the mass balance correction model for the IRT shows better results than the linear model. However the correlation coefficient shows comparable results as achieved when applying the linear model. The mass balance model is recommended when significant changes of the recovery are expected.

Further investigations will be performed with a glucose sensor placed at the outlet of the MD probe allowing a continuous online monitoring of the dialysate glucose ($\mathrm{Gluc_{Dia}}$). The findings of this thesis will be implemented in the design of the online sensor evaluation.

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List of Abbreviations

AGES	Austrian Agency for Health and Food Safety
B&A	Bland and Altman
BMI	Body Mass Index
CV	Coefficient of Variation
EGA	Error Grid Analysis
GCP	Good Clinical Practice
$Gluc_{Blood}$	Blood plasma glucose concentration
$Gluc_{Dia}$	Dialysate glucose concentration
iv	intravenous
$Ions_{Blood}$	Conductivity of blood (assumed to be like the conductivity of NaCl 0.9%)
$Ions_{Dia}$	Conductivity of dialysate (in % of conductivity of NaCl 0.9%)
IRT	Ionic Reference Technique
ISF	Interstitial Fluid
LLOQ	Lower Limit of Quantification
LSM	Least Squares Method
MARD	Mean Absolute Relative Difference
M2ARD	Median Absolute Relative Difference
MD	Microdialysis
MUG	Medical University of Graz
%PRESS	Predicted Error Sum of Squares
Q	Flow rate
R	Mass transfer resistance
Rec_{Gluc}	Relative recovery of glucose
Rec_{Ions}	Relative recovery of ions
SD	Standard Deviation
SE	System Error
WHO	World Health Organization

List of Definitions

[mg/dl]	common used measurement unit for the glucose concentration, 1 mmol/l = 18.0182 mg/dl
[IU]	international unit, a unit of measurement for the amount of a specific substance in pharmacology (e.g. 1 IU insulin = 45.5 μg pure crystalline insulin)
[Dalton]	unified atomic mass unit (1.660539 * 10^{-27} kg)
relative Recovery	ratio between concentration in the dialysate and concentration on the outside of the membrane (e.g. blood).
molecular Weight Cut Off	lowest molecular weight of a molecule in which 90% is retained by the membrane.
line of identity	In a 2-dimensional cartesian coordinate system, the identity line is the y = x line
1.96SD	1.96 * Standard Deviation, in case of a Gaussian distribution 95% of all values lie in this confidence interval.

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Statutory Declaration

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

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Date Signature