Clinical Evaluation of Novel Methods to Determine Dialysis Parameters Using Conductivity Cells

Dissertationsschrift zur Erlangung des
naturwissenschaftlichen Doktorgrades
der Bayerischen Julius-Maximilians-Universität
Würzburg

vorgelegt von

Rainer Goldau

Würzburg 2002

Eingereicht am:

1. Gutachter: Prof. Dr. R. Benz

2. Gutachter: Prof. Dr. H. Lange

Table of Contents

Hinweis: *Eine Zusammenfassung in Deutsch findet sich auf Seite 144.*
Variablennamen, die im Text verwendet sind, werden in Kapitel 8 / S.118 erklärt.

Variable names are introduced in chapter 8 / page 118.

1. Introduction

Nowadays dialysis has become an irreplaceable part in the therapy of end stage renal disease (ESRD). Compared to the first human experimental dialysis treatment that has been performed in 1924 by Haas in Gießen [Ha1] which was hardly attributed with life preserving capability, further regarding the conditions under which Kolff [Ko1] did his first life saving dialysis in 1945 with a large rotating drum dialyser, hence the early era of coil dialysers within Ringer solution drums in the '50s, looking back to the early '60s when dialysis slightly became available to a minority of ESRD patients sometimes selected by commissions of physicians to decide who is to be dialysed [Mur1] [Lin1] we now after the turn of the millennium can appreciate the enormous effort of clinical investigations that have developed dialysis to be a great art of preserving the lives of millions of patients in more or less industrialised countries. Today the artificial kidney has abolished the fright of terminal kidney insufficiency completely. In combination with kidney grafts the clinical ESRD outcome is encouraging. But it is still not satisfactory yet. There is no reason to rest with endeavour. Why is it not satisfactory ?

The first point is that it is still an 'artificial' kidney, far below the performance of our natural kidneys. The clearance, which in simple words is the mass flux of a particular solvent from the body to a pure drain divided by its concentration in the body, is still applied insufficiently and periodically by hemodialysis and not high effective and continuously as demonstrated by nature. The composition of the dialysed diuretic toxins at the drain of the artificial kidney and the way of extraction differs completely as it mainly comprises passive diffusive and convective effects. Its original within the glomerula and Henle's loop works with an extremely intelligent cascade of membrane types and different concentration gradients which are actively controlled and supported by metabolic and hormonal processes, not yet completely understood. Hence the electrolyte and fluid homoeostasis regulatory effects are not matched by the artificial kidney. Endocrinological aspects completely are excluded from being modelled by the artificial kidney. Aspects related to gene technology and xenotransplants will not be considered here but do not attenuate the statement that more than 75 years after the first dialysis mankind still is far from having created an adequate substitute for the human kidney. We still have to learn a lot from nature.

The second point is the money. Nature supplies us free of charge with high quality "dialysis". Artificial life preserving dialysis – with all its present disadvantages – is still a privilege of the rich. This can easily be understood if we face the tremendous costs dialysis is accompanied with. There are costs of hospitalisation with all its staff and care facilities, of the dialysis machines, the filters and also the follow up costs for treatment of ESRD comorbids which are manifold.

Both aspects can be expected to remain for a unforeseeable period. Even if xenotransplants recently more and more seemed to rise hope for sustaining improvement of the therapy "conventional" hemodialysis will continue to play a major role in ESRD treatment throughout at least the next decade. It can be expected that in spite of the long and narrow course of the past hemodialysis development has potential to become more physiological. To supply a maximum number of patients in

the world with hemodialysis today the primary goal of the investigations in this field today can not be to perfectly copy the kidney in all its integral functions but to determine and to model those fractional functions and mechanisms that are either essential to preserve a high long term life quality for the patients as also a reasonable compromise on costs. In a mass business as hemodialysis is today costs predominate the discussions. Scientists and engineers working in the field will strongly be judged by cost effectiveness and any new feature or treatment alteration will only succeed widespread if it fulfils this criterion. This work is committed to this maxim without loosing the focus on the major goal: the benefit of the patient.

Its the intention of this work to elucidate the potential and the limitations of conductivity based dialysate measurements during hemodialysis as a mean to get more online information on the status of the patient and the treatment effectiveness without involving any additional efforts or costs for either the patient, the physician or the hospital like blood loss for samples, handling of syringes, laboratory chemistry or just time. It will be demonstrated that a simple conductivity cell has potential to supply a lot of information about clearance, about aqueous distribution volume of the patient and about plasma sodium. It will be emphasised the conductivity cells capability to control the correct 'dosage' of dialysis and therefore ensure sufficiently low long term mortality rates. The proportional costs of the conductivity cell per treatment is approaching zero.

The use of conductivity cells moreover is a comfortable mean to assure quality of the treatment. Quality assurance is not only mandatory to keep the high dialysis standard and to decrease the patients mortality rates but soon will become a mandatory component of the surgery accountancy.

Considering the disadvantages patients in some countries have to stand as there is short term dialysis with its high blood flow rates and subsequent risk of high recirculation, arterial cannula suction or fistula problems, hence reuse of dialysers, economised attendance of the physician and insufficiently maintained dialysis machines we can state that conductivity based dialysate measurements are a powerful tool to online detect any irregularities that correlate to dialysis ineffectiveness. Moreover it is to deplore that in a time when dialysis is an economic matter and has become subject of commerce there might also arise a certain need to protect the patient from improper dialysis.

1.1. Motivation of the investigation

Towards the end of the sixties of the last century dialysis became available to numerous patients and was no longer a privilege of only a few. The lack of mandatory standards that describe how to perform not only lifesaving but high quality dialysis became obvious. In addition to the fundamental question on the nature of the mortal mechanisms associated with uraemia - which are not yet understood today - in particular the dialysis quantification problem became apparent. No physician was able to predict the amount of dialysis that is required to keep an ESRD patient not only alive but preserve a high long term quality of his life. To address this problem in 1975 a conference was held in Monterey, CF, USA [Go2] on which it has been realised that there is an urgent need to conduct a prospective, randomised

multicenter study to determine the adequate dose of dialysis. The National Cooperative Dialysis Study (NCDS) was funded to fulfil this need. First results of this study have been published in [Lo1].

The NCDS – now more than 20 years of age - had strong impact on the scientific efforts to understand the mass kinetics that stand behind the detoxification dialysis performs. One of the initiators of the first kinetic modelling were Sargent and Gotch [Sar3]. They first introduced an equation system for calculating the concentration of toxic substances on basis of mass transfer from and into the solvent volume of the human body via the artificial kidney, the residual function of the native kidneys and the generation of new toxic solutes by the liver. One of the basic premises of this model was that urea would be a convenient marker substance to describe the behaviour of small molecules in dialysis even if a single toxic target solute to be responsible for uraemia has not been identified up to now. It was the first time a model purely based on mass balance had potential to quantify dialysis. Initially this model was only focused on the prediction of the normalised treatment ratio Kt/V of a dialysis treatment as the quantity that matters, where K is the clearance for urea, t the treatment time and V the distribution volume for urea within the patient. Implicitly it was assumed that urea clearance based Kt/V would be a valid marker able to express the success and failure of dialysis. This is to understand on the background that the human kidney in some aspect has only been modelled to be a simple filter for solutes that does little more than to remove them diffusively. In this terms success of a treatment would mean to remove an adequate amount of urea and other solutes and it would only have to be considered what amount is 'adequate' in this sense. It can be argued if this is a technocratic reduction of the broad functional spectrum of the human kidney or if it is a permissible simplification of the complicated but finally limited functionality of the kidney [Lo2, Go3]. However the nephrologic community felt the captivating simplicity of the concept that could express dialysis success in only one value and even if not commonly accepted and surely not finally or sufficiently discussed Kt/V today is more and more accepted –predominantly in the US- as a measure for long term dialysis mortality and outcome, regardless of the partially lacking scientific basement and the turn of dose calculations to mortality predictions. Concomitant to uraemia there are manifold alterations of the physical condition of an ESRD patient: deviations in concentrations of numerous other solutes like creatinine, β2 microglobuline, electrolytes, not only a minority of the patients suffers from diabetes, blood pressure problems, severe vascular degeneration and malnutrition as only to mention a few. It is not easy to imagine that dialysis outcome and its interaction with all these different clinical characteristics can simply be expressed by a simple number. Nevertheless statistics draws another image: It seems to be possible, although there are some discrepancies that are not clearly understood [Lo2, Go3].

It is beyond this scope to discuss the Kt/V – mortality relation extendedly. How ever this discussion may end, the premise of this work is just to give a tool to the hand of the nephrologist to reliably measure Kt/V without value judgement. The interpretation of the measurement will ever be left to the clinician's responsibility. Some of the relevant facts considering clearance and Kt/V are explained in the following section.

1.1.1. The Clearance and the Kt/V concept

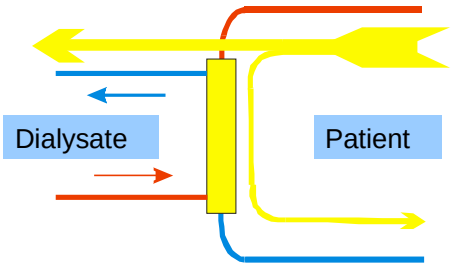


Figure 1.1.1.a: *In dialysis the artificial kidney is the link between patient and dialysis machine that permits diffusive and convective substance transfer from the patients blood to the rinse fluid, the dialysate.*

To understand the use of Kt/V as a dialysis quantifier it is necessary to first define some technical expressions like V, the distribution volume, K, the clearance and some further definitions.

V describes the volume of the aqueous solvent that is available in the human body for distribution of a particular substance.

In this context the substance is urea or sodium. All aqueous compartments in the human body are available to solve urea, as there is the intravascular, the interstitial and the intracellular fluid (ICF). The intravascular and interstitial fluid can be summarised as extracellular fluid (ECF). Sodium essentially has only ECF available for distribution, because the sodium pump maintains a physiological sodium concentration gradient across the cell membrane. The implications for the externally visible distribution space for sodium will be discussed later.

The clearance in general is a quantity that describes how efficient a biological or artificial system like the kidney or a dialysis process is able to clear its target fluid, which will always be the patients blood in this context (see figure 1.1.1.a). During dialysis the blood has very close contact to the rinsing fluid, the dialysate. The link is embodied by the artificial kidney, which consists of thousands of hollow fibres, at their outside being flushed by the dialysate and having a sum surface of usually more than $1m^2$. Each fibre has an outer diameter of typically 200µm, the inner diameter, which allows the passage of blood is 140µm. The fibre wall consists of a porous material (e.g. polysulfone) manufactured by a special spin procedure that generates a pore size gradient starting with the smallest pore diameter of approximately 3-5nm at the lumen wall (figure 1.1.1.b). These pores are completely passable for water and small molecules. As soon as molecule size rises, the diffusive transfer is more and more impeded and the molecules can only pass the membrane by pressure gradients in a convective flow. Diffusiveness is decreasing for

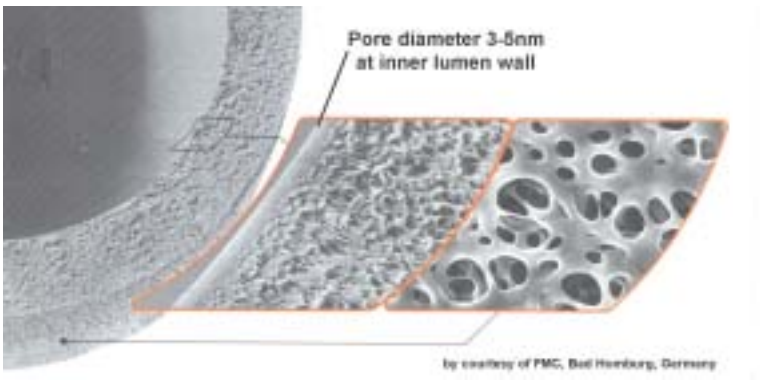


Figure 1.1.1.b: *Cross section of a single capillary of a dialyser.*

heavy molecules and on a logarithmic scale a cut-off around molecular weight of 65000 is realised to avoid albumin loss to the dialysate (figure 1.1.1.c). Substances with higher molecular weight than the cut-off limit can hardly pass the membrane neither by diffusion nor by convection. It is clear that the passage of small molecules like electrolytes, urea or water is predominated by diffusive effects, the transfer of more complex molecules like β2 microglobuline (β2m) or inulin is forced purely by convection, that means water molecules will drag them across the membrane by hydrostatic or osmotic pressure. Further it is to emphasise that there is not only a superficial contact but an intensive mixing of plasma water and dialysate water along the fibre. Dialysis clearance can be defined as follows:

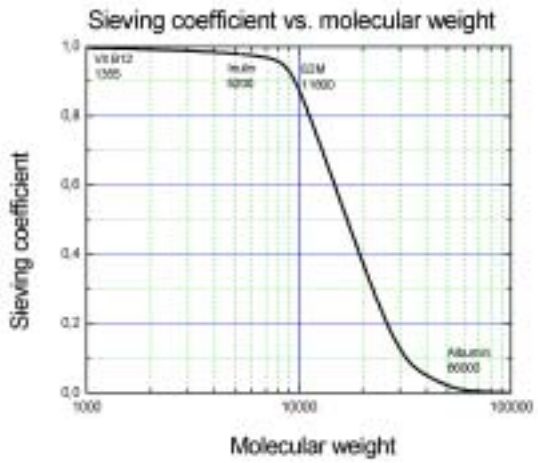


Figure1.1.1.c: *The dependence of the sieving coefficient from the molecular weight. Typical curve for a modern high flux polysulfone membrane (by courtesy of FMC, Bad Homburg).*

The clearance is that flow rate fraction of the blood water flow rate that is completely cleared from a particular substance. As long as the sieving coefficient of this substance is 1 the convective flow rate of filtered blood water across the membrane can be added directly to the clearance because the patient could replace it by pure water.

A sieving coefficient of 1 means the molecule can pass the membrane as easy as water in a convective fluid stream. The clearance definition is only valid for substances like urea, whose concentration in the inlet dialysate is zero. For sodium the inlet concentration in the dialysate is not zero but typically 140 mmol/l. Another expression is used for this case, the dialysance:

The dialysance is that flow rate fraction of the blood water flow rate that is completely brought to the dialysate inlet concentration of a particular substance. As long as the sieving coefficient of this substance is 1 the convective flow rate of filtered blood water across the membrane can be added directly to the clearance because the patient could replace it by water of the dialysate inlet concentration.

In the definition the *blood water flow rate* is mentioned, to be distinguished from the whole blood flow rate. The difference is that only the aqueous solute fraction of the blood is available as solving space. Also the blood cells contain solving space. The rest like proteins and complex structures like cell walls is passive space in this context. The difference is about 10% of volume. From this definitions it is to emphasise that the clearance can never exceed the blood water flow rate.

The time t expresses the duration of the dialysis treatment. If any interruptions of the treatment like alarms or dwelling of the pumps leads to a clearance interruption, t is not interrupted.

According to the clearance definition Kt or more precisely $\int K_{eff}(t)dt$ is the absolute amount of fluid that is completely cleared from a substance. This volume should be at least the volume of fluid that has to be cleared, which is the patients distribution volume for this substance. This implies a simple dosage definition: The quantification of dialysis should assure that the aqueous volume of the patient is cleared at least once. This expressed in mathematical terms means: $1 \leq$ Kt/V. In particular if the substance is urea the catabolic generation during dialysis should also be taken into account. A patient who catabolizes protein to 10mg of urea per minute will produce 2.4 g of urea during a 4h dialysis session that was not present at dialysis start and will have to be cleared additionally. A typical hemodialysis regimen will schedule him every 2 days for 4h of dialysis. That means his dialysis time ratio would be 4h divided by 44h, approximately 9% of his time he is under dialysis and therefore he produces 9% of the urea during the treatment. One might estimate that the dose therefore should be increased for at least this 9%. Further there is evidence to expect urea generation rate being increased during and towards the end of treatment [Bo1, Sha1, Fa1] for some amount (50-100%), so that from a purely mass transfer based aspect one should recommend the Kt/V for urea to be at least approximately 1.15 in a linear consideration for patients with no residual kidney function. This is not a precise calculation but a stringent argument how Kt/V should be prescribed.

1.1.2. Kt/V and Mortality

The ongoing discussion about mortality and Kt/V can be condensed to three different question complexes that can not clearly be separated or even be solved:

1.) Why must urea and other small molecules like creatinine and pseudouridin be removed and can not be left in the body? What is their toxic concentration and mechanism? What is “the” uremic toxin? Is there any?

2.) What minimum value of Kt/V(urea) is sufficient to remove urea and other small solutes with similar removal pattern sufficiently?

3.) Is Kt/V as a dose parameter for small solutes a valid marker to predict the patients outcome? Is only Kt/V, or is Kt or t alone of mortality predictive character?

In table 1.1.2.a is depicted a summary of the major solutes the human kidney clears from the blood and their toxicity properties.

The discussion about these questions is reported here to only that extend it needs to understand the context and the background of this work. A comprehensive review of the present state of discussion is found in [SemDial].

The first question – as it seems to the author – has not really found an answer yet. It is not the question if urea has to be removed. The healthy kidney as the shining example removes small solutes including urea, and it does it very effectively and continuously. There is no evidence to believe that natural urea removal is just a side product of the evolution because it is well known that urea is the final protein

Solute	*Toxicity*	*Removal parallel to urea*
Small water soluble compounds		
Urea	±	
Creatinine	-	+
Pseudouridine	-	+
Uric acid	±	+
Potassium	+	+
Xanthine	+	-
Hypoxanthine	+	-
Phosphorus	+	-
Middle molecules:		
β2 Microglobuline	+	-
AGE	+	-
Parathyroid hormone	+	-
Leptin	+	-
Small protein bound compounds		
P-cresol	+	-
Indoxyl sulfate	+	-
CMPF	+	-
Hippuric acid	+	+
Homocysteine	+	-

Table 1.1.2.a *Uremic solutes and their biological impact and removal pattern [Van] AGE: Advanced glycation end products CMPF:Carboxy- methyl-furan propionic acid. [Van, modified]*

catabolite. So it is likely urea must be removed due to osmotic reasons. Protein intake is essential to life and a non-removal of urea would result in a strong overload of urea and high plasma urea concentrations. An essential enzymatic process like protein catabolism can be expected to be independent to some extent from the concentration of its products but it can not be imagined it would work properly in the presence of extremely high, non-physiological concentrations of urea. In a consequence non removal of urea would lead at least to a high osmotic impairment. This would prevent the cells from keeping their natural osmotic equilibrium and water homeostasis. Water as the solute for every reaction in the body will be shifted into the cells with high urea concentration. This will affect enzymatic processes. A good example are the mitochondrial multi-enzyme systems located on the mitochondrial membrane [Gil, See]. Their activity is dependent on the spatial distance of the active reaction centre on the membrane. Water influx will influence membrane distance and deteriorate the reaction rate of multiple enzymes and therefore lead to multifunctional perturbation. This and other mechanisms may lead to a severe uremic syndrome. The consequences are well known from the time dialysis was not available.

This may lead us to the second and third question about the dosage of dialysis. The stringent physical consideration that Kt/V must be 1.1 or 1.15 is only one aspect. The background is that today Kt/V no more is just a quantification but is increasingly interpreted as a measure for clinical outcome and morbidity. Since the NCDS study [Low2] at the end of the seventies there are many publications that discuss if Kt/V is a valid and independent parameter to predict the long term survival and the treatment success in terms of quality of life for the patients. It can only be listed here a few of them representing the major topics of the discussion. The interested reader may find numerous further quotations within these publications [DOQI, Go1, Go2, Go3, Go5, Ley, Low, Ow, Par, Sar4, USRDS, Wo]. In particular in [SemDial] several experts of the field give a broad overview on the state of discussion.

The discussion in this field currently has reached a more differentiated view. There are those authors who completely disagree that Kt/V is a measure for mortality at all, although they seem to be in the minority. But they have strong arguments because the body size itself seems to be correlated to morbidity. Small and tiny patients with a

small V usually experience a high Kt/V. If Kt/V is a mortality predictor they should show lowest mortality under dialysis. The opposite is the case. Their long term survival is worse than that of the patients with high V and therefore lower Kt/V. Malnutrition since years is known as an dialysis associated problem. Obese patients to some extent seem to be more tolerant of underdialysis. The treatment time seems also to be correlated to mortality [SemDial]. A common sense however seems to exist among most of the authors that in a 3x4 hours per week dialysis schedule the urea based Kt/V should not fall below 1.2, which is in good accordance with the initial consideration. The widely accepted DOQI guidelines recommend this value to be the minimum. Also the older NCDS study [Go1, Lo1,Lo2] supports this opinion.

Conclusively it must be stated that the discussion has not yet decided if Kt/V is an independent predictor for mortality. But is has been decided that Kt/V may not fall below a particular minimum. To assure this is the central matter of this work.

1.1.3. Urea and Sodium Chloride

	Diffusion coefficient in free solution *[10^{-5} cm^2/s]*
Urea	1.808
Sodium	2.156

Table 1.1.3. a:- *The diffusion coefficients of urea and sodium chloride [Klein]*

Both urea and sodium are implicitly used as a substitute for each other in this work. This is on the background of similar diffusion coefficients that can be utilised for technical purposes (table 1.1.3.a):
Urea is not very accessible to a quantifying measurement unless a precise enzymatic sensor is available. The enzyme splits urea to ammonium-ions that can be detected as an electrical potential across ion-specific membranes, needing further sophisticated electronics to amplify the current. Therefore the use of enzymatic sensors is accompanied with some disadvantages: The enzyme must be kept cold until its contact with the urea solution. Further the sensor must be calibrated frequently to keep its accuracy. This requires a standard solution of known urea concentration. This calibration solution also needs sterile and cool conditions to not decay in concentration.

The situation is different for sodium. Sodium can easily be measured using conductivity cells. If the diffusion coefficients for sodium and urea are similar, they will behave similar at a membrane and in particular show similar clearance. The idea is to measure sodium as a substitute for urea. Conductivity cells are technical standard and present in any dialysis machine. The question is if in presence of the other accompanying solutes in the dialysate the analogy is maintained so that the behaviour of sodium and urea at the dialysis membrane is sufficiently comparable. If this analogy is acceptable a valid substitute for urea clearance measurement is found.

2. Theoretical Considerations

2.1. The effective dialysance and the effective clearance

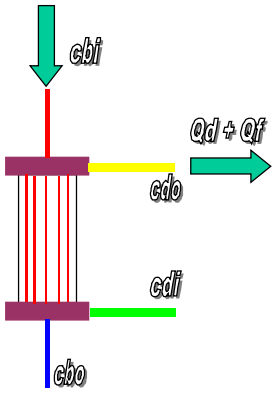


Figure 2.1.a: *The description of the concentration and flow variables at the filter.*

A more formalistic but equivalent definition of the dialysance refers to the mass transfer of a given solute via the filter per unit of time, divided by the blood and dialysate inlet concentration gradient of this particular substance (figure 2.1.a):

$$K_{eff} = \frac{\text{extracted mass flow}}{\text{concentration gradient}} = \frac{(Q_d + Q_f)cdo - Q_d\,cdi}{cbi - cdi}$$

E 2.1.a

If the cd values do not stand only for conductivity but for a concentration in general, this is the fundamental formal definition of a effective dialysance, valid for any substance that is imaginable to be observed. Also the ultrafiltration Q_f is included which makes the flow rate out of the filter higher than the incoming flow rate. Clearance and dialysance have already been defined in words. Dialysance is the more general expression E 2.1.a. Formally clearance describes the subgroup if cdi is zero:

$$K_{eff} = \frac{cdo\,(Q_f + Q_d)}{cbi}$$

E 2.1.b

This is the case for urea clearance, because the dialysis machine does not supply the dialysate inlet with urea but with sodium and other ionic substances. Therefore the expression ‘dialysance’ is correct for the electrolytic dialysance.

2.2. Relation of ionic Dialysance and Urea Clearance

One of the fundamental questions of these studies is the equivalence investigation of urea clearance and ionic dialysance. For a comparison the mathematical expressions for both must be defined. This needs some further considerations on the mass transfers that are measured. If we use sodium as a substitute for urea, of course the absolute mass transfers are different. So the comparison does not apply to sodium masses or urea masses directly but only to clearance and dialysance. This difference must be emphasised clearly: According to the dialysance definition we can only compare the ability of the patient-filter-dialysis machine- system to clear a certain fraction of available mass of urea or sodium. The need of suited formulas to insert

those values that are available is evident and the following sections will provide the necessary information.

The index ‘eff’ in E 2.1.a,b indicates the reference to the ‘effective’ clearance including the cardiopulmonary and fistula recirculation. This is an important distinction because the possible clearance of the filter is reduced by the direct reflux of a fraction R of the freshly dialysed blood to the filter without having passed the tissue areas where it could be loaded with uremic toxins again. The effective clearance can be measured from both sides of the filter, blood-side and dialysate side. We will consider the blood side first.

2.2.1. The Role of Recirculation

When the patient is connected to the dialysis machine with its blood pump running there is no access to systemic blood at the arterial and venous blood lines, which connect filter and patient. Only systemic blood is of the representative urea concentration that is needed to calculate the effective clearance. The recirculation causes the arterial blood at the entrance of the dialysis filter to be consisting of a – usually large – fraction of systemic blood that has passed the capillary system of the patient and a minor fraction of recirculated blood that did not. After leaving the dialysis filter through the venous bloodline and entering the arterio-venous fistula the minor fraction has passed the lung circuit or has directly found its way back to the arterial needle. Both pathways did not load the blood with uremic toxins, so this blood would not need to be dialysed again immediately. Typically this fraction R is in the amount of 0.05-0.07 of Q_b. If it would rise to 0.9 still a high urea filter clearance could be measured using the urea concentrations and flows at the arterial and venous connectors of the filter. Without external notice in fact nearly all blood has recirculated and the effective clearance would be dramatically decreased, because only 10% systemic blood participate. An online clearance measurement system therefore would need to be able to measure the effective clearance, not the filter clearance. A blood-side reference as it is needed for the study must also reflect the effective clearance, based on concentration measurements at the arterial and venous connectors, because only those are available.

If systemic blood would be accessible the blood-side clearance could be written directly as the effective blood side urea clearance:

$$K_{eff} = KeUB = \frac{Q_{Systemic} c_{Systemic} - c_{ven}(Q_{Systemic} - Q_f)}{c_{Systemic} - cdi} = \frac{Q_{Systemic} c_{Systemic} - c_{ven}(Q_{Systemic} - Q_f)}{c_{Systemic}}$$

E 2.2.1.a

Again this is the extracted mass flow divided by the gradient. With no urea in the dialysate inlet cdi is zero and the right term is equivalent. Assuming the fraction of recirculation, R, is known, the concentration of the arterial blood at the filter inlet would mix with the venous outlet blood according to

$$c_{art} = (1-R)c_{Systemic} + Rc_{ven}$$

$$c_{Systemic} = \frac{c_{art} - Rc_{ven}}{1-R} \qquad \text{E 2.2.1.b}$$

The systemic blood flow is decreased by the recirculated fraction:

$$Q_{Systemic} = (1-R)Q_e \qquad \text{E 2.2.1.c}$$

Q_e is the effective blood flow. This refers to the blood water flow that is available to urea. It is not the total volume that is available to solve urea. The volume is decreased by solid and protein fractions. The relation of Q_e and Q_b, the whole blood flow as measured by the blood pump, is:

$$Q_e = Q_b\left[FP - hct/100(FP - PWF)\right] \text{ and } FP = 1 - 0.0107\,TP$$

(FP= fraction of protein, TP = total protein, PWF = Plasma water fraction = 0.8 in this study, hct = Hematocrit)

Roughly estimated $Q_e \approx 0.9Q_b$. Both expressions E 2.2.1. b,c can be inserted into E 2.2.1.a :

$$KeUB = \frac{(1-R)Q_e \dfrac{c_{art} - Rc_{ven}}{(1-R)} - c_{ven}((1-R)Q_e - Q_f)}{\dfrac{c_{art} - Rc_{ven}}{(1-R)}} = (1-R)\frac{\left[Q_e c_{art} - c_{ven}(Q_e - Q_f)\right]}{c_{art} - Rc_{ven}}$$

E 2.2.1.d

Analogously the effective clearance with dialysate side concentration values can be written:

$$KeUD = (1-R)\frac{cdo(Q_d + Q_f)}{c_{art} - Rc_{ven}} \qquad \text{E 2.2.1.e}$$

The recirculation can be measured utilising E 2.2.1.b. Typically Q_b is more than 200ml/min. A reduction to 50 ml/min would decrease the effective clearance and also the recirculation and consequently would not allow to measure the clearance, but gives access to systemic blood. The systemic concentration does not change by blood flow variations. The shunt flow rate which is the flow rate of the blood the heart maintains in the arterio-venous fistula is typically 800-1000ml/min. A transient blood flow reduction to 50ml/min for 2 min decreases R to negligible values, because 50ml/min is small compared to 1000ml/min. This means $c_{Systemic}$ will be available in the fistula and therefore in the arterial connection. Technically 3 samples have to be drawn: c_{art} and c_{ven} at full dialysis blood flow rate to calculate the clearance according to E 2.2.1 d, e and c_{art} at a 50ml/min blood flow rate which is regarded to be $c_{Systemic}$. Now E 2.2.1.b can be resolved for R:

$$\frac{c_{art} - c_{Systemic}}{c_{ven} - c_{Systemic}} = R \qquad \text{E 2.2.1.b'}$$

Every dialysis treatment at least one time the recirculation fraction has been measured according to E 2.2.1.b'. Due to the blood loss no additional systemic samples have been drawn. Instead at least one further measurement was performed by using the BTM (blood temperature monitor, Fresenius Medical Care AG), which uses a thermodilutive method to measure R directly.

2.3. Mass Balance

Equations E 2.2.1 d,e can be utilised to control if the mass balance across the filter is correct. This helps to identify erroneous laboratory measurements. The mass balance error relates the difference in mass flux that has been found by a corresponding blood side and dialysate side measurement to the mean mass flux of both measurements:

$$MBE[\%] = 200 * \frac{[Q_e c_{art} - c_{ven}(Q_e - Q_f)] - cdo(Q_d + Q_f)}{[Q_e c_{art} - c_{ven}(Q_e - Q_f)] + cdo(Q_d + Q_f)} \qquad \text{E 2.3.a}$$

Some of the evaluations have been made in two different data subsets: In the first only measurements with MBE < 5% have been taken into consideration, in the second an unrestricted MBE was accepted. This helps to distinguish if a result only seems erroneous due to erroneous references or if it is truly erroneous.

2.4. Automatic access to ionic effective dialysance

Since a correlation of the dose of dialysis in terms of urea based Kt/V and morbidity has become apparent [Go1, Hak, Low2, Par, USRDS 97 Chpt.III and 99 Chpt.V], there is a rising demand for precise, online dialysis dose quantification to ensure that no hemodialysis session fails in dose delivery, which would result in accumulation of uremic toxins. The principle has been investigated by various other groups and today there is only few doubt that conductivity (CD) based dialysance measurements precisely match dialysis dose analysis with less than 5% absolute difference [DF3, Ku, Ku2, Loc, Manz, Pe1, Pe3, RG2].

The technical equipment necessary to perform the measurement is simple. A standard dialysis machine will always include a single conductivity cell for supervision of the incoming dialysate that will penetrate the filter. The machine must assure that no dialysate of inconvenient or hazardous sodium concentration may enter the filter to prevent the patient from being violated. This conductivity cell can also be adopted for sodium balancing purposes if only a second, identical cell is inserted downstream the dialysis filter. Assuming that conductivity is predominated by sodium chloride - in fact more than 97% of the dialysate conductivity is due to sodium chloride – the inlet-

outlet conductivity cell configuration is able to measure the sodium mass balance across the filter. The configuration principle is sketched in figure 2.4.a.

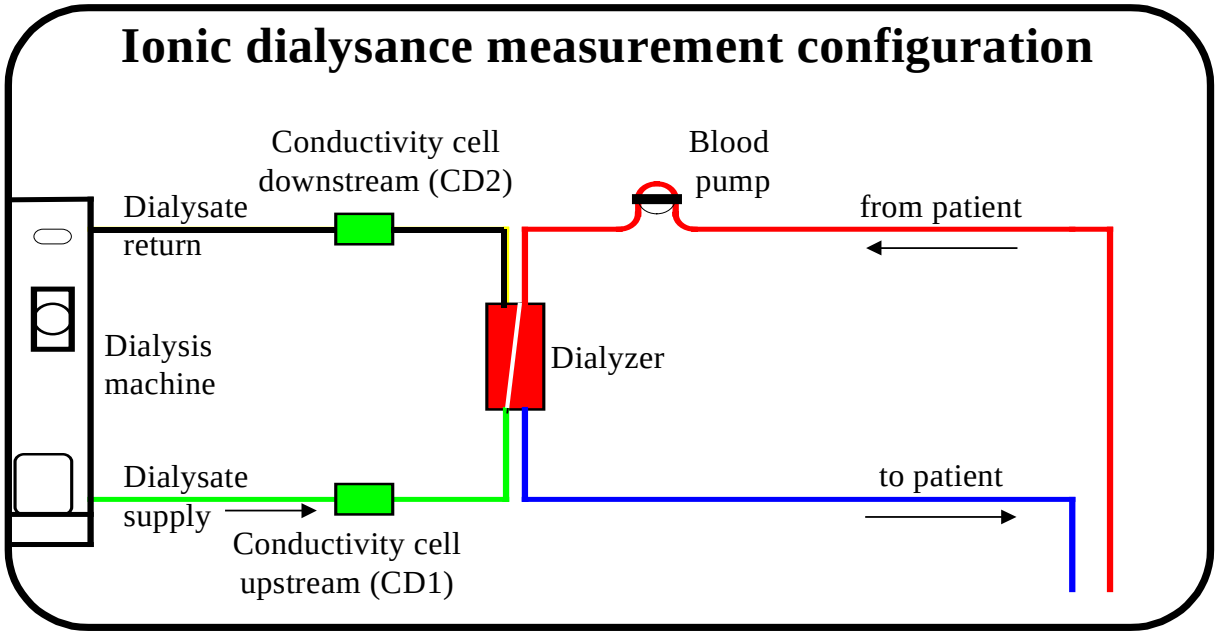


Figure 2.4.a: *To measure the ionic dialysance it is necessary to implement a downstream conductivity cell. No further alterations of the standard dialysis circuitry is necessary. The measurement is regarded to be non-invasive, although sodium is directly transferred into the patients vascular system.*

Internally within the controlling software the machine must be modified additionally to be able to apply conductivity variations. It will soon become clear why this is necessary to measure ionic dialysance. Practical problems may arise from the protection system of the dialysis machine which usually is implemented to independently supervise the control system and stop the dialysate flow if the control system computer works erroneously. In standard dialysis machines the software of both systems must be changed in that way that the control system can generate acceptable and not too high sodium concentration variations and the protective system will accept them for a certain amount of time before it alerts. This is a severe intervention to the security system and takes high effort of testing before the modification can be accepted to be safe for the patient, because a bolus of sodium will always be also a bolus of potassium. In case of malfunction in severe cases hemolysis or cardiac episodes could be the consequence. Therefore it is necessary to have the protection system be alert during the conductivity variations.

To conduct the studies it was necessary to provide 4 machines for the MI study and 2 machines for the MII study that fulfil this demands and further have been certified to comply with all relevant regulations that apply on medical devices in Europe. The machines were no trial versions but had fully been approved according to the CE certification procedure before they were operated with the patients. This means no additional attention of the physician to supervise the machine was necessary during the studies.

The conductivity variations to measure ionic dialysance have been performed in two different manners. The first principle made use of steady state profiles. This means the conductivity was varied like a step function from one constant value to another. A third constant conductivity level was adjusted afterwards, initially by the intention of equilibrating the sodium transfer that occurs during an increase of conductivity. There have been found some disadvantages of this principle, as it will be described. Therefore a second principle was involved that made use of dynamic profiles and never reached a steady state. In total it transferred much less sodium. Both principles will be explained in detail here because the major question of the study was if one of the principles works better than the other. The result should be relevant for deciding which principle is going to be implemented into the series machines afterwards.

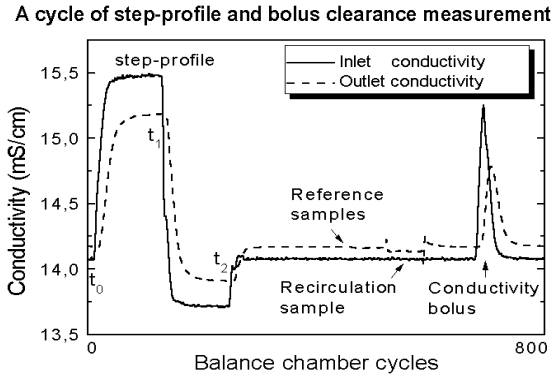


Figure 2.4.1.a: *The comparative dialysance measurements have been conducted in a consecutive order beginning with the step-profile measurement of effective ionic dialysance, followed by the reference urea clearance measurement, the recirculation measurement to calculate from dialyser clearance to effective clearance and finally the bolus dialysance measurement. The times t_0,t_1,t_2 referred to in the text are indicated. Frequently the last bolus of the third cycle was omitted because dialysis time was over. The time is given in balance chamber cycles to normalise for dialysate flow. At typical dialysate flow the balance chamber cycle lasts 3s.*

2.4.1. The step-profile

To perform the measurement using steady states usually a step-profile similar to that shown in figure 2.4.1.a is applied, which increases dialysate inlet CD at time t_0 , lasting 7min until t_1. Usually it was followed by a decrease until t_2 and a final return to baseline. Meanwhile the CD of the dialysate at filter inlet (cdi) and outlet (cdo) using the newly introduced CD cell, the dialysate flow (Qd) and the UF-rate (Qf) was recorded. The transition to the next step is performed as soon as the outlet conductivity has achieved sufficient stability or its stability value can be anticipated. It should be emphasised that all quantities are measurable non-invasively on the dialysis machine side and do not need any additional patient access. The electrolytic dialysance $KeCn_{I,J}$ according to [Pe1] can now be calculated by

$$K_{eff} = KeCn_{IJ} = \left(1 - \frac{cdo_I - cdo_J}{cdi_I - cdi_J}\right)\left(Qd + Q_f\right) \quad \text{with } I = 0..2;\ J = 0..2;\ I \neq J \qquad \text{E} \quad 2.4.1.a$$

For variables see chapter 8 "Equations and abbreviations".

where I,J indicate the time couple from times (t_0,t_1,t_2) when the CD stability values have been measured. This expression will be derived in the following chapter. It is well accepted in the literature and many authors rely on it [DF1, DF3, Ku, Ku2, Ku3,Pe1,Pe2, Ste]. Nevertheless by analysing the data of this study we found some

inconsistency with using step-functions and E 2.4.1.a. This in particular was the reason why the second dynamic principle has been invented during the study.

We now will focus on the problem that occurs using E 2.4.1.a. It has been found that the three permutations in (I,J) are not equivalent but establish a ranking within the results: $KeCn_{01} < KeCn_{12} < KeCn_{02}$ [DF1,DF2, Lind, RG2]. Each difference is about 2-5% and, furthermore, a difference versus the urea reference remains. Both results are not explained satisfactorily and seem to indicate an additional process that is taking effect. In addition, there are contributions that a membrane type specific effect could be responsible [Pe2]. Another theoretical reason could be an inadequate correction for ultrafiltration because ultrafiltration is partially neglected in the derivation of E 2.4.1.a (see also expressions 11-14 in Sar1). Finally there is evidence that a violation of plasma sodium stability – which is essential to apply E 2.4.1.a – by the measurement itself predominates the questionable inaccuracy rather than the membrane type or the neglected ultrafiltration rate. Some authors assign this to cardiopulmonary recirculation (CPR) [DF2]. Our study should elucidate this context.

2.4.2. The dynamic bolus measurement

Viewed from a biophysical aspect the system consisting of patient, extracorporeal circuit and dialysis machine is very inert to sodium variations due to large participating volumes. This in particular is the reason why the conductivity-based dialysance measurement operates in this manner at all. Reflecting that sodium dialysance works in both directions across the membrane, a simplified linear example may roughly demonstrate that sodium transfer is not negligible: Applying a +10% conductivity step at an effective dialysance of 200 ml/min also means that an equivalent of 200 ml blood water per minute is increased by +10% if the dialysate sodium matched the plasma sodium prior to the measurement. This amount of sodium will be mostly loaded to extracellular fluid (ECF) and not enter the cells. For simplification we consider a 60kg patient with 33l distribution volume, 10l of them extracellular fluid. Due to the duration of flows and rebound onset no intracellular fluid interference should return to the dialyser within the observation time of the first two minutes after the change. A replacement of 2min*200ml/min=400ml of plasma water with the new concentration will result in a new ECF concentration of (9,6l*c0+0,4l*1.1c0)/10l = 1.004*c0, thus ECF would be increased by 0.4% of its initial concentration. Actually the concentration will follow an exponential curve, but the linearity error should be negligible within the first 2 min. At 140 mmol/l the increase is 0.56 mmol/l. To understand the consequences it is necessary to go into the derivation of E 2.4.1.a now inserting the difference in cbi . E 2.4.1.a is based on a simple pure mass balance formulation over the filters blood and dialysate side assuming nothing more than that any particles could vanish or appear:

$$J_B = D\left(1 - \frac{Qf}{Qe}\right)\left(\alpha_D cbi - cdi\right) + Qf * \alpha_D cbi$$

$$J_D = \left(cdo - cdi\right)Qd + Qf * cdo$$

If we neglect that the filter is a comparable small buffer, mass balance must exist whatever the concentrations are at t=I or t=J:

$$J_{B,I} = J_{D,I};\ \ J_{B,J} = J_{D,J} \Rightarrow J_{B,I} - J_{D,I} = J_{B,J} - J_{D,J} = 0$$

$$\begin{aligned} & D\left(1-\frac{Qf}{Qe}\right)\left(\alpha_D cbi_I - cdi_I\right) - \left(cdo_I - cdi_I\right)Qd \\ & + \left(\alpha_D cbi_J - cdo_J\right)Qf \\ = \ & D\left(1-\frac{Qf}{Qe}\right)\left(\alpha_D cbi_J - cdi_J\right) - \left(cdo_J - cdi_J\right)Qd \\ & + \left(\alpha_D cbi_I - cdo_I\right)Qf \end{aligned}$$

$$\begin{aligned} \Rightarrow D \ = \ & \frac{\left[\left(cdo_I - cdi_I\right) - \left(cdo_J - cdi_J\right)\right]Qd}{\left(1-\frac{Qf}{Qe}\right)\left[\left(\alpha_D cbi_I - cdi_I\right) - \left(\alpha_D cbi_J - cdi_J\right)\right]} \\ + \ & \frac{\left[\left(\alpha_D cbi_J - cdo_J\right) - \left(\alpha_D cbi_I - cdo_I\right)\right]Qf}{\left(1-\frac{Qf}{Qe}\right)\left[\left(\alpha_D cbi_I - cdi_I\right) - \left(\alpha_D cbi_J - cdi_J\right)\right]} \end{aligned}$$

Therefore for the ionic dialysance we obtain:

$$\begin{aligned} KeCn_{IJ} \ = \ & D\left(1-\frac{Qf}{Qe}\right) + Qf \\ = \ & Q_f + \frac{\left[\left(cdo_I - cdi_I\right) - \left(cdo_J - cdi_J\right)\right]Qd}{\left(\alpha_D cbi_I - cdi_I\right) - \left(\alpha_D cbi_J - cdi_J\right)} \\ + \ & \frac{\left[\left(\alpha_D cbi_J - cdo_J\right) - \left(\alpha_D cbi_I - cdo_I\right)\right]Q_f}{\left(\alpha_D cbi_I - cdi_I\right) - \left(\alpha_D cbi_J - cdi_J\right)} \end{aligned} \qquad \text{E 2.4.2.a}$$

If cbi persists during the application of the step, it could be cancelled and E 2.4.2.a turns to E 2.4.1.a, with the Donnan factor α_D being of no influence. α_D represents concentration shifts in the presence of proteins on one side of the membrane. If cbi is actually varied by the step, the Donnan factor is of moderate influence because it applies only to the cbi difference. Considering typical values yielding KeCn=200ml/min using E 2.4.1.a (e.g. cdi_0= 140mmol/l, cdo_0=140mmol/l, cdi_1=154mmol/l, cdo_1=148.4mmol/l, Qd=500ml/min, Qf=0), an insertion to E 2.4.2.a with $\alpha_D(cbi_1 - cbi_0)$= 0.56mmol/l results in an actual $KeCn_{01}$ of 208.33 ml/min. The measured dialysance of 200ml/min would be –4% erroneous. This could explain the magnitude of the error.

If this consideration is correct it has two implications: The first is that the error should be related to the interfering volume and might indicate a way to measure the sodium distribution volume and even V(urea). The second is that it is recommended for precise dialysance results to keep the sodium transfer as low as possible. The minimum step-function duration is restricted by the minimum time to achieve stability at the dialysate outlet, therefore the transfer can not fall below a particular minimum. An important improvement therefore would be to avoid stability and measure dynamically, e.g. using boli. The transition to dynamic profiles permits us to minimise the inlet variation to a value depending only on the technical resolution of the system

and therefore the plasma sodium stability condition is much less violated compared to step profiles. Boli should be understood here as a physical representation of a theoretically infinite short test spike, just as telecommunication engineers use voltage spikes to characterise the properties of electronic systems by analysing the response to the spike, which is called the transmission function g(t). Knowledge of g in this context permits us to calculate the response cdo(t) of the patient-machine system to any arbitrary inlet conductivity signals according to the convolution integral:

$$cdo(t) = \int_{-\infty}^{+\infty} cdi(t'-t_f)\,g(t-t')\,dt' \qquad \text{E 2.4.2.b}$$

Figure 2.4.2.a demonstrates what the operator does: It delays and widens the inlet signal and varies the area beneath the response curve. $\int g(t)$ is in the same way related to the dialysance as the term $(cdo_I\text{-}cdo_J)/(cdi_I\text{-}cdi_J)$ in E 2.4.1.a and we

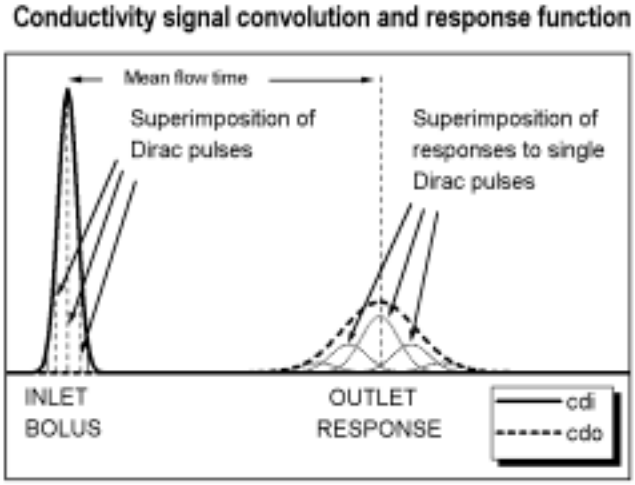


Figure.2.4.2.a: *This figure illustrates E 2.4.2.b,c: Many arbitrary inlet dialysate sodium variations, e.g. boli, can be regarded as superimposition of an infinite number of infinite short, scaled "Dirac" pulses, which are only a theoretical consideration. Any measured corresponding response is the addition of the associated responses to the single Dirac pulses. The mathematical operation that converts inlet to outlet is called a convolution.*

can write instead:

$$K_{eff} = KeCn_{Bolus} = \left(1 - \int g(t)dt\right)\left(Qd + Q_f\right) \qquad \text{E 2.4.2.c}$$

The equivalence of E 2.4.1.a and E 2.4.2.c can be demonstrated if we apply E 2.4.2.c to a cdi conductivity step starting at t=0 of infinite duration to demonstrate the equivalence of.

$$cdi(t<0) = cdi_0 = const; cdi(t \geq 0) = cdi_1 = const$$

The step should be of only moderate height so that the superposition principle is still valid and E 2.4.2.b can be applied also to differences of conductivities. E 2.4.2.b therefore describes the change of cdo(t) if we consider aqueous solutions:

$$\Delta cdo(t) = \int_{-\infty}^{\infty} \Delta cdi(t'-t_f)\, g(t-t')\, dt'$$

For simplicity we will regard g as being symmetrical: g(x) = g(-x). This is only a formal simplification that allows us to keep the integral boundaries symmetric. Of course a non-symmetric g would yield the same result but does not help to understand why E 2.4.2.c and E 2.4.1.a are equivalent. Further we must take for granted that causality is valid which guarantees us that no fraction of the step response can appear in cdo prior to the step. Mathematically this means we can limit the boundaries of the integral to the minimum flow time:

$$\Delta cdo(t) = \int_{-tf}^{+tf} \Delta cdi(t'-t_f)\, g(t-t')\, dt'$$

For an ideal step at t_0 we have for all $t > t_0$ Δcdi(t) = constant and therefore can be shifted out of the integral:

$$\Delta cdo(t > tf) = \int_{-tf}^{+tf} \Delta cdi(t)\, g(t-t')\, dt'$$

$$\frac{\Delta cdo(t > tf)}{\Delta cdi(t > tf)} = \int_{-tf}^{+tf} g(t-t')\, dt'$$

This in fact is the same expression we have used in E 2.4.1.a before:

$$KeCn_{I;J} = \left(1 - \frac{cdo_I - cdo_J}{cdi_I - cdi_J}\right)(Qd + Q_f) \quad \text{with } I = 0..2; J = 0..2; I \neq J \qquad \text{E 2.4.1.a}$$

and we can conclude for stable – and only for stable- cbi :

$$KeCn_{I,J} = KeCn_{Bolus} = \left(1 - \int_{-tf}^{+tf} g(t-t')\, dt'\right)(Qd + Qf)$$

2.4.3. True continuous electrolytical dialysance measurement

As we have described above a possible technical realisation to measure g(t) could apply boli that are sufficiently short to have the character of transient spikes compared to the involved time constants of the system under observation, regarding the response to these boli as the transmission function g itself. Of course this is a concession to practicability while true indefinitely short boli (‘Dirac pulses’) are not possible per se, they are of only theoretical character and only defined by their integral. The data presented in this work are based on the use of this discrete boli that have been used within our studies.

For the patients it is more advantageous to use the fully dynamic principle: g(t) can continuously be calculated from arbitrary conductivity profiles bearing sufficient frequency spectra (e.g. sinuous composition, ramp, sawtooth, boli...) by applying the convolution theorem of Laplace transform [Coh,Abram,Church]. Knowledge of g is knowledge of the dialysance according to E 2.4.2.c. Thus, calculating the time behaviour of g(t) permits true continuous online dialysance analysis, not only restricted to discrete measurements as performed today.

Every dynamic signal of filter inlet and outlet conductivity that bears these frequency characteristics can be transformed to its corresponding Laplace transforms. Laplace transforms have an advantageous property which is called the convolution theorem that reduces the calculation of g(s) to a simple division in the Laplace space. Like in other integral transforms s is a complex variable that corresponds to t in time space or to the complex frequency $i\omega$ in Fourier transforms. It seems straightforward to use mathematical procedures for reducing calculations to basic arithmetical operations, but this is possible only at the cost of a non-simple inverse transformation of g(s) to g(t). Starting with E 2.4.2.b at a time t>0 with

$$cdo(t) = \int_0^t cdi(t'-t_f)\,g(t-t')\,dt'$$

we apply the Laplace transformation to both sides of the equation:

$$L.T.\left[\Delta cdo(t)\right] = L.T.\left[\int_0^t \Delta cdi(t'-t_f)\,g(t-t')\,dt'\right]$$

According to the convolution theorem this equals:

$$L.T.\left[\Delta cdo(t)\right] = L.T.\left[\Delta cdi(t-t_f)\right]\ \ L.T.\left[g(t)\right]$$

which is a simple product in Laplace space and therefore we yield:

$$L.T.\left[g(t)\right] = \frac{L.T.[\Delta cdo(t)]}{L.T.[\Delta cdi(t-t_f)]}$$

There are different ways to transform ∆cdo and ∆cdi. Again it is straightforward but not very practical to numerically apply the transform and the corresponding inverse transform to the measured signals. The transform rules are [Coh, p.190]:

$$L.T.\left[\Delta cdo(t)\right] = \int_0^\infty \Delta cdo(t)e^{-st}dt \qquad \text{E 2.4.3.a}$$

and by analogy

$$L.T.\left[\Delta cdi(t)\right] = \int_0^\infty \Delta cdi(t)e^{-st}dt$$ for the transformation and

$$g(t) = \frac{1}{2\Pi i} \int_{c-i\infty}^{c+i\infty} \frac{L.T.[\Delta cdo(t)]}{L.T.[\Delta cdi(t - t_f)]} e^{st} ds \qquad \text{E 2.4.3.b}$$

for the inverse Laplace transform, which is an integration across the positive complex half-plane with the argument being analytical in $\mathrm{Re}(s) \geq c$.

The transformation of cdi and cdo is not difficult to automate due to its evaluation of the integral E 2.4.3.a. To determine g(t) vice versa we can use Cauchy's theorem that converts the integration over the complex plane to the integration along a path around this plane, once the function is proved to be analytical in this plane. This means a closed contour must be constructed that contains the path along the integral boundaries of the inverse transform on the positive complex half-plane. All this in general can only be done automatically by a sophisticated software algorithm that will hardly fit to the computer of a dialysis machine.

In presence of limited calculation power and if numerical input values are subject of the transformation it is more convenient to adapt a better software design that makes use of the predefined transformations of analytical functions and fractions. It is helpful to break down an arbitrary input signal to a sum of scaleable sinuous or preferably exponential functions, which will always be possible. Both have very simple analytical transforms that are scheduled in the literature [Bron, Abram, Spi]. The analytical inverse transform sometimes is laboriously but will always work by applying partial fraction analysis. The single fractions can be re-transformed separately because the Laplace transform is a linear transformation that can be superimposed.
The amplitude, kurtosis and steepness of the underlying analytical functions are to be scaled by the software using a least square fit that fixes the set of parameters fitting the input signal best. These parameters must be part of the analytical inverse transform as a variable, so that the transformation itself is not done by the software at runtime. The remaining task for the software would only be to fit the parameters and to insert the values – and even this is a strain for a small computer. Nevertheless it can be imagined that today's dialysis machine processing units could bear this burden.

The result would be a permanent calculation of g(t) by a sliding substitution of older conductivity measurements by the latest incoming data. A clearance decay therefore should be detectable within the response time of the patient-machine system, which should be only a few minutes.

2.4.4. Virtual continuous electrolytical dialysance estimation

A more practical solution that does not involve prolonged numerical calculations but can be implemented on machines with restricted calculation resources is to measure the clearance using boli without a continuous signal analysis as described in the last paragraph. This method releases boli after fixed periods of time. These boli are analysed by Laplace transform or equivalent algorithms like described. The

calculation interpolates the clearance for the intermediate time with respect to the operational parameters of the dialysis machine like bloodflow, dialysate flow and alarm status, without actually measuring it continuously. This is possible by making use of the assumption that the filter does not change its diffusive properties substantially during the time between two boli measurements. Of course this is a criterion to define the length of the period that should be appropriate without a true change of the filter. The experience meanwhile shows that a period of 30 – 60 min is convenient to assure that the properties of the filter during a standard hemodialysis do not change unacceptably. The clearance of the intermediate phases can be calculated according to the following considerations [RG1]:

Basically it is further assumed that any dialysis filter normally is operated under conditions that not the filter itself will be the absolutely limiting device for urea mass transfer rate but the operational parameters, thus an increase of Q_d or Q_b will always lead to a further increase of clearance. This assumption does not mean that the filter does not restrict the transfer but it means the transfer is not at its absolute upper limit as it would be at infinite bloodflow and infinite dialysate flow when no further increase could increase urea mass transfer. If this assumption is not valid the dialysis process is malfunctioning and the filter

- is not appropriately selected for the individual dialysis situation or
- the filter is extremely occluded by any cause.

Both will be detected by the next bolus measurement and therefore can not exist for longer than the intermediate period between two boli measurements.

The clearance corresponding with this absolute upper limit is called D_{max}. The expression E 2.4.4.a for D_{max} can be found in the literature [Dep2], sometimes it is called KA, the mass transfer area coefficient in (ml/min), because theoretically it is constituted by a mass transfer resistance (=1/K) multiplied with the membrane area where it takes effect. More general theoretical considerations on exchange properties of counter current membrane devices are found in the fundamental work of Sigdell [Sig] or in [Klein2]. Using D_{max} in mathematical terms the upper condition corresponds to the actual clearance of the filter $K < D_{max}$ being valid.

$$D_{\max} = \frac{Q_b Q_d}{Q_b - Q_d} \ln\left[\frac{1 - D/Q_b}{1 - D/Q_d}\right] \qquad \text{E 2.4.4.a}$$

The relation of clearance and bloodflow or dialysate flow across a dialyser in a good approximation is of exponential character [Diet], assuming each with the other flow at infinity, see figure 2.4.4.a:

$$D(t) = D_{\max}\left[1 - \exp(-kQ_d(t))\right] \qquad \text{E 2.4.4.b}$$

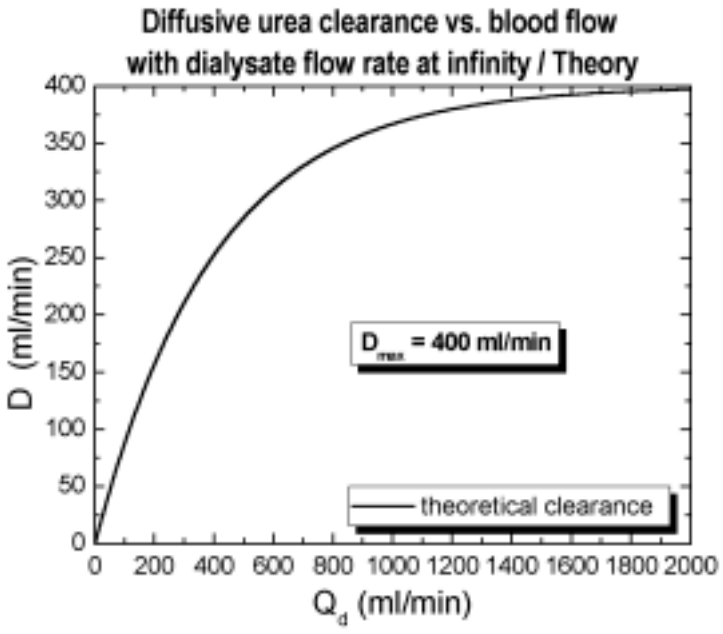


Fig. 2.4.4.a: *Theoretical curve of the diffusive urea clearance versus dialysate flow Q_d at a standard dialysis filter operated in counter-current flow. The clearance curve would be of the same shape if it was drawn versus the blood flow Q_b with the dialysate flow rate at infinity because the ideal filter can be mirrored in his properties. Practical filters show differences in D_{max} if they are connected vice versa. This is due to their membrane structure and internal flow mechanics. This means the figure is rather an idealistic demonstration than a true reflection of the filter properties.*

This can be resolved for k

$$k = -\frac{1}{Q_d(t)} \ln\left[1 - \frac{D(t)}{D_{\max}}\right] \qquad \text{E 2.4.4.c}$$

In a real dialysis situation of course nor the bloodflow neither the dialysate flow are infinite. Even by increasing the dialysate flow rate to infinity the clearance of the system can not overcome the blood flow rate. Vice versa an infinite blood flow rate could not let the clearance of the system overcome the finite dialysis flow rate. Thus with fixed blood flow Q_b at a time t_{meas} E 2.4.4.b,c turn to

$$D(t_{meas}) = Q_b(t_{meas})\left[1 - \exp(-k_1 Q_d(t_{meas}))\right]$$

$$k_1 = -\frac{1}{Q_d(t_{meas})} \ln\left[1 - \frac{D(t_{meas})}{Q_b(t_{meas})}\right]$$

$$\Leftrightarrow D(t_{meas}) = Q_b(t_{meas})\left[1 - \exp\left[\frac{Q_d(t_{meas})}{Q_d(t_{meas})} \ln\left[1 - \frac{D(t_{meas})}{Q_b(t_{meas})}\right]\right]\right] \qquad \text{E 2.4.4. d}$$

for the function $D(Q_d)$. With fixed dialysate flow Q_d at a time t_{meas} E 2.4.4.b,c turn to

$$D(t_{meas}) = Q_d(t_{meas})\left[1 - \exp(-k_2 Q_b(t_{meas}))\right]$$

$$k_2 = -\frac{1}{Q_b(t_{meas})} \ln\left[1 - \frac{D(t_{meas})}{Q_d(t_{meas})}\right]$$

$$\Leftrightarrow D(t_{meas}) = Q_d(t_{meas})\left[1 - \exp\left[\frac{Q_b(t_{meas})}{Q_b(t_{meas})} \ln\left[1 - \frac{D(t_{meas})}{Q_d(t_{meas})}\right]\right]\right] \qquad \text{E 2.4.4. e}$$

for the function $D(Q_b)$. E 2.4.4 d, e are dependent and do not allow to determine D alone if they are combined, but once D is measured they allow to conclude from

$D(Q_b(t_{meas}))$ to any $D(Q_b(t))$ if Q_d (and, exactly D_{max}) has not changed meanwhile (E2.4.4.d). And also they allow to conclude from $D(Q_d(t_{meas}))$ to any $D(Q_d(t))$ if Q_b has not changed meanwhile (E2.4.4.e). It is just to go along the exponential curve if we use E 2.4.4.e , because k_2 has not changed:

$$D(Q_b(t_2)) = Q_d(t_{meas})\left[1-\exp\left[\frac{Q_b(t_2)}{Q_b(t_{meas})}\ln\left[1-\frac{D(t_{meas})}{Q_d(t_{meas})}\right]\right]\right] \qquad \text{E 2.4.4. f}$$

This is the clearance if we keep Q_d constant and vary $Q_b(t_{meas})$ to $Q_b(t_2)$. E 2.4.4.f alone therefore would allow to calculate a value for D at any bloodflow within the accuracy tolerance of the exponential curve if once a bolus clearance measurement was performed at a known bloodflow. Up to now we have just involved the inaccuracy of the assumption that the clearance changes on an exponential curve defined by the k_2 coefficient. This is not exactly the case for sodium because the outlet concentration of the dialyser is not zero [Diet] but it is a tolerable deviation within small Q_b variations of a few percent as it is usual in dialysis sessions.

A change of Q_b is not the only change that could occur during dialysis. How can superimposed changes of Q_d that might occur simultaneously between two bolus measurements be reflected ? The operational conditions (Q_b) have already changed now and a new k_1 must be calculated because the system has left the initial exponential curve that was established using k_1. The new clearance $D(Q_b(t_2))$ can be used for this purpose:

$$k_1' = -\frac{1}{Q_d(t_{meas})}\ln\left[1-\frac{D(Q_b(t_2))}{Q_b(t_2)}\right]$$
$$\Leftrightarrow D(t_2) = Q_b(t_2)\left[1-\exp\left[\frac{Q_d(t_2)}{Q_d(t_{meas})}\ln\left[1-\frac{D(Q_b(t_2))}{Q_b(t_2)}\right]\right]\right] \qquad \text{E 2.4.4.g}$$

This is a useful expression that allows to calculate the diffusive clearance for any operating condition within the tolerance of the exponential curves that could occur between two subsequent bolus clearance measurements. By the experience of the author the flow rate changes should not exceed 15% to keep the error of $D(t_2)$ compared to a true bolus measurement within 3%.

The effective clearance including the ultrafiltration is calculated according to E 2.4.2.a:

$$KeCn_{t2} = D(t2)\left(1-\frac{Qf}{Qe}\right)+Qf$$

During the data analysis with no regard whether it is single pool or two pool comparison or reference and machine side measurements all clearances that are measured at discrete times during dialysis have been interpolated in the described manner to get also clearance values for other times. This was of substantial importance in particular when calculating K for the Kt/V comparison from only few instant urea blood sample measurements. Always the previous successful bolus

measurement or blood sample was used to generate a factor f for every second of the dialysis treatment:

$$f(t) = KeCn_t \Big/ KeCn_{t_{meas}} \quad \text{with} \quad t > t_{meas}$$

The mean clearance for the Kt/V comparison was then calculated according to the average integral

$$K_{Mean} = 1 \Big/ T \int_0^{end} K_{t_{meas}} f(t) dt \qquad \text{E 2.4.4.h}$$

whereas K_{tmeas} was always the previous valid measurement or sample relative to the integration variable t.

2.4.5. The dialysance measurement is profile type sensitive

The expression to derive the three electrolytic dialysances from a conductivity step profile should be presented again here. There are three permutations:

$$KeCn_{I,J} = \left(1 - \frac{cdo_i - cdo_j}{cdi_i - cdi_j}\right)(Qd + Qf) \qquad \text{Timeindex I} \neq \text{J} \qquad \text{E 2.4.1.a}$$

In the study MI it was apparent that the choice of I,J was of relevance. It can be anticipated here that at nearly all measurements $KeCn_{01} < KeCn_{12} < KeCn_{02}$ was found.

A series of measurements had to be started first in the laboratory to determine the quality of the effect and also its quantification. It was necessary to understand if it is a systematic error, if it is inherent to the measurement, patient dependent or if it is only an artefact. Further it had to be decided during the ongoing study if the protocol should be changed to correct it.
Emphasis is put here to the aspect of the difference of the four possibilities to measure the ionic clearance and not to its absolute mean error. The four methods are:

$KeCn_{01}$	$KeCn_{02}$
$KeCn_{12}$	$KeCn_{Bolus}$

For definition $KeCn_{Bolus}$ see E 2.4.2.c. The bolus is also included because from its nature it seems not to be prone to extended sodium shift from or to the patient. According to the study protocol also in the MI study a pulse clearance measurement has been performed but it was found to be of high standard deviation compared to

the step profile. In the MII study the boli were designed differently with the experience of the MI study. These data directly led to the development of the theory of distribution volume measurement that will be described in the following chapter.

2.5. Measurement of the urea distribution volume

All efforts planning both online clearance studies MI and MII never had the intention to find a solution for measuring the missing link in the chain leading to conductivity based complete Kt/V assessment, this is to measure V. Nevertheless the study design is suited to have access to the reference V_{Urea} by direct quantification (DQ) and other methods without an idea of an electrolytical approach to measure V_{Na} or V_{Urea}. The considerations made during the data analysis led to this approach, a conductivity based principle to measure the sodium distribution volume.

In-vivo urea distribution volume comparisons comprise a fundamental problem. It is the complete lack of an absolute volume reference. There is no container precisely filled with a known amount of water that could be employed as a reference. In-vivo the patients distribution volume can only be derived from more or less accurate urea kinetic modelling, single- or double pool or at least from anthropometric variables using the body weight, the Hume or Watson formula. All these are not satisfactory and may lead to wrong references and high standard deviations in reference V_{urea}. In-vitro the alternative could be to dialyse a container of exactly known volume. But how to handle the influence of the two or even more pools and their internal mass exchange, how to model the shunt- and cardiopulmonary recirculation correctly ? To the knowledge of the author no method that is reliable, precise and handy in clinical practice has been found yet. Some authors label the urea isotope dilution method as the golden standard [Klop] in spite of this method being not very practical. Many authors regard the direct quantification of urea by sampling spent dialysate as the method of choice for individual assessment of urea distribution volume [Kop, Ba, Dep1].

2.5.1. V from direct quantification

Direct quantification describes a method that samples the total amount of urea that is removed by dialysis. In its simplest form it would sample the whole quantity of spent dialysate from the drain of the dialysis machine. This is a practical problem because the total amount of spent dialysate – approximately 120l-150l per treatment - usually can not be handled. The alternative is to sample only a representative fraction of the total amount. The consequence is to involve valves with precise nozzles that permit to split the flow into a minor fraction that is to be sampled and the major fraction bound for the drain. This valve can easily be obstructed by detritus and thin bacterial layers if it is not continuously rinsed. The correct function must be controlled during

the treatment by measurement of the split volume and control of the correct fraction. If the fraction varies during the treatment in comparison to the total flow it must be assumed that the valve has failed, the sampled amount is not representative and the measurement must be aborted.

To circumvent these problems within the investigations it was made use of having access to the software of the dialysis machine. Because the dialysis devices that were to participate in the study had a dedicated software for data acquisition and time control of the sampling unit it was easy to integrate an algorithm that performed a time control of a splitter valve at the drain of the machine. This valve was switched by a connected computer in fixed mark space ratio: 1.5s on, 58.5s off. During alarms or bypass periods of the machine the valve was not released to avoid dividing of fluid that has not passed the dialyser and therefore was not loaded with urea. Approximately 2% of the total amount were sampled in a small container as a representation for the mean urea concentration of the total amount of dialysate. It was of no importance to match exactly 2% rather than to be representative for the total amount. Thus the only condition to fulfil was to have the valve not changing its properties during the treatment significantly. The inner diameter was selected such that obstructions were extremely improbable and a sliding occlusion would not take affect within a single treatment. The uncertainty of a small diameter splitter nozzle with its occasional obstructions during the treatment was therefore converted to a time splitting that could precisely be performed by the computer involved.

The urea distribution volume according to direct quantification is calculated using the expression 2.5.1.a. To calculate the urea distribution volume at the start of the treatment this equation compares the extracted urea mass, corrected for that amount of urea contained in the fluid that has been extracted during the treatment, to the concentration change in the body the extraction of the mass has generated. The final urea mass in the body differs from the initial urea mass by the amount of the urea mass generation – urea removal by dialysis - residual renal clearance:

$$V_{t30}U_{PW,t30} = V_0U_{PW,t0} - R_{Dialyse} + G - R_{res}$$

$$V_{t30}U_{PW,t30} = V_0U_{PW,t0} - \Delta M_{Urea}$$

$$V_{t30} = V_0 - Q_f t_{Dial}$$

$$\Rightarrow V_0 = V_{DQ} = \frac{\Delta M_{Urea} - Q_f\ t_{Dial}U_{PW,t30}}{U_{PW,t0} - U_{PW,t30}}$$

E 2.5.1.a

The urea concentrations of the plasma water at times t_0 (start of dialysis) and t_{30} (end of rebound) are inserted. M_{Urea} is the total urea mass found in the spent dialysate. Using the single pool model by lack of availability the residual urea removal and the urea generation are neglected in this analysis and ΔM_{Urea} is calculated by

$$M_{Urea} = V_{sample}c_{Urea}\frac{V_{Total}}{V_{sample}}$$

E 2.5.1.b

This neglect may be responsible for a –10 to -12% error because the liver of the patient produces 10-12% of the total urea generation during dialysis. This means

either the $U_{PW,t30}$ is higher than it would be without generation or in the sample there is additional urea that would not be seen if the generation would have been subtracted. Both would lead to an erroneous, too high V_{DQ}. This must always be remembered if a comparison to V_{DQ} is made. The basic problem is that the generation rate is not available when observing only a single treatment. At least the previous or subsequent interdialytic phase must be available for a better calculation.

In the two pool urea kinetic model both quantities are not neglected because they result only from two pool analysis which has been exceeded to contiguous dialysis treatments. It is clearly seen by E 2.5.1.b that the error in the measurement of V_{sample}, which is the volume that has been sampled by the split valve, is of no interest because it cancels. In contrast V_{total} must be known exactly. By software access to the balance chamber counts of the machine it is possible to reduce its error to 0.1%, therefore the predominating error is in c_{Urea}. A certified laboratory as it has been involved is expected to have ±3% SD in urea concentration measurements. This means the error of the volume – being 1/30 relative to the concentration error - can be neglected within this context.

This formula should be understood as a tribute to practical demands. There is a more elaborated formula for the urea distribution volume to be found in the literature [Steg], but with the disadvantage that data of subsequent or preceding dialysis sessions are needed. The observation of subsequent dialysis treatments was not included in the single pool model, which is confined to a unique dialysis session. The background is that patients did not continuously participate in the study in every treatment. Many treatment sequences that have been acquired are interrupted by off-study dialysis treatments. This reduces the data pool for two pool analysis significantly and is one of the reasons the single pool analysis has been performed at all.

2.5.2. Anthropometrical methods to estimate urea distribution volume

In clinical practice the handling of enormous amounts of fluid or the installation of splitting valves at the rear of dialysis machines that are not to be manipulated without loss of operating certification and safety warranty claims is hardly possible. The clinical experience has found some general rules to calculate the urea distribution volume of a particular patient from his anthropometrical characteristics. These rules are handy and widespread known and can be applied by the clinician in place if accuracy and scientific interest are not in the foreground but a rough estimate of the patients urea distribution volume. The emphasis is on ‘estimate’ because they are not true analytic measurements. These formulas are known as the Watson- formula, the Hume-formula and the %bodyweight formula for urea distribution volume in litre:

Watson formula [Wat]:

$$\text{Females}: -2.097 + \text{Body height}\,[\text{cm}] * 0.1069 + \text{"dry weight"}[\text{kg}] * 0.2466)$$
$$\text{Males} \;: \; 2.447 - \text{Age}[\text{years}] * 0.09516 + \text{Body height}\,[\text{cm}] * 0.1074 + \text{"dry weight"} * 0.3362$$

E 2.5.2.a

Hume formula [Hum]:

$$\text{Females}: 0.344547 * \text{Body height}[\text{cm}] + 0.183809 * \text{"dry weight"}[\text{kg}] - 35.270121$$
$$\text{Males} \;: 0.194786 * \text{Body height}[\text{cm}] + 0.296785 * \text{"dry weight"}[\text{kg}] - 14.012934$$

E 2.5.2.b

%BW formula [Kop]:

$$\text{Females}: 0.55 * \text{dry weight"}[\text{kg}] \qquad \text{Males} \;: 0.58 * \text{"dry weight"}[\text{kg}]$$

E 2.5.2.c

The comparison of these formulas to V_{DQ} and V from two pool urea kinetic model is made in chapter 4.2.3.2. to give an impression of the relation of results from anthropometric and analytical methods of urea distribution volume determination.

2.5.3. Conductivity based sodium distribution volume measurement

It will be discussed in this chapter if there is also an electrolytical approach to measure V, the missing quantity in Kt/V, by utilising the conductivity cells. Initial to the following considerations there was the observation that it is not equivalent which of the permutations I,J with I≠J in E 2.4.1.a are used. The explanation of this neglected effect was unclear in the beginning but during analysis of the data it became clear that there must be a dilutive effect to the plasma of the patient during the clearance measurement using step profiles (see chapters 4.1.1.1 and 4.1.1.2.2). The theoretical aspects of this effect will be analysed here.

In theory we found the difference of $KeCn_{IJ}$ from E 2.4.1.a and E 2.4.2.a to be dependent on the difference of cbi_I-cbi_J. A given amount of sodium transfer during the step-profile will modify cbi more in small than in weighty patients. If there is no net sodium transfer the volume should not affect cbi. Thus we could compare a symmetric step profile measurement with substantially lower cbi variation like $KeCn_{12}$ to a measurement with unbalanced sodium transfer like $KeCn_{01}$, where we only evaluate the positive half-wave of the complete profile. If the sodium transfer imbalance is causative for the difference we can further suppose that $KeCn_{12}$ with its symmetric profile is more accurate, and the difference of $KeCn_{12}$-$KeCn_{01}$ should

reflect the interacting volume behind the dialysis filter. We will regard $KeCn_{12}$ and its diffusive fraction D_{12} as correct in this consideration, even if this actually is a simplification again, because symmetric profiles will increase cbi in its time averaging, too. To avoid this the low phase should last longer than the high phase so as not to alter cbi at an average during the profile duration. This simplification may bring an offset to both $KeCn_{01}$ and $KeCn_{12}$ that we can accept since we evaluate their difference. To test our hypothesis on the basis of the existing data we will have to find the expression for the distribution volume, $V(KeCn_{12}\text{-}KeCn_{01})$.

As demonstrated the electrolytic clearance KeCn is calculated by

$$KeCn_{I,J} = \left(1 - \frac{cdo_i - cdo_j}{cdi_i - cdi_j}\right)(Qd + Qf) \qquad \text{Timeindex } I \neq J \qquad \text{E 2.4.1.a}$$

where i, j are the time indices for t0, t1 and t2. Anticipating the experimental results it will be demonstrated that the three possible permutations in I,J to calculate KeCn are not equivalent but establish a ranking in the results: $KeCn_{01} < KeCn_{12} < KeCn_{02}$. Each difference is at least 3% and further a difference versus urea clearance still remains. This result is not explained satisfactory in the literature and seems to indicate the existence of an additional process that is taking effect [DF1]. In [Pe1] Petitclerc discusses a membrane type specific effect could be responsible. It is also to consider the cancelling of ultrafiltration because ultrafiltration therefore is partially neglected in the derivation of E 2.4.1.a [expressions 11-14 in Sar1], but the rates of ultrafiltration adjusted in the course of the studies do not explain the difference. Only a third and stronger condition is capable of producing the inaccuracy. In this study it has been investigated if the sodium transfer itself could be responsible for the difference of KeCn we observe using the different permutations in I,J.

An important condition in the derivation of E 2.4.1.a is the stability of plasma sodium during the course of the step profile measurement. There is reason to doubt that this condition is respected by the step profile measurement and the predominating quantity that produces the questionable inaccuracy neither is the membrane type nor ultrafiltration rate neglect in the formula derivation but the sodium transfer that is induced by the measurement itself due to the necessary variations of the dialysate inlet sodium concentration. On the assumption that the plasma sodium stability condition is violated a set of equations will be derived now which in the end give an expression for KeCn and also for the virtual sodium distribution volume that serves as a solute sink behind the dialyser. In a first attempt the simplified basic mass equation for the variation of cbi will be used assuming there is only a single compartment behind the filter, no rebound and a short term complete

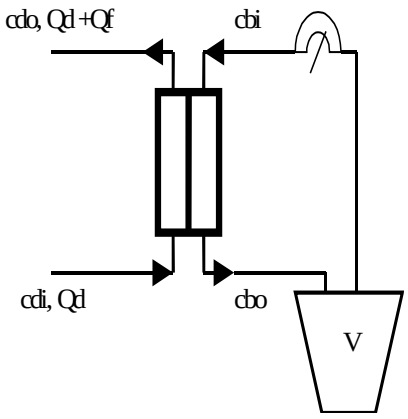


Fig 2.5.3.a:
A simplified model to demonstrate mass flux into and from the patient who is reduced to a single pool volume without internal structure or recirculation.

distribution of the solutes in the body will occur (see figure 2.5.3.a):

$$\int_{t}^{t+\Delta t} Qd\ cdi(\tau) - (Qd + Qf)cdo(\tau)d\tau = V(t+\Delta t)\ cbi(t+\Delta t) - V(t)\ cbi(t) \qquad \text{E 2.5.3.a}$$

This can also be expressed as a differential equation after limΔt has approached zero:

$$Qd(cdi(t) - cdo(t)) - Qf\ cdo(t) = \frac{d(V(t)cbi(t))}{dt} = V'(t)cbi(t) + V(t)cbi'(t) \qquad \text{E 2.5.3.b}$$

With V'(t) = -Qf:

$$\Rightarrow cbi'(t) = \frac{Qd\,cdi(t) - (Qd + Qf)cdo(t) + Qf cbi(t)}{V(t)} \qquad \text{E 2.5.3.c}$$

cbi' can also be derived from the effective clearance definition:

$$KeCn = Qd\frac{cdi(t) - cdo(t)}{cdi(t) - \alpha cbi(t)}\left(1 - \frac{Qf}{Qe}\right) + Qf$$

$$\Rightarrow cbi = \frac{1}{\alpha}\left[cdi(t) - \frac{\left(1 - \frac{Qf}{Qe}\right)Qd(cdi(t) - cdo(t))}{KeCn - Qf}\right]$$

$$\text{Assuming stable conditions } \frac{dQd}{dt} = 0, \frac{dQf}{dt} = 0, \frac{dKeCn}{dt} = 0, \text{ the derivation is:}$$

$$cbi'(t) = \frac{1}{\alpha}\left[cdi'(t) - \frac{\left(1 - \frac{Qf}{Qe}\right)Qd(cdi'(t) - cdo'(t))}{KeCn - Qf}\right] \qquad \text{E 2.5.3.d}$$

Equating E 2.5.3.c and E 2.5.3.d yields for V(t):

$$V = \alpha \frac{Qd\ cdi(t) - (Qd + Qf)cdo(t) + Qf\ cbi(t)}{cdi'(t) - \frac{\left(1 - \frac{Qf}{Qe}\right)Qd}{KeCn - Qf}(cdi'(t) - cdo'(t))} \qquad \text{E 2.5.3.e}$$

E 2.5.3.e is an equation that in principle allows to measure V because also cbi can be derived from a pure machine-side dialysance measurement, but by practical aspects it might be inconvenient to measure the differentials cdi' and cdo'. In a technical system like the concentrate preparation hydraulics of a dialysis machine always short term variations and slight instabilities of conductivity might be generated that could affect the result of E 2.5.3.e by making the numerical evaluation of the differentials inaccurate. Therefore a better solution would completely avoid the use of differentials and directly refer to the observed difference in $KeCn_{01}$, $KeCn_{12}$. To derive the equations that express V in terms of the difference $KeCn_{12}$ - $KeCn_{01}$ from a step profile dialysance measurement again some essential conditions have to be fulfilled to avoid the differential equation. These conditions confine the validity of the derivation to the very first minutes after the conductivity change, but normally a step profile measurement of ionic dialysance does not last longer than a few minutes and it can be expected that these restrictions are acceptable. The restrictions are:

- The measurement is performed by applying a conductivity variation with the shape of a step-function (up and down step) at the dialysate inlet to the filter. The shape of the whole step profile is designed to keep the integral balance of sodium transfer at zero. No net sodium should remain in the patient after complete termination of the profile.
- All states, the baseline, at high level and at low level, have achieved stability. Stability is accepted if cdo change per minute is less than 5% of the total cdi change.
- The change in cdi is approximately 20 times higher than the change of cbi that will result from the variation of cdi. This in equivalence means the time-dialysance-product is less than 1/20 of the distribution volume. All calculations are linear to the initial condition.
- Only a single pool model is considered. The internal structure of the distribution volume is neglected. This recommends the intra-extra-cellular clearance of the participating fluid compartments of the patient to be significantly higher than the clearance applied by the dialysis.
- Recirculation is neglected and should be limited to only a few percent (<6%).

Due to the linearisation also an extension to prolonged periods of conductivity variation would recommend to use the complete differential equation that also includes the feedback on cbi which is not included here. The longer the change sustains the less the following equations are valid.

Starting with the basic assumption that the sodium transfer is responsible for the difference in $KeCn_{I,J}$, there are two different expressions for $KeCn_{01}$, (E 2.4.1.a and E 2.4.2.a), the first with neglect of cbi change but it can be applied directly because all variables are known, and the second with respect to the cbi change but the disadvantage of non-applicability because cbi is not known:

$$KeCn_{C,IJ} = D\left(1-\frac{Qf}{Qe}\right)+Qf$$
$$= Q_f + \frac{\left[\left(cdo_I - cdi_I\right)-\left(cdo_J - cdi_J\right)\right]Qd+\left[\left(\alpha_D cbi_J - cdo_J\right)-\left(\alpha_D cbi_I - cdo_I\right)\right]Q_f}{\left(\alpha_D cbi_I - cdi_I\right)-\left(\alpha_D cbi_J - cdi_J\right)}$$

The index C refers to the “correct” value with respect to the variation of cbi. Further the diffusive fraction of the effective dialysance will be of interest:

$$D = \frac{\left[\left(cdo_I - cdi_I\right)-\left(cdo_J - cdi_J\right)\right]Qd+\left[\left(\alpha_D cbi_J - cdo_J\right)-\left(\alpha_D cbi_I - cdo_I\right)\right]Qf}{\left(1-\frac{Qf}{Qe}\right)\left[\left(\alpha_D cbi_I - cdi_I\right)-\left(\alpha_D cbi_J - cdi_J\right)\right]}$$

Applying a transition from inlet conductivity cdi_0 to cdi_1 for time t the ‘correct’ diffusive dialysance D_{12} replaces a fraction of plasma water of concentration cbi_0 with the new concentration cdi_1 while the convective term $Q_f t$ only extracts plasma water with cbi_0. Neglecting recirculation we get for the new systemic concentration cbi_1:

$$cbi_1 \cong \frac{V_0 cbi_0 - D_{1,2} t cbi_0 + D_{1,2} t cdi_1 - Q_f t cbi_0}{V_0 - Q_f t} \qquad \text{E 2.5.3.f}$$

This equation only holds for the very first minutes, as long as the product $D_{1,2}$*t is small compared to V_0 . With V_t=V_0-Qf t we now have an expression for the change of blood concentration:

$$cbi_0 - cbi_1 \cong \frac{D_{1,2} t(cbi_0 - cdi_1)}{V_t} \qquad \text{E 2.5.3.g}$$

$$KeCn_{C;0,1} = \frac{\left[\left(cdo_0 - cdi_0\right)-\left(cdo_1 - cdi_1\right)\right]Qd+\left[cdo_0 - cdo_1 - \frac{D_{1,2} t(cbi_0 - cdi_1)}{V_t}\right]Qf}{\frac{D_{1,2} t(cbi_0 - cdi_1)}{V_t} - cdi_0 + cdi_1} + Qf$$

E 2.5.3.h

To find the difference Y= $KeCn_{C;01}$ – $KeCn_{01}$, we subtract (E 2.5.3.h – 2.4.1.a) :

$$Y = \frac{\left[\left(cdo_0 - cdi_0\right) - \left(cdo_1 - cdi_1\right)\right]Qd + \left[cdo_0 - cdo_1 - \frac{D_{1,2}t(cbi_0 - cdi_1)}{V_t}\right]Qf + \left[\frac{D_{1,2}t(cbi_0 - cdi_1)}{V_t} - cdi_0 + cdi_1\right]Qf}{\frac{D_{1,2}t(cbi_0 - cdi_1)}{V_t} - cdi_0 + cdi_1} - \frac{\left[(cdi_1 - cdi_0) - (cdo_1 - cdo_0)\right]}{(cdi_1 - cdi_0)}(Qd + Qf)$$

Collection and cancelling yields:

$$Y = \frac{-\left[\left(cdo_0 - cdi_0\right) - \left(cdo_1 - cdi_1\right)\right](Qd + Qf)\frac{D_{1,2}t(cbi_0 - cdi_1)}{V_t\left(cdi_1 - cdi_0\right)}}{\left(cdi_1 - cdi_0\right)\left(1 + \frac{D_{1,2}t(cbi_0 - cdi_1)}{V_t\left(cdi_1 - cdi_0\right)}\right)} = \frac{-KeCn_{0,1}\frac{D_{1,2}t(cbi_0 - cdi_1)}{V_t\left(cdi_1 - cdi_0\right)}}{\left(1 + \frac{D_{1,2}t(cbi_0 - cdi_1)}{V_t\left(cdi_1 - cdi_0\right)}\right)}$$

$$= \frac{KeCn_{0,1}}{\left(\frac{V_t\left(cdi_1 - cdi_0\right)}{D_{1,2}t(cdi_1 - cbi_0)} - 1\right)}$$

E 2.5.3.i

E 2.5.3.i can be solved for V_0 , the pre-dialysis sodium distribution volume of the patient:

$$V_0 = V_t + Q_f t \cong \left(\frac{KeCn_{0,1}}{KeCn_{1,2} - KeCn_{0,1}} + 1\right)\frac{D_{1,2}t(cdi_1 - cbi_0)}{\left(cdi_1 - cdi_0\right)} + Q_f t$$ E 2.5.3.j

This linear expression can be applied to in-vitro and in-vivo situations, because the value for cbi_0 can be derived from the dialysance measurement itself, therefore all variables are known. E 2.5.3.j has been applied to the different data sets available in the studies also including some laboratory measurements where the absolute V was known exactly. To understand the character of E 2.5.3.j it is helpful to consider the particular case when the initial baseline cdi_0 of the step profile exactly matches cbi_0, further $Q_f = 0$ and therefore KeCn=D. In that case we yield:

$$V_0 \cong \frac{KeCn_{1,2}^2 t}{Y} \Leftrightarrow Y \cong \frac{KeCn_{1,2}^2 t}{V_0}$$ E 2.5.3.k

because of $V_0 >> D_{1,2}$ t according to the initial conditions. That means in general the difference $KeCn_{1,2} - KeCn_{0,1}$ that we observe is in inverse proportion to the distribution volume of the patient and depends by power of two of the measured ionic dialysance. That means the higher the absolute clearance is the more important it is to be aware of sodium shift that could falsify the measurement. It can be understood now that the utilisation of a simple low-high step profile will not automatically result in a correct dialysance measurement, especially with patients of low urea distribution

volume. On one hand E 2.5.3.j and E 2.5.3.k are easily applicable formulas to measure V with a symmetric step profile of inlet conductivity if the restrictions made for their derivation can be accepted, but on the other hand it should be noticed that the result is critical to errors in measurement of $KeCn_{0,1}$ because they interfere with power of two.

2.5.4. Single pool urea distribution volume

The urea distribution volume can also be derived from single pool urea kinetic model. To understand the derivations it is necessary to first consider the single pool urea kinetic model in total which will be explained in chapter 2.6. The calculation of the single pool urea distribution volume is possible according to equation E 2.6.1.e' of the next chapter, which results from this model.

2.6. Urea kinetics – Evaluation of Kt/V

The considerations on the distribution volume suggest that the human body could be regarded as a coupled combination of different compartments – 'pools' - where uremic toxins, in this context urea, are stored. Urea kinetics makes the attempt to describe and simulate theoretically the mass transfer processes and transfer rates between the pools which are regarded as different compartments that interact with each other and the external compartment. In ESRD patients the external interaction is mediated by the dialysis machine.

Urea is a highly soluble, free diffusing solute that can cross nearly any membrane that is passable by water. In the healthy patient without kidney malfunction urea is distributed throughout the whole body water in nearly total equilibrium. Rarely there are found urea gradients within intra- and extra-cellular space. The only exception in the healthy patient is the urea concentration gradient from cortex to medulla of the kidney which helps along Henle's loops to conserve water from primary urine that is filtered from plasma water in the glomeruli [Si]. This concentration ratio of urea in the kidneys papilla to plasma urea can be greater than 100:1 [Dep2] and therefore it can substantially contribute to the urea distribution volume. This is not the case in the ESRD patient, whose kidney has nearly completely lost the ability to clear the plasma from uremic toxins. The osmotic gradient from cortex to medulla is gradually or totally lost and no longer a separate urea pool in the kidney exists.

The extracellular fluid (ECF) and the intracellular fluid (ICF) are commonly regarded to be the two important pools for urea. The question is if it is necessary to distinguish between these in clinical urea quantification. Urea kinetic modelling usually is performed only on ESRD patients, in particular during dialysis. Healthy patients do not show significant urea gradients and if their urea kinetics should be analysed it will be completely sufficient to model them as a single volume if not the kidney with its high urea concentration is of particular interest. ESRD patients however will experience a high urea gradient during dialysis. Within intermittent hemodialysis which does not work as continuous as the kidney but in confined periods the clearance is 7-10 times the clearance of the natural kidney. Urea exclusively is extracted from the ECF. Initially to dialysis a gradient between ECF and ICF is established that never exists in healthy patients. Urea kinetic modelling must predict the behaviour of the mass fluxes correctly even with the gradient present. This is the major reason why ESRD patients – in particular in combination with a scientific interest – are often modelled using two compartments.

The post-dialytic urea concentration curve – the rebound curve - is characterised by a mass flux from ICF to ECF to equilibrate the gradient. Duration and total mass that is transferred during rebound is determined by the inter-compartment clearance, the participating volumes and the urea generation rate. Concentrations of ECF that have been measured immediately after termination of dialysis can not directly be used to derive any whole body mean concentrations or total treatment clearance values. Further there might exist additional, poorly vascularised or weakly circulated body regions that show prolonged rebound phases until they reach post dialysis concentration equilibrium. A model that neglects these rebound effects can not show

accurate results. Aside from the impractical blood samples and the costs, the measurement of the rebound is time-consuming and patients are not willing to wait 30-45 min to have the rebound elapsed just to assure that they have been dialysed adequately.

To circumvent this problem many improvements have been made that involve clinical experience and statistical knowledge. The most accepted empirical formula is the 'second generation' formula of Daugirdas [Daugd], but there are many other less empirical variations of the single pool model that regard also intra-dialytic fluid shifts, several blood urea nitrogen (BUN) measurements, variable volume and equilibration processes [Dep3, Sar5, Fro, Go6]. A valuable summary is also found in [Dep2].

Due to its closed analytic solution and mathematical simplicity within this study the 'equilibrated single pool variable volume urea kinetic model' (eSPVV) has been used in the primary data analysis. To give a clear information on what was calculated within the data analysis the eSPVV will be explained to some extent in the next chapter.

2.6.1. Single pool urea kinetic model

In its straightforward adaptation the single pool urea kinetic model (SPUKM) models the patients urea distribution volume as fixed compartment with *constant* volume V. This compartment is containing a fixed amount of urea at dialysis start t_0 which is cleared from urea by K_{eff} and not filled by any urea generation. The time dependent urea concentration under clearance taking effect in this model is identical to that of an isotope decay and can be described with the same simple rate equation:

$$\frac{d(Vc(t))}{dt} = -Kc(t) = V\frac{dc(t)}{dt}$$
$$\Leftrightarrow c(t) = c_0 e^{\frac{-Kt}{V}} \Leftrightarrow \ln\left(\frac{c_0}{c_t}\right) = \frac{Kt}{V} \qquad \text{E 2.6.1.a}$$

Analogously the half life can be introduced with $c_t=c_0/2$:

$$\ln\left(\frac{c_0}{c_t}\right) = \ln 2 = \frac{Kt_{Halflife}}{V} \Leftrightarrow t_{Halflife} = \frac{V}{K}\ln 2$$

When assuming dialysis will reduce the urea concentration to 10% of its initial value, we can compare $t_{Halflife}$ to $t_{10\%}$ and will find the ratio

$$\frac{t_{10\%}}{t_{Halflife}} = \frac{\ln 10}{\ln 2} = 3.32$$

This means in a rough calculation that after approximately 30% of dialysis time 50% of urea is removed.

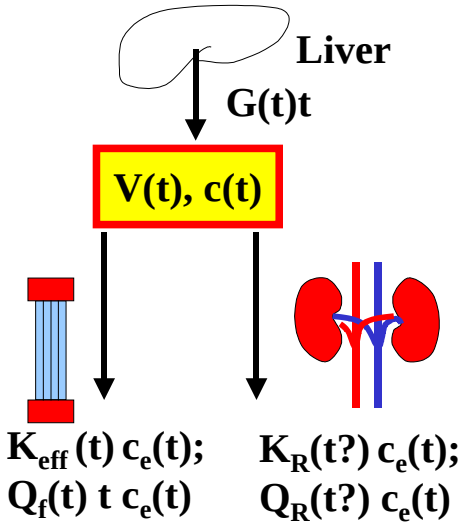


Figure 2.6.1a:*The single pool model describes the interference of the patient, dialysis and urea concentration in systemic plasma water using a model that is based on the assumption that the patients urea kinetic can sufficiently be described with a single internal urea distribution volume.*

The outstanding attractiveness of this model is its simplicity: There are only two urea plasma measurements necessary to calculate the dose parameter Kt/V.

With only a few additional considerations the model and its accuracy can be enhanced. The single pool urea kinetic model with variable volume assumes the patients body to be consisting of a single internal urea distribution compartment of the *non-constant* volume V(t) and urea concentration c(t). The initial dialysis conditions are assigned to V_0, c_0. Dialysis continuously extracts urea with the effective urea clearance $K_{eff}(t)$ and plasma water with the ultrafiltration rate $Q_f(t)$ from the urea distribution volume. Additionally in some patients a residual clearance K_R and an residual filtration of Q_R exists. The liver generates urea with the urea generation rate G(t) (see figure 2.6.1.a.).

For simplification it is possible to summarise the residual clearance and the generation rate to a resulting G'(t), the virtual generation rate, further the residual ultrafiltration can be included in the ultrafiltration rate, which is regarded to be constant throughout dialysis to keep the resulting mathematical expressions compact. The mass flux balance again can be described by a differential equation similar to the previous but now enhanced with a variable volume and G':

$$\frac{d\left((V_0 - Q_f t)c(t)\right)}{dt} = G' - K_{eff}\,c(t) \qquad \text{E 2.6.1.b}$$

In words this means that the momentary change of urea mass content (V(t)*c(t)) of the single pool is determined by the gain of urea from (virtual) generation and the loss of urea by dialysis activity. The analytic integration is straightforward and results in

$$c(t) = \frac{G'}{K_{eff} - Q_f}\left[1 - \left(\frac{V_0 - Q_f t}{V_0}\right)^{\frac{K_{eff}}{Q_f}-1}\right] + c_0\left(\left(\frac{V_0 - Q_f t}{V_0}\right)^{\frac{K_{eff}}{Q_f}-1}\right) \qquad \text{E 2.6.1.c.}$$

This can be resolved to yield $V(t) = V_0 - Q_f t$ and the dialysis start volume V_0, both formulated in dependence of two different concentrations:

$$V(t) = \left(\frac{c(t) - {G'}/{K_{eff} - Q_f}}{c_0 - {G'}/{K_{eff} - Q_f}} \right)^{\frac{Q_f}{K_{eff} - Q_f}} V_0$$

$$\Rightarrow V_0 = \frac{Q_f t}{1 - \left(\frac{c(t) - {G'}/{K_{eff} - Q_f}}{c_0 - {G'}/{K_{eff} - Q_f}} \right)^{\frac{Q_f}{K_{eff} - Q_f}}}$$

E 2.6.1 d,e

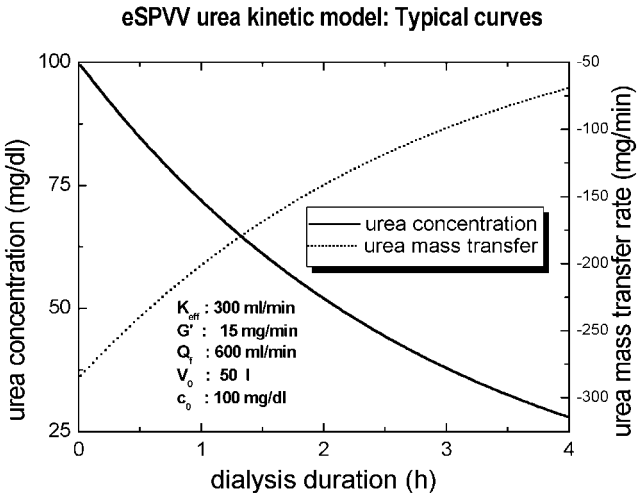


Figure 2.6.1b: *Typical shape of a single pool modelled urea concentration and urea mass transfer rate curves during hemodialysis. The rebound is not included in the model. The values used for the calculation are depicted in the figure.*

E 2.6.1.e can directly be adopted to calculate the initial urea distribution volume by inserting the c_0 and c_{t30} concentrations. The c_{t30} value is beneficial to be inserted for higher accuracy due to the inclusion of the rebound phase. Within the single pool analysis of this study G' has been set to 0 and the expression

$$V_0 = \frac{Q_f t}{1 - \left(\frac{c_{t30}}{c_0} \right)^{\frac{Q_f}{K_{eff} - Q_f}}}$$

E 2.6.1.e'

has been evaluated. Again the neglect of G' will be responsible for an error around 10%, like explained for the urea distribution volume in chapter 2.5. A theoretical plot of E 2.6.1.b,c is shown in figure 2.6.1.b. The exponential curves are typical for the model.

For analysis of the study data it is necessary to have an independent reference for Kt/V. Thus we will have to derive not only V_0 but also K. Formally this can be done by using the fully equilibrated Kt/V according to E 2.6.1.a inserting the rebound value c_{t30} and dividing it by t_{Dial}/V_{Mean}:

$$KeUB_{eSPVV} = \frac{KeUB_{eSPVV}\, t_{Dial}}{V_{Mean}} * \frac{V_{Mean}}{t_{Dial}} = \ln\left(\frac{c_0}{c_{t30}} \right) \frac{V_o c_o - \left(V_o - Q_f t_{Dial} \right) c_{30}}{\left(c_0 - c_{30} \right) t_{Dial}}$$

E 2.6.1.f

This is only a formal consideration: There is a need of further input because V_0 is not independent but a function of K_{eff}. This problem can be solved by using KeUB from E 2.2.1.d to calculate V_0, thus taking the reference samples that have been sampled during the treatment. Therefore the equilibrated single pool Kt/V is calculated according to:

$$\left[\frac{Kt}{V} \right]_{eSPVV} = \frac{KeUB_{eSPVV} t_{Dial}}{V_o}$$

E 2.6.1.g

This equation has been used for the eSPVV urea kinetic model evaluations.

2.6.2. Kt/V from Direct quantification

The principle of direct quantification is described in chapter 2.5.1 leading to E 2.5.1.a for the urea distribution volume, V_{DQ}. Similar to the derivation of E 2.6.1.f we multiply V_{DQ}/t_{Dial} by $\ln(c_0/c_{t30})$:

$$K_{DQ} = \frac{V_{DQ}}{f_{corr} t_{Dial}} \ln\left(\frac{c_0}{c_{t30}}\right) \quad \text{with } f_{corr} = 1 - \frac{t_{Bypass}}{t_{Dial}}$$

$$\left[\frac{Kt}{V}\right]_{DQ} = \left[\frac{Kt}{V}\right]_{eSPVV} = \ln\left(\frac{c_0}{c_{t30}}\right)$$

E 2.6.2.a

The dialysis time here is corrected for the amount of time that has elapsed with the machine being in bypass, t_{Bypass}, due to alarms or pressure holding tests the machines use to perform every 12min. During this periods the sampling unit was programmed not to sample dialysate, because dialysate was not in contact with the patients blood. Consequently the bypass time must be reflected in the calculation.
Comparing E 2.6.1.g and E 2.6.2.a we find that from a theoretical aspect both must yield the same result, except for internal differences in K, t and V. Further differences may occur due to different data sets, because not for every reference principle the same subsets of data were available. Sometimes the DQ reference failed for technical reasons. This diminishes the data pairs to compare. Nevertheless DQ has been selected because in general it is accepted as the gold standard. If Kt/V is derived from DQ like described it may not really differ from the equilibrated variable volume single pool urea kinetic model results.

2.6.3. Daugirdas second generation Kt/V formula

Daugirdas has introduced an empirical formula of clinical relevance that is very easy to use and widely accepted, even though it is partially based on statistical analysis and not purely on individual measurements. To show the relation of the results of this studies to other results the ‘second generation’ Daugirdas formula, which is the most elaborated of the Daugirdas formulas, has been used for analysis [Daugd]:

$$\left[\frac{Kt}{V}\right]_{\text{Daugirdas 2nd gen}} = -\ln\left(\frac{c_{t0}}{c_{tEnd}} - 0.008\, t_{Dial}\right) + \left(4 - 3.5\frac{c_{t0}}{c_{tEnd}}\right)\frac{\text{total Ultrafiltrate}}{\text{initial patient weight}}$$

E 2.6.3.a

2.6.4. Double pool urea kinetic model

A single pool model can only describe the urea kinetics correctly if the patient is in complete equilibrium. This is fairly correct within the intermediate phases of two dialyses. During dialysis this is completely incorrect because the patient from this aspect can be regarded as composed of two fractional urea distribution volumes: The intracellular fluid (ICF) and the interstitial and intravasal fluid, both forming the extracellular fluid (ECF). The urea concentration within both of these aqueous distribution spaces of the body of the ESRD patient is inhomogeneous during dialysis. This is due to strong concentration gradients generated by dialysis. Dialysis has only access to the ECF, because the patient is connected via the cannula positioned within his arterio-venous fistula or graft. Urea once removed by dialysis from extracellular space will be refilled by an osmotic gradient across the cell membrane. This is a non-instantaneous process. Typically it takes 30min to equilibrate 95% of an initial urea gradient over the cell membrane if the gradient is not perpetuated by further dialysis. This effect, usually referred as rebound, is the measurable trace of the delayed urea mass transfer across the cell membrane. Any single pool model applied to a data set of a dialysis session will fail to predict intra- and extracellular urea concentrations correctly because the rebound is not taken into account. Further a gradient from ICF to ECF is maintained during active dialysis by continuous urea mass drain from extracellular pool to the dialysate, which is not included with single pool models. The lack of a second pool makes ECF urea concentration measurements not representative for the whole distribution volume and therefore total body urea can not be quantified by analysis of ECF urea concentrations during dialysis. In contrast to the single pool analysis the mathematical evaluation with both intra- and extracellular pool is not very handy and usually will not be performed by the clinical staff.

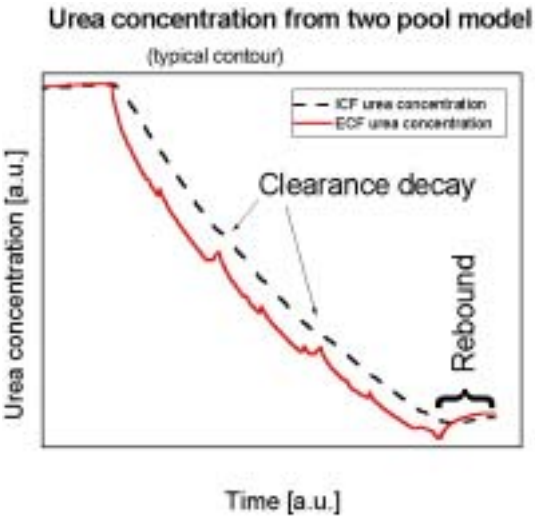


Figure 2.6.4 a: *Typical concentration decay of urea concentration in ICF and ECF compartments of an ESRD patient under dialysis on base of recorded data. Clearance deterioration takes primary effect only on the ECF urea concentration.*

A typical concentration vs. time profile during dialysis is shown in figure 2.6.4 a .

2.6.5. Two pool urea kinetic model : Mathematical description

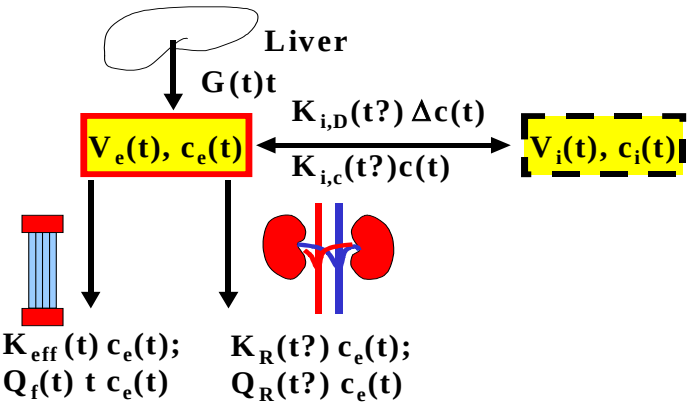


Figure 2.6.5.a: *A simplified representation of the participating constituents of the two pool urea kinetic model of an ESRD patient.*

To make it accessible to mathematical considerations the urea mass kinetics must be simplified to an interaction of the urea generating component, which is the liver, both the intra- and extracellular pool each with a given buffer volume and the urea eliminating processes like a residual clearance of the native kidneys and the urea removal performed by the dialysis machine. Figure 2.6.5 a illustrates the interaction. The liver excretes urea with a time dependent generation rate G(t) to the ECF space of volume Ve(t) and concentration ce(t). The extracellular volume interferes with the intracellular volume by the inter-compartment clearance $K_{i,D}$ (diffusive fraction) and $K_{i,c}$ (convective fraction), driven by osmotic pressure and expressed as a fluid shift rate per concentration gradient [ml/(mmol/ml)/s)]. Both can be subject to time dependent alterations that are induced by the vessel constriction, electrolyte balance, membrane properties and other physiologic or pathologic processes. Further it is not straightforward to measure a realistic value for both of these quantities. $K_{i,D}$ is reported to be in the order of 760-1000 ml/min [Sar1, Bell, Go4, Hei] and has been selected to be 800ml/min. Some ESRD patients may have retained a fraction of residual clearance and filtration, expressed by K_R and Q_R that is only effecting ECF. Dialysis as the life-preserving treatment of the ESRD patient is the only path a substantial amount of urea can leave the body via the extracellular space. Dialysis is not applied continuously and frequently interrupted by alarms, therefore $K_D(t)$, the diffusive clearance applied by dialysis is time dependent, as $Q_f(t)c_e(t)$, the amount of urea extracted by convection is. Q_f sometimes is superimposed by time dependent ultrafiltration profiles. All these interactions of the different processes, overlaid by a time dependence of the variables linked to each other like clearance, volume and concentration, make the mathematical description sophisticated and the analytical mathematical solution extremely complicated if not impossible. The numerical approach with assistance of a computer is much more practical and has been chosen to integrate the equations.

The problem can be described by the mass balance formulation assuming no particle can vanish or appear in a compartment without entering or leaving another. The consideration is restricted to urea and can not in general be transferred to other solutes with different kinetics and excretory mechanisms. Even for urea a trans-cutaneous excretory process can not totally be excluded but is neglected here. There is no selective trans-membrane transport process for urea that shifts exclusively urea against its concentration gradient to ECF or ICF, therefore for the outer pool (= ECF) can be concluded:

$$\#\,\text{Urea molecules} = \#\,\text{at t0} \pm \text{diffusive loss to ICF} \pm \text{convective loss/gain to ICF} + \text{urea generation since t0 - loss by dialysis - loss by residual clearance.}$$

The same expression is made for the ICF, except that there is only transfer out of the body via the ECF:

$$\#\,\text{Urea molecules} = \#\,\text{at t0} \pm \text{diffusive gain from ECF} \pm \text{convective gain/loss from ECF}$$

In mathematical terms using the variables of figure 2.6.5.a we find the equations be linked to each other, thus an independent solution for one of them does not exist:

$$V_e c_e = V_{e,t0} c_{e,t0} + \int_{t0}^{t} K_{i,D}(c_i - c_e)dt' + \int_{t0}^{t} K_{i,c}(c_e - c_i)c_e\theta(c_i - c_e)dt' + \int_{t0}^{t} K_{i,c}(c_e - c_i)c_i\theta(c_e - c_i)dt'$$
$$+ G(t - t_0) - \int_{t0}^{t} (K_{eff} + K_R)c_e dt'$$

$$V_i c_i = V_{i,t0} c_{i,t0} - \int_{t0}^{t} K_{i,D}(c_i - c_e)dt' - \int_{t0}^{t} K_{i,c}(c_e - c_i)c_e\theta(c_i - c_e)dt' - \int_{t0}^{t} K_{i,c}(c_e - c_i)c_e\theta(c_e - c_i)dt'$$

E2.6.5 a,b

with θ being the Heaviside function, which is 0 for x<0, 1 for all other x. $K_{i,c}$ is the specific convective inter-compartment flow rate that describes fluid transfer from ECF to ICF or vice versa driven by the osmotic pressure. The unit of $K_{i,c}$ is [ml/(mmol*s)]. Though some authors [Go4] do expect variations for the generation rate G - in particular during dialysis – for simplicity G is regarded to be constant over time and can fairly be calculated from blood urea concentration at the end of the previous and at the beginning of the present dialysis treatment and further from the urea distribution volume, which itself is not known prior to the integration of equation system E 2.6.5a,b. This means G will also be a result of the integration, not a variable that is known initially. Another variable that is not known precisely is the residual clearance. For most ESRD patients it is zero. However many of the patients who had participated in this studies still revealed residual clearance because their dialysis treatment has begun only a few month prior to the study. A calculation of G from the final blood urea concentration of the previous treatment, the initial concentration of the present treatment and the distribution volume will include the residual clearance. This will be indicated by using the apostrophized G', the equivalent generation rate which is never larger than G. The relation of G and G' is

$$G(t_2 - t_1) - \int_{t1}^{t2} K_R c_e dt' = G'(t_2 - t_1) \qquad \text{E2.6.5 c}$$

A numerical calculation needs an initial guess for G' that can be obtained from the following consideration: During the interdialytic period a patient gains weight due to his fluid intake. The gain of urea from treatment n to treatment n+1 should therefore be

$$\left[(V_e + V_i)c_e\right]_{t30,n} + G'(t_{0,n+1} - t_{30,n}) = \left[(V_e + V_i)c_e\right]_{t0,n+1} \qquad \text{E2.6.5 d}$$

It is an acceptable inaccuracy to assume that the rebound has equilibrated completely and V_e and V_i are of the same concentration if 30min have elapsed since the end of treatment n. This means we neglect the slight interdialytic concentration difference of ICF and ECF due to urea generation that is loading only to ECF. Another simplification to obtain an initial guess of the equivalent generation rate G' is to assume that the intradialytic fluid loss $\int_0^{tend} Q_f dt$ by ultrafiltration is performed such that the patient will always keep the same weight at the end of each dialysis n, n+1. This permits to write

$$\left[(V_e + V_i)_{t0} - Q_f(t_{end} - t_0)\right]_{n+1} c_{e,t30,n} + G'(t_{0,n+1} - t_{30,n}) = \left[(V_e + V_i)c_e\right]_{t0,n+1}$$

$$G' = \frac{\left[(V_e + V_i)c_e\right]_{t0,n+1} - \left[(V_e + V_i)_{t0} - \int_{t0}^{tend} Q_f dt\right]_{n+1} c_{e,t30,n}}{(t_{0,n+1} - t_{30,n})}$$

$$G' = \frac{\left[(V_e + V_i)_{t0} - \int_{t0}^{tend} Q_f dt\right]_{n+1} c_{e,t30,n} - \left[(V_e + V_i)c_e\right]_{t0,n+1}}{t_{30,n} - t_{0,n+1}}$$

$$G' = \frac{(V_e + V_i)_{t0,n+1}(c_{e,t30,n} - c_{e,t0,n+1}) - \left[\int_{t0}^{tend} Q_f dt\right]_{n+1} c_{e,t30,n}}{t_{30,n} - t_{0,n+1}}$$

E2.6.5 e

whereas the value of $\int_0^{tend} Q_f dt$ is not an unknown variable, because $Q_f(t)$ is recorded by the dialysis machine continuously and therefore the amount of ultrafiltrate is known permanently throughout dialysis. The aim of this consideration is to find a solution of $c_e(t)$ and $c_i(t)$, the time dependent concentration of urea in the ECF and ICF. For clarity we have to rearrange and simplify E2.6.5 a,b . We will abbreviate

$$S1 = \int_{t0}^{t} K_{i,c}(c_e - c_i)dt'$$

Sum 1: Amount of fluid that has been shifted from ICF to ECF (>0) or vice versa (<0).

$$S2 = \int_{t0}^{t} K_{i,D}(c_i - c_e)dt')$$

Sum 2: Amount of urea that has been shifted by diffusion from ICF to ECF (>0) or vice versa (<0).

$$S3 = \int_{t0}^{t} K_{i,c}(c_e - c_i)c_e\theta(c_i - c_e)dt'$$

Sum 3: urea loss from ECF by convection to ICF (<0)

$$S4 = \int_{t0}^{t} K_{i,c}(c_e - c_i)c_i\theta(c_e - c_i)dt'$$

Sum 4: urea loss from ICF by convection to ECF (>0)

$$S5 = \int_{t0}^{t} K_{eff}c_e dt'$$

Sum 5: urea loss from ECF by dialysis

$$\overline{Q_f} = \frac{\int_{t0}^{tend} Q_f dt}{t_{end} - t_0}$$

Mean value of Q_f during dialysis

and find for ECF at any particular time t_+=t+dt during dialysis n:

$$\begin{aligned}\left[V_{e,t0} - \overline{Q_f}t + S1 + K_{i,c}(c_e - c_i)dt\right]c_e = {} & V_{e,t0}c_{e,t0} + S2 + K_{i,D}(c_i - c_e)dt + G'_{n-1,n}\,t \\ & + S3 + K_{i,c}(c_e - c_i)c_e\theta(c_i - c_e)dt \\ & + S4 + K_{i,c}(c_e - c_i)c_i\theta(c_e - c_i)dt \\ & - S5 - K_{eff}c_e dt\end{aligned}$$

and for ICF:

$$\begin{aligned}\left[V_{i,t0} - S1 - K_{i,c}(c_e - c_i)dt\right]c_i = {} & V_{i,t0}c_{i,t0} - S2 - K_{i,D}(c_i - c_e)dt \\ & - S3 - K_{i,c}(c_e - c_i)c_e\theta(c_i - c_e)dt \\ & - S4 - K_{i,c}(c_e - c_i)c_i\theta(c_e - c_i)dt\end{aligned}$$

E2.6.5 f,g

These expressions conduct us from the situation at t to the situation at t+dt of the integration if the situation at t is known from a former integration – which is the sequence of all previous steps - by adding the contribution of an infinitesimal step, which of course in the numerical simulation is a discrete and not infinitesimal one. Usually the integration can be started at an initial situation as the beginning of the intermediate non-dialysis phase or the beginning of dialysis and will provide numerical information about time dependence of c_e and c_i during the period of integration. To achieve an expression for c_e(t+dt) and c_i(t+dt) once c_e(t), c_i(t) is known we will have to solve the quadratic equations in c_e, c_i, that are enclosed in E2.6.5 f,g. If we separate the terms to quadratic, linear and constant parts in c_e, c_i, it is not more than to apply the well known root of the homogeneous quadratic equation

$$ax^2 + bx + c = 0 \quad \text{with} \quad x_{1,2} = \frac{-b}{2a} \pm \sqrt{\frac{b^2 - 4ac}{4a^2}}$$

E2.6.5 h

to E2.6.5 f,g. The separation yields

$$
\begin{aligned}
& c_e{}^2\left[K_{i,c}dt - K_{i,c}\theta(c_i - c_e)dt\right] \\
& + c_e\left[V_{e,t0} - \overline{Q_f}t + S1 - K_{i,c}c_i dt + K_{i,D}dt + K_{i,c}c_i\theta(c_i - c_e)dt - K_{i,c}c_i\theta(c_e - c_i)dt + K_{eff}\,dt\right] \\
& + S5 - V_{e,t0}c_{e,t0} - S2 - K_{i,D}c_i dt - G'_{n-1,n}\,t - S3 - S4 + K_{i,c}c_i{}^2\theta(c_e - c_i)dt \qquad = 0
\end{aligned}
$$

and

$$
\begin{aligned}
& c_i{}^2\left[K_{i,c}dt - K_{i,c}\theta(c_e - c_i)dt\right] \\
& + c_i\left[V_{i,t0} - S1 - K_{i,c}c_e dt + K_{i,D}dt - K_{i,c}c_e\theta(c_i - c_e)dt + K_{i,c}c_e\theta(c_e - c_i)dt\right] \\
& + S2 - V_{i,t0}c_{i,t0} - K_{i,D}c_e dt + S3 + K_{i,c}c_e{}^2\theta(c_i - c_e)dt + S4 \qquad = 0
\end{aligned}
$$

E2.6.5 i,j

As a result we have to insert these expressions prior to every integration step within the loop of the calculation algorithm to E 2.6.5.h :

$$
\begin{aligned}
a_1 &= K_{i,c}dt - K_{i,c}\theta(c_e - c_i)dt \\
b_1 &= V_{i,t0} - S1 - K_{i,c}c_e dt + K_{i,D}dt - K_{i,c}c_e\theta(c_i - c_e)dt + K_{i,c}c_e\theta(c_e - c_i)dt \\
c_1 &= S2 - V_{i,t0}c_{i,t0} - K_{i,D}c_e dt + S3 + K_{i,c}c_e{}^2\theta(c_i - c_e)dt + S4 \\
a_2 &= K_{i,c}dt - K_{i,c}\theta(c_i - c_e)dt \\
b_2 &= V_{e,t0} - \overline{Q_f}t + S1 - K_{i,c}c_i dt + K_{i,D}dt + K_{i,c}c_i\theta(c_i - c_e)dt - K_{i,c}c_i\theta(c_e - c_i)dt + K_{eff}\,dt \\
c_2 &= S5 - V_{e,t0}c_{e,t0} - S2 - K_{i,D}c_i dt - G'_{n-1,n}\,t - S3 - S4 + K_{i,c}c_i{}^2\theta(c_e - c_i)dt
\end{aligned}
$$

E2.6.5 k-p

There are some formal aspects to be discussed about the discriminator of E2.6.5 h concerning negative roots that could occur. We have to identify the physical conditions that relate to a negative root. The root will only be negative if $b^2<4ac$. We will consider here only the case during dialysis, usually when $c_i>c_e$. In a similar consideration this can be shown also for the intermediate phase.

$$
\begin{aligned}
4a_1c_1 &= 4K_{i,c}dt[S2 - V_{i,t0}c_{i,t0} - K_{i,D}c_e dt + S3 + K_{i,c}c_e{}^2 dt + S4] \\
b_1^2 &= [V_{i,t0} - S1 - 2K_{i,c}c_e dt + K_{i,D}dt]^2 \\
b_1^2 - 4a_1c_1 &= V_{i,t0}{}^2 - 2V_{i,t0}S1 - 4V_{i,t0}K_{i,c}c_e dt + 2V_{i,t0}K_{i,D}dt + S1^2 + 4S1K_{i,c}c_e dt - 2S1K_{i,D}dt \\
&\quad + 4(K_{i,c}c_e dt)^2 - 4K_{i,c}c_e dtK_{i,D}dt + (K_{i,D}dt)^2 \\
&\quad - 4K_{i,c}dtS2 + 4K_{i,c}dtV_{i,t0}c_{i,t0} + 4K_{i,c}dtK_{i,D}c_e dt - 4K_{i,c}dtS3 - 4K_{i,c}dtK_{i,c}c_e{}^2 dt \\
&\quad - 4K_{i,c}dtS4
\end{aligned}
$$

$$b_1^2 - 4a_1c_1 = (V_{i,t0} - S1)^2 + 2V_{i,t0}K_{i,D}dt - S1^2 + (S1 - K_{i,D}dt)^2 + 4K_{i,c}dt(V_{i,t0}(c_{i,t0} - c_e) + S1c_e - S2 - S3 - S4)$$

E2.6.5 q

It can be seen that as soon as the algorithm has left the very first steps the new fraction that is added by the time interval dt is of only minor relevance compared to the sum that has yet been accumulated during the previous steps. Neglecting their magnitude E 2.6.5 q converts to the simple expression

$$b_1^2 - 4a_1c_1 \cong (V_{i,t0} - S1)^2$$

E2.6.5 r

This is a mathematical condition every physician strictly avoids without knowledge to prevent the patient from cardiac insult: The physical condition we have identified to avoid negative roots in the 2PUKM is that we should never shift more fluid from the patients ICF during dialysis than it contains at the beginning of dialysis. It is clear that even a distant approach to this point would not be compatible with the patients life and the root will always be positive by reasons of biology.

The step width dt should be selected appropriate to the resolution desired: During the intermediate periods between the dialysis sessions it should be convenient to choose a dt of 15min because there is no additional information expected if the time resolution is increased to higher values. This period lasts for 2-3 days, thus approximately less than 0.5% of the total amount of urea is expected to be generated within dt. From this aspect the model is overcautious here because there are no data recorded from the patients during this phase. Any time related deviations of urea production the patients experienced by their nutritional habits have not been recorded and can therefore not be interpreted by the analysis. In our model the intermediate urea production is based on linearity. Further analysis would take more effort by drawing additional blood samples and registration of the meal composition and timing. It is questionable if this can be practically performed on a patient collective that is not hospitalised.

The situation is completely different during dialysis when we have data from the dialysis machine for every second that all are reflected in this evaluation. The blood samples that are currently drawn during dialysis as well as the permanent data recording stream gain the amount of available data. Even transient dialysis interruptions of a few minutes are recorded and could have their correlation in blood urea concentration. Thus the recalculation interval dt has been reduced to 60s during dialysis.

2.6.6. Data analysis of subsequent dialysis sessions

The accuracy of the model can be improved if the duration of numerical analysis is extended to not only a single dialysis session. The analysis software is programmed to scan the whole data set if there are any two sessions of the same patient recorded and less than 4 days have elapsed meanwhile. This is assumed to be a criterion that the patient did not experience dialysis from another dialysis centre. These two dialysis sessions have been linked together and are regarded to represent a single set of continuous data that is accessible to two pool modelling.

The benefit of integrating the intermediate phase into consideration is a more precise calculation of the generation rate. A balance of the total amount of urea that has left the patient by residual clearance and dialysis on the one hand and the amount of urea that has been generated during the same interval on the other hand must result in an identical blood urea concentration at the same time relative to dialysis treatments unless the patient has changed weight. If the post-rebound c_{t30} value and the patients weight is exactly the same after previous and present dialysis we can assume that urea removal = urea generation. Therefore it is acceptable to determine G' according to E2.6.5 e.

2.6.7. Source code of the numerical algorithm kernel

The source code for analysing the data of the studies is written in object oriented C++ language and encloses about 850 pages of program code, including all attempts to find a valid algorithm for the bolus clearance calculation. Additionally there are included another 150 pages of external code that was involved for visualisation and presentation of the data. The reader may understand that it is not possible to give a full documentation of this code within this context. Nevertheless some particular parts that represent the kernel of the numerical two pool analysis are given in the appendix. The variables names should be telling their meaning. The code is encapsulated in a function that is called

void CFileDoc::TwoPoolCalcer(double t, double *SetOfParams, double *Y_at_t, double *dY_dParams, int NrOfParams) .

This function does not more than a single run of the integration described above. The integration starts at the end of the previous dialysis with a first loop that is indicated by the code

for (int runner = 0; runner <= InbetwStepnumber ; runner++)

This loop covers the intermediate time until the following dialysis begins. 'runner' is the variable that counts the integration intervals of lenght dt. Arrays (CI[], CE[], VE[], VI[], S1..5[]) for all corresponding c_e and c_i values in time steps of dt=15min are filled

and prepared for return of the routine. The names directly correspond to the variables names in the theory section. Once the algorithm has reached the start time of the second dialysis it switches to dt=60s and starts a second loop during the dialysis session recorded. This is indicated by the code

for (runner; runner < SumOfSteps ; runner++).

Again the arrays are filled with the results of the integration. After having reached the time of termination of dialysis the function passes control to its mother-process until it is called again. The mother-process is responsible to select all parameters for minimal deviation of laboratory and model values before it calls the integration again. The relevant properties of the mother-process will be explained here.

2.6.8. The target variable

The target variable of the numerical calculation is the time behaviour of c_e during the observation interval. This interval is fixed to start 30 min after the end of the previous dialysis and is enduring until 30 min after termination of the present dialysis to include the rebound. c_i has not been chosen as the target variable because there is no direct access to c_i and therefore no reference available. The goal of the analysis is to minimise the difference of c_e from the model and as measured at the various times reference samples are available. References are available after rebound of previous dialysis, at the start, during dialysis when the blood samples for clearance calculation have been drawn, at the end and after the rebound of the present dialysis. Therefore within the embedding mother-process the parameters like generation rate, inter-compartment clearance $K_{i,D}$ (diffusive fraction), $K_{i,c}$ (convective fraction), V_i , V_e and the clearance as applied by dialysis can be continuously varied to find the minimum of a least square fit in c_e. The parameters that are varied and even the method of minimum search can be modified by the programmer, because in this context it is not useful to vary all of them, as will be discussed in the next paragraph. In principal the mother-process searches the particular set of the above variables that fits best the actually measured values of c_e from all times references are available. The selection is done in discrete steps, in conclusion the results will be discrete and not continuous. The resolution however is selected appropriately and is increased when approximating a minimum. All c_e have been equally weighted to calculate ε. The fit terminates if an additional variation does not reduce the remaining ε. ε was calculated according to

$$\varepsilon = \sqrt{(\Delta c_{e,to})^2 + \sum_{i=1}^{n} (\Delta c_{e,ti})^2 + (\Delta c_{e,tend})^2 + (\Delta c_{e,t30})^2} \qquad \text{E 2.6.8.a}$$

whereas the Δ indicates the difference of the c_e the algorithm has found and the c_e that has been measured in the blood sample of the referring time, n refers to the number of blood samples related to bolus clearance measurements and t_i are the times the samples have been drawn during dialysis. The value of the previous $c_{e,t30}$

is not included because it is set as the starting point of the integration and therefore it is always exactly matched.

The c_e in E 2.6.8.a that are related to bolus clearance measurements during dialysis can not be applied directly due to cardiopulmonary and fistula recirculation that decreases the urea concentration of ECF artificially. These samples were drawn with the blood pump running so that systemic concentration is diluted to some extend with the reflux from the filter that has not passed the capillaries. To have access to the systemic ECF urea concentration c_e was corrected according to:

$$\Delta c_{e,ti} = c_{e,ti,Model} - c_{e,ti,Systemic} \quad \text{with}$$

$$c_{e,ti,Systemic} = \frac{c_{in} - c_{out} * R}{1 - R} \qquad \text{E 2.2.1.b}$$

with c_{in}, c_{out} being the urea concentrations at filter inlet and outlet as reported from the laboratory. R is the recirculation in %/100. Using this method a set of 6 reference values for the c_e versus time curve are available. This means not more than 5 independent parameters can be evaluated by the model theoretically.

Termination of the calculation:
The lack of an analytical solution of the two pool model made it impossible to find an expression for the absolute minimum of ε . It is a general problem of non-analytical calculations that they can not always assure that a minimum once found by numerical least square fit is the absolute minimum on the domain of the input variables. Thus the calculation could not be continued until ε has reached an absolute minimum, enclosing the risk of finding an only relative minimum. This in particular means that there is a theoretical chance that a completely different set of input parameters gives better results. To minimise this risk a two stage calculation was implemented: a coarse pre-scan on the full range of plausible data was prefixed to the analysis. The absolute minimum of the pre-scan was taken as the initial guess of the fine scan. This procedure assures that solutions totally out of the typical clinical range of any variable are discarded.

2.6.9. The set of input parameters for two pool modelling

The mathematical model applied has potential to vary numerous input variables during the calculation. The list of variables and their major and minor relation to other variables is shown in table 2.6.9.a. Nevertheless, it is of poor benefit to vary all of them just because the model allows to do so. This would not only vastly expand the necessary calculation effort so that even modern PC system is overloaded (a variation of 4 parameters takes 4h of calculation per dialysis session on a 350 MHz system). Essentially it would not give additional information due to an over-determination of the system. Another criterion is that the variation of some of these parameters may of course affect the c_e versus time curve with except of those

periods we have references about. The additional information – even if correctly imaged - therefore could not be verified on the data. Other variables may have controversially effect on c_e and therefore the variation of one of them would automatically shift its antagonist, because they are not independent. We will discuss this to some extend here to have a justification for the method of model application to the data that was chosen in this work.

Variable	Major influence on calculation result of	Minor influence on
G (Generation rate)	• Difference of previous c_{t30} and present c_{t0} • total urea removal • initial c_e and c_i at start of dialysis	• clearance • residual clearance • V_i and V_e
V_e (ECF volume)	• change rate of c_e and c_i during dialysis • change rate of c_e and c_i in the intermediate phase • V_i • Rebound	• generation rate • residual clearance • clearance
V_i (ICF volume)	• change rate of c_e and c_i during dialysis • change rate of c_e and c_i in the intermediate phase • V_e • Rebound	• generation rate • residual clearance • clearance
$K_{i,D}$ (Inter-compartment diffusive clearance)	• Rebound • Coupling of c_e and c_i	• clearance • residual clearance • $K_{i,c}$
$K_{i,c}$ (Inter-compartment convective clearance	• Rebound • Coupling of c_e and c_i • Coupling of V_i and V_e	• generation rate • residual clearance • clearance
c_i (Urea concentration of ICF)	• $K_{i,c}$ • $K_{i,D}$ • Rebound	• clearance • residual clearance • sum of V_i and V_e
K_{eff} (Effective urea clearance during dialysis)	• ce • ci • Rebound	• residual clearance • 2 treatment mean generation rate • sum of V_i and V_e
Q_f (ultrafiltration rate during dialysis)	• V_e	• residual clearance • generation rate
K_R (residual clearance)	• V_i+V_e • G' according to E 2.6.5.c • ce • ci	• $K_{i,c}$ • $K_{i,D}$

Table 2.6.9.a: *List of variables and their major and minor influence on the calculation result of other variables.*

The benefit of a variable variation is determined by

- the grade of independence from other variables. The ideal set would be composed of those variables that establish a complete coordinate system to describe the situation, each independent from one another.
- The quality of the fixed parameters. A parameter that is not varied should be accurately known.
- Even the aim that is prosecuted by the calculation has influence on the selection of the varied parameters. It appears as we are leaving the scientific pathway if the

data analysis is influenced by the interest of the investigator. This is certainly not the case: If we are interested in a comparison of the clearance that the patient has experienced according to the model versus the clearance the dialysis machine has displayed, we are obliged to vary the clearance and can not fix it. Vice versa fixing a variable could make some sense if it is relative precisely known like the generation rate to confine the model to the intrinsic scatter caused by the variable of interest. Further in the case when the model result for urea distribution volume is to be compared to the result of direct quantification the volume has to be varied.

We will discuss the variables in detail in this paragraph.

$K_{i,C}$ and $K_{i,D}$

Table 2.6.9.a allows to identify those quantities whose variation will affect mainly the variables we have no or insufficient data about. These variables are $K_{i,C}$ and $K_{i,D}$. Their effect can hardly be measured because only c_e is visible from outside the patient, but they act at the interface of ECF and ICF, the inner cell membranes. To achieve information on their magnitude it would be necessary to have access to c_i directly. This is very impractical -maybe even impossible- because the distribution of urea may show gradients and inhomogeneity in particular tissue regions. Even if a tissue sample from the patient could be taken each session the sample can not be regarded as representative and therefore the mean whole body tissue urea concentration could not be calculated.

A rough estimate of the common effect of both, $K_{i,C}$ and $K_{i,D}$, could be achieved by precise measurement of the rebound versus time curve. To record this curve 5-7 additional blood samples would be necessary. This is not practical: The 10 session overall duration of the studies, each with 9 blood samples let it appear to be an unacceptable burden to the patient and the hematocrit. Therefore the rebound curve was not time resolved. The samples have only been drawn at onset and termination of rebound. The only reference information on $K_{i,C}$ and $K_{i,D}$ therefore is lost. If these data were available, the model would have to put emphasis to the rebound period. Only during the rebound the c_e differences of model and measurement can be minimised with essential effect on $K_{i,C}$ and $K_{i,D}$.

The first three quarters of the rebound curve are predominated by $K_{i,C}$ and $K_{i,D}$. Near the end of the rebound the gradient of c_e to c_i is stronger influenced by the generation rate that more and more takes effect and impedes resolving the inter-compartment clearance. Therefore a precise measurement of $K_{i,C}$ and $K_{i,D}$ by rebound analysis would only be possible if the generation rate is exactly known. A questionable feedback of dialysis to the protein catabolic rate and the generation rate [Gior1, Sar2] must further be included into the consideration. These arguments led to the conclusion that there is no corresponding data base to assess a variation of $K_{i,C}$ and $K_{i,D}$ and their effect on the result. It is also clear that their inner relation could not be resolved by our study not only due to the lack of data. The principal problem is to distinguish how many urea molecules have passed the cell membrane by convection or by diffusion. What criteria could apply to distinguish the effects? Within the literature no value for $K_{i,C}$ alone has come to the authors attention. Thus it has been

decided to regard $K_{i,C}$ and $K_{i,D}$ as a single quantity. For technical reasons $K_{i,C}$ has been set to zero and $K_{i,D}$ in general has been set to 800 ml/min, independent of the patient. Other authors have found this value to be 760-1000ml/min [Bell, Go4, Hei]. The coupling of the concentrations of ECF and ICF therefore will not correctly be reflected by our calculations.

G', V_i, V_e

The only true uncertainty in calculation of the equivalent generation rate G' according to E2.6.5.e is the urea distribution volume of the patient. Further both V and G' depend on each other because a greater volume normally corresponds to a weighty patient with higher absolute urea metabolic rates. At given concentrations c_e at the end of previous and at the start of the present dialysis a fixed volume fixes G' and vice versa. This is the reason letting float both of them during the calculation and let the model find the best corresponding values.

Another question is if the inner relation of V_i and V_e . The ECF fraction of total urea distribution volume may vary to some extent. Obese patients may have a different relation than tiny ones. Is it justified to keep a constant ratio ECF/ICF throughout the calculations and which is the best value for this ratio ? To decide this we have carefully to observe the phase when the effect of the ratio can be tracked: again it is the rebound phase and therefore again we have a lack of data by the same reasons as mentioned in the discussion of the inter-compartment clearances. Because the inter-compartment clearance and the volumes involved on both sides of the cellular membranes are linked by the calculation an inverse effect is the result: Large ICF volume and small ECF volume at a given inter-compartment clearance will result in short rebound phases with high amplitude and vice versa small ICF volume and large ECF volume at the same clearance will result in a weak, prolonged rebound phase that is continuously proceeding to the inter-dialysis curve of urea concentration. The duration and the amplitude of rebound is the criterion which would allow to make a better fit to the internal ratio of ECF/ICF. The rebound duration has not been recorded, because this again would be accompanied by additional blood samples which is not acceptable. The time the rebound blood sample was drawn has been fixed to 30 min, independent of any patient individual scattering. In conclusion the data available do not allow to model the ECF/ICF ratio. Any chosen ratio is purely arbitrary and not supported by the model. On the other hand a fixed ratio could not easily be selected due to the lack of a valid criterion. Because the ratio the model would select is predominated by the fixed inter-compartment clearance that has been set to 800ml/min it was decided to have the ECF/ICF ratio not fixed so that the model would select a convenient value corresponding to the fixed inter-compartment clearance. Fixing both would mean to cut the degrees of freedom because the model could not shape the rebound curve through both known urea concentrations at t_{end} and t_{30}. This is not acceptable.

K_{eff}

K_{eff} is one of the most important quantities of this investigation. The fundamental goal of the studies is to determine if the method K_{eff} has been measured is technically and

theoretically correct, in particular on the data of the MII study, where the bolus clearance has been adopted. Therefore its the natural consequence of the considerations that K_{eff} must be varied by the model to determine its accuracy. In all calculations K_{eff} has been left floating to be selected by the model. The model fits K_{eff} by multiplying the K_{eff} the machine has recorded with a constant factor in the range of 0.70 to 1.30 in discrete steps to find the factor that fits best the data in combination with the other parameters. This means the K and Kt/V results will also be only in discrete steps. The resolution has been selected to be sufficient. The variation of K_{eff} during the dialysis session due to dialysate and blood flow variations is also reflected because the data set with the operation parameters of the dialysis machine was included in steps of 1min.

K_R

The residual urea clearance has not been observed in the studies because initially it was not intended to evaluate a period longer than a single dialysis session. The occasion of having sufficient data addressed the interest to the two pool model analysis. The lack of data has been compensated by using the residual creatinine clearance which was recorded every treatment. Creatinine has similar or slightly lower clearance than urea. No fixed typical ratio of urea and creatinine clearance was found in the literature to be implemented here. It was decided to set them equal, even if this is accompanied by some concern. In consequence K_R was not varied but kept constant at the creatinine clearance.

Summary:

For practical reasons the two pool urea kinetic model (2PUKM) analysis has been made in two subsequent steps:

Step A: The parameter ECF, ICF and KeCN (KeCNBolus in MRII study) have been varied.
Step B: The parameter ECF, ICF, KeCN (KeCNBolus in MRII study) and G' have been varied.

The variation of both V_i and V_e has been implemented because a fixed ratio is not selectable a priori. The variation of $K_{i,C}$ and $K_{i,D}$ has not been performed because there are no data that would reflect the consequences and therefore the result would not give any information. Both in sum have been set to 800ml/min. On the one hand the variation of G' has been omitted in the first run because a full variation of 4 parameter takes an enormous amount of calculation resources. This was not practical in the first analysis. The variation of only 3 parameters takes just a few minutes on a modern PC and could be done in clinical practice to get results in short terms. On the other hand in this case G' can only be calculated using expression E2.6.5.e, but with the uncertainty of the distribution volume from direct quantification influencing the calculation. The uncertainty in the correct G' led to step 2., where all parameters have been varied. It is of some interest for in-time calculations directly during a dialysis session if the extended analysis can be omitted without loss of essential information about the concentration behaviour.

2.7. Determination of Plasma Sodium

It is helpful to know the plasma sodium concentration of the patient when dialysis starts. Most but not all of the patients can be dialysed conveniently if their intrinsic plasma sodium is not altered during dialysis. Others may have to follow a distinct sodium variation profile. In both cases a sodium balancing is necessary. Disturbances initiated by dialysis may lead to severe cardiovascular problems, nausea, vomiting, violent headache or at least to more or less severe general discomfort of the patient. Even a slight sodium overload, although it tends to stabilise the cardiovascular functions, may increase post dialysis thirst perception and therefore influence fluid intake and weight of the patient. Usually the thirst control mechanism of the ESRD patients still works correct and the imperative character of thirst forces the patients to drink more than the clinician has recommended to do. Further the clinician can not be aware of a sodium over- or under- load if he does not measure plasma sodium of his patients regularly prior to every dialysis. A permanent sodium control is corresponding with high costs for laboratory analysis and therefore will normally not be practised in most standard hemodialysis treatments. Also those patients that have been prescribed deviating dialysate sodium concentration need strict control of sodium transfer. This means sodium balancing is necessary and would be a support to the clinician in his efforts to increase the patients quality of life. The clearance measurements, once they are performed in a technically correct way, also permit to conclude from simple conductivity measurements to the plasma sodium value and sodium balance.

The basic assumption is that the dialysate conductivity is predominated by sodium-chloride ions. This in fact is the case, only 2-3% of the conductivity is contributed by other constituents. A typical composition of bicarbonate- and acetate dialysis fluid is depicted in table 2.7.a. The studies have been conducted using bicarbonate dialysate.

Constituent	**Bicarbonate dialysate** *(mmol/l)*	**Acetate dialysate** *(mmol/l)*	**Normal plasma level** *(mmol/l)*
Sodium	137-144	132-145	136-145
Potassium	0-4	0-4	3.5-5.0
Calcium	1.25-2.0	1.5-2.0	2.2-2.6
Magnesium	0.25-1.0	0.5-1.0	0.8-1.2
Chloride	98-112	99-110	98-106
Acetate	2.5-10	31-45	<0.1
Bicarbonate	27-38	-	21-28
Glucose	0-11	0-11	4.2-6.4

Table 2.7.a: *Levels of electrolytes and buffers in typical dialysis fluids and in normal plasma [Gra]*

To apply sodium balancing in a simplification a proportionality is assumed to be true:

$$cd(NaCl)_{mS/cm} * 10 + 0.5 = [NaCl]_{mmol/l} \qquad \text{E. 2.7.a}$$

This means the concentration of sodium chloride in mmol/l is calculated from the conductivity of the dialysate. This is only an empirical formula on the base of laboratory measurements and it has been established in clinical practice over the years without any regard to the influence of internal shifts of the minor constituents like potassium, calcium and magnesium that contribute to conductivity and even without regard to the Donnan factor α_D . The Donnan factor reflects equilibrium concentration shifts in the presence of proteins at only one side of the filter membrane. Therefore on the one hand a measurement in its literal sense of course is not performed, but the indirect way to measure concentrations via a conductivity measurement is much more than a estimation on the other hand. In practice the relative dialysate constituents do not vary in a wide range at the majority of the patients, in particular not within single dialysis centres with central concentrate supply. This enables the clinician to become a feeling for the relation of conductivity and concentration. Many clinicians simply multiply by 10 in practice. The underlying considerations to measure blood sodium chloride concentrations are depicted here. According to E 2.4.2.a a mass balance again is the start of the derivation:

$$J_B = D\left(1-\frac{Qf}{Qe}\right)\left(\alpha_D cbi - cdi\right) + Qf * \alpha_D cbi$$

$$J_D = \left(cdo - cdi\right)Qd + Qf * cdo$$

Both mass fluxes must be equal and the terms can be resolved for cbi:

$$\alpha_D cbi = \frac{\left(Qd+Qf\right)cdo/cdi + \left[D\left(1-\frac{Qf}{Qe}\right) - Qd\right]}{D\left(1-\frac{Qf}{Qe}\right) + Qf} cdi \qquad \text{E. 2.7.b}$$

This is an equation without unknown variables and therefore can be resolved purely on the machine side without access to the blood of the patient. This in fact is the advantage of this approach: Without laboratory analysis or any effort of the clinic staff it is possible to measure the patient's plasma sodium concentration and to adjust the machine to the correct prescribed dialysate sodium for highest benefit of the patient.

An uncertainty still remains not only in α_D but also in Q_e , the blood water flow:

$$Qe = Qb\left[FP - hct/100(FP - PWF)\right] \text{ and } FP = 1 - 0.0107\,TP$$

The variables hct (hematocrit), FP (fraction of protein), PWF (plasma water fraction) and TP (total protein) are not known. In patients with normal blood composition numerical insertion of the laboratory measured concentrations shows that the blood flow Q_b - the "geometrical" or whole blood flow as measured by the blood pump- must be multiplied with a factor in the range of 0.87 – 0.91, mainly dependent on the hematocrit. Due to ultrafiltration which extracts water during dialysis the hematocrit is slowly increasing during dialysis. These uncertainties must all be covered by an empirical factor of $Q_e = 0.9 * Q_b$ if the sodium concentration analysis is done in practice on a dialysis machine. The data of the MII study are analysed to test the hypothesis that E 2.7.b can be used to calculate plasma sodium.

3. Concept and experimental protocol of the Studies

3.1. Study Design

The MI study was designed as an anonymous dicentric approval study. It was performed at two different centers:

1.) Centre of Internal Medicine, Clinic of Nephrology – University of Marburg -, Baldinger Straße, Marburg, Germany. Principal Investigator : Prof. Dr. H. Lange; Practical measurements from May 7th, 1997 to June 24th, 1998; 22 patients, 206 dialysis sessions. Early data analysis beginning in 1997, detailed analysis was terminated in September 2001.

2.) Divisione Nefrologia e Dialisi, Ospedale di Lecco, via Ghislanzoni, 22053 Lecco – Italy. Principal Investigator : Prof. Dr. Francesco Locatelli. Practical measurements from July 14th, 1997 to Mai 15th, 1998; 20 patients, 200 dialysis sessions. The report on this study is not included in this work. Some aspects of this study are reported in [DF2].

Parts of the monitoring, the technical engineering and full data analysis has been done by the author using a dedicated software that has been written for this purpose in particular. The study was initiated and supported by the R&D department of Fresenius Medical Care (FMC), Bad Homburg, Germany. The author was member of the department during the study period and responsible for the study as the project manager for the implementation of dialysis quantification tools to Fresenius 4008 series dialysis machines.

The studies have been planned with the benefit of the patient in mind and with agreement of the responsible ethical committees. Not only the scientific intention to validate a device that is able to quantify the dialysis by simple means is expected to be of high benefit for the patient. Also in the practical handling of the devices should be developed. To minimise the burden for the patients all blood samples have been reduced to the absolute minimum and all relevant physiological parameters have been observed with maximum care to avoid any critical situation. Regularly the sequence of participation of a patient was interrupted by organisational reasons. If the responsible physician who performed the clinical part of the study was not on the shift the patient was scheduled for the sequence was interrupted. To not influence the confidentiality of patient and physician it was avoided to confront the patients permanently with different physicians just for not interrupting the sequence. Also it was avoided to alter the dialysis schedule of the patient in duration and frequency due to study requirements. No patient ever has shifted a dialysis once scheduled due to requirements of the studies.

3.1.1. Ethical and legal regulations:

This study was performed according to the principals of the revised declaration of Helsinki (Hongkong 1989) (see appendix).

A risk-benefit-comparison was performed prior to the study. The therapeutically benefits were rated higher than the potential risks of the patients being part of the study. The patient will be informed by the physician of the meaning, the extent and the specifications of the study with an information sheet. The patient was also informed of his right to withdraw his agreement at any time during the study without drawback. If the patient agreed to participate in the study he had to sign-off his written consent in two forms provided. One of the two forms remained with the patient, the second form with the Hospital for the period of fifteen years.

3.1.2. General participation criteria

Willingly cooperative patients who have given their written informed consent and are:

- Age $\geq$ 18 years
- On regular hemodialysis for $\geq$ 1 months
- Hemodialysis schedule: 1-3 times a week
- Length of dialysis sessions $\geq$ 180 minutes
- With discrete to good dialysis tolerance (frequency of symptomatic session lower than 25%).

3.1.3. Exclusion criteria:

- Currently in an acute care or chronic care hospital
- Previous leg amputation
- Scheduled for a renal transplantation from a living donor within the period of study
- Severe congestive heart failure after maximal therapy (NIHAU class IV)
- Unstable angina pectoris
- Active systemic infections (i.e. TBC or systemic fungal infection or AIDS)
- Uncontrolled glycemic levels (> 200 mg/dl) in diabetic patients
- Involvement in other studies
- Unable to follow the main requirements of the protocol
- Unwillingness to accept the procedures of the protocol
- Participation of patients showing an Hb lower than 8.5 has been suspended for a period necessary to reconstitute a convenient Hb

3.1.4. Written and informed consent:

Those patients that were selectable for inclusion have been informed of the purpose of the study, the protocol and if desired on any detail of their interest to be able to form a free decision if to participate or not. They were informed in particular that their participation is absolutely voluntary and withdrawal from the study would be possible at any time without any disadvantage or interference to their regular treatment.

3.1.5. Insurance of the patients:

Any hazard for patient caused by malfunction of the dialysis machine or one of its modules is insured by the FMC liability insurance as far as installation, configuration, calibration, reparation and the Online Clearance Measurements (OCM) are executed by authorised personnel that underwent the manufacturers training. FMC has effected an additional insurance.

3.1.6. Function of the monitor:

During the study the data recording and collection was supervised and technically directed by a staff member of FMC, to whom the following tasks are incumbent :

- Control of the completeness of all written documents.
- Guaranty of reliable function of all technical data recording devices.
- Collection and conservation of all data recorded except those to allow the identification of the patient.
- Coincidence control of study proceeding and study protocol.
- Instruction for use of the OCM option delivered to the personnel involved.
- Participation in the measurements as far as necessary by technical reasons if there is no irritation for the patient or disadvantage for dialysis treatment expected.

The hospital was liable to mark all documents and files in a consistent manner to allow internal re-identification for further investigations in the patients hospital report, even after an extended period.

3.1.7. Ethical and administrative supervision:

At any time authorised personnel of the administration had access to all information necessary to supervise the monitoring and study proceedings. Prior to the study the protocol was approved by the responsible ethical committee.

3.1.8. Technical engineering:

The studies were under close technical supervision of the responsible development group of FMC. All functions of the technical equipment have been continuously monitored, checked and maintained.

Both studies that are reported here were conducted using 4 (MI) and 2 (M2) dialysis machines that were taken from the standard serial production but have been modified within their software to control the timing of the measurements, the conductivity variations and to transfer all relevant data to the computer. The second conductivity cells downstream the dialyser that have not been part of the serial machines but which are essential for conductivity based dialysance measurements have been mounted. In particular the machines had been carefully observed if they would not fail to apply the conductivity variations correctly. Severe failure to do so could be hazardous to the patients health due to sodium imbalance and, even more serious, due to uncontrolled potassium transfer because all electrolytes were increased in the same proportion. Only dialysis machines who successfully underwent the full CE certification procedure have been in operation.

3.1.9. Major goal of the MRI Study:

The study was intended to prove the step function clearance measurement principle as described on a series dialysis machine. Both aspects the principle itself and its clinical practicability on such a device as an instance of technical realisation were of fundamental interest. The goal was to make experiences for achievement of good clinical practicability and the technical approval of a valid conductivity based clearance measurement principle. The bolus principle was not yet in the focus but has already been implemented.

The goal of the study recommends a statistical selection of patients representative of a typical population of dialysed patients willing to participate in the study with regard to the exclusion criteria. The selection of the patients is done by the Principal Investigator of the study with respect to a statistical principle, the individual aetiology and the inclusion criteria.

The MRII Study:

The study is designed to be a randomised anonymous mono-centric approval and investigative study.

3.1.10. Major goals of the MRII study:

- The major goal of the MRII study was the investigation and definitive decision if non-steady, dynamic conductivity signals such as boli allow to measure the urea clearance with accuracy comparable to that known from step-profiles and if the boli can be regarded as an improved substitute for them.
- A further approval if accurate results for urea based Kt are achievable using conductivity-based techniques.
- Determination if the urea distribution volume is of considerable influence on the ionic clearance measurement results and if it can even be measured.
- The data obtained during the study were intended to be the base of the technical approval process of the online clearance measurement option of the dialysis machines.

3.2. Subjects and Methods

3.2.1. The MI Study

3.2.1.1. Patient collective and hemodialysis parameters

A total of 20 informed patients (14 male and 6 female, age 58.7 ±16.1 years, body weight 73.9 ±11.0 kg, height 1.74 ±0.08 m) with ESRD treated by standard hemodialysis (dialysate: Na 140 mmol/l, K 3.0 mmol/l, Ca 1.5 mmol/l, Mg 0.5 mmol/l, bicarbonate 28.0 mmol/l, glucose 2 g/l, mean ultrafiltration rate 8.6ml/min) at a mean of 9.13 months were enrolled in the study. Underlying diseases were chronic glomerulonephritis (11 pat.), diabetic nephropathy (5 pat.), obstructive nephropathy (1 pat.), APCKD (2 pat.) and plasmocytoma (1 pat.). The remaining kidney function corresponded to interdialytical residual Kt/V fraction of 0.45. All patients were dialysed thrice weekly by 214 ±17 min plus 30min ultrafiltration at the end of hemodialysis with the dialysis machine 4008 (Fresenius Medical Care) and the hollow fibre polysulfone membrane (F8 HPS, 1.8 m^2,, Fresenius Medical Care). Blood flow was adjusted at 201±9.7 ml/min. Dialysate flow was maintained at 553±16.5 ml/min. The ultrafiltration was 1.99 ±0.6 l per dialysis. Drug therapy was not varied during the study period. Each patient was subjected to OCM during 10 hemodialysis treatments. They did not participate every subsequent session, occasionally the next study session was delayed by organisational reasons. A total of 206 dialysis sessions has been recorded. A detailed summary of the patient and treatment parameters is given in table 10.2.a,b.

3.2.1.2. On line clearance measurements (OCM):

The details of electrolytic clearance measurements based on two conductivity probes have been described in detail by Polaschegg [Pol], Steil [St] and Petitclerc[Pe1, Pe2]. A brief summary of the basics is found in chapter 2.4; 2.4.1. The dialysis machine has been modified by providing a second standard conductivity cell downstream of the dialyser in addition to the conventional upstream cell, according to the description of chapter 2.4.. No other hardware modification was made. A typical impulse of a delta +10/-7% dialysate CD variation of about 7 minutes above and 7 minutes below standard conductivity was applied 2-3 times per session of dialysis. In cases when the prescribed baseline was too close to the lower machine limit, the low-part of the step profile was weaker, similar to that shown in figure 2.4.1.a. The data of CD pre/post dialyser and blood/dialysate/ultrafiltration flow were collected online by an additional personal computer. Eight minutes after termination of the automatic clearance measurement, blood and dialysate samples were drawn from the arterial and venous blood lines as well as from the inlet and outlet dialysate lines. The samples were submitted to the laboratory for urea tests (Hitachi 747 Automatic Analyser) [Neu] immediately after dialysis. The blood and dialysate flow meters of the dialysis machine were under frequent volumetric control by a sound velocity meter (Transsonic HD01) to compensate the sliding drift of the peristaltic pump, the pressure sensors and bloodlines. All bloodlines originated from a single production batch. Additionally, a 2% computer-controlled dialysate sample was taken from the dialysate drain with a dedicated valve. To exclude phases of inactive dialysis from sampling, all bypass and alarm times were automatically recorded and taken into account by interruption of valve release. The dialyser clearance was corrected for recirculation three times per session by the thermodilution device [St] of the blood temperature monitor (BTM, Fresenius Medical Care) in order to obtain the effective clearance for determination of Kt/V by the OCM. The complete setup and procedure to record the data is demonstrated in figure 3.2.1.2.a.
Using the conductivity-based clearance the correlation of total momentary (diffusive and convective) clearance with the reference of momentary blood side urea clearance and dialysate side urea clearance was determined. In order to consider the mass balance error (MBE), in a first calculation only those blood and dialysate side urea samples were used as reference, which varied by less than 5% in MBE. In a further step all results have been included. Kt/V based on

1. the single pool model according to E 2.6.1.a
2. the equilibrated single pool variable volume kinetic model (E.2.6.1.g)
3. Daugirdas equation (E 2.6.3.a)
4. direct quantification (E 2.6.2.a)
5. two pool urea kinetic model (chapter 2.6.4 – 2.6.9)

has been calculated as reference. All results from these five references were compared to electrolyte based Kt/V of the OCM monitor. Urea generation during dialysis treatment and the patients' residual urea clearance were neglected except in evaluation 5. All mathematical derivations are described in the related chapters.

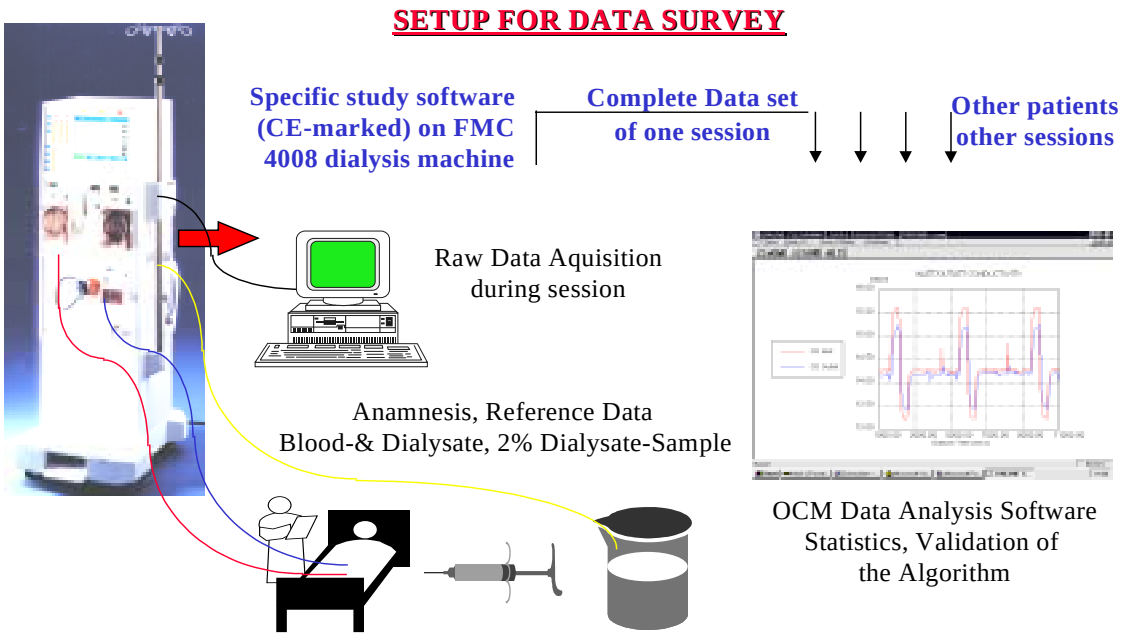


Figure 3.2.1.2.a: *Prior and post to dialysis the patient has been interviewed by a skilled physician concerning irregularities. All technically relevant data have been recorded by the computer that was connected to the dialysis machine. The machine was provided with a drain valve that was controlled by the computer on base of the operational data transmitted. The anamnesis and reference data were written to the computer by hand afterwards to complete the data set of one patient to a single data object. All data from all patients were collected in a dedicated software unit that was able to analyse the whole data of all patients by the different aspects running under batch control. Modifications during software development period allowed to easily integrate algorithms based on further knowledge to evaluate all topics of this work.*

Data collected in Case Report Form			
ANAMNESIS	AETIOLOGY	AGE	GENDER
$UREA_{BLOOD}$	$UREA_{DIALYSATE}$	NA, K	$UREA_{2\%SAMPLE}$
WEIGHT	HEIGHT	HCT	TOTAL PROTEIN
Blood pressure	TEMPERATURE	DIALYSATE UREA CONCENTRATION	RECIRCULATION
Permanent computer assisted data collection during dialysis			
MEASUREMENTS /CURVE SHAPES	CONDUCTIVITY	ALARM STATUS	MACHINE STATUS
DIALYSATE TEMP	DIALYSATE FLOW	BLOODFLOW	UF RATE
BLOODLINE PRESSURE	2% DIALYSATE SAMPLING	TIMING	SODIUM BALANCE

Table 3.2.1.2.a: *Parameters that have been acquired during a dialysis session. Those that were accessible to automated data recording have been directly written to the data base of the evaluation software during dialysis, others – in particular any laboratory results - had to be typed in afterwards.*

A list of all data that have been recorded automatically or by the physician is shown in table 3.2.1.2.a.

3.2.1.3. Monitoring of clinical outcome

To assess subjective feelings every patient was interviewed by a skilled physician concerning thirst, muscle cramps, headache, nausea and vomiting after every study session. Serum sodium at start of each hemodialysis and serum sodium prior to and 8 minutes after the impulse were measured to detect sodium imbalance. Electrolytic balance – calculated by the product of the area under the curve and the dialysate flow - was calculated as a parameter of sodium balance. Fluid homeostasis was monitored by body weight, breathing and heart rate at rest and arterial pO_2 and pCO_2 at the start as well as by the blood pressure at the start and the end of hemodialysis.

3.2.2. The MII Study

The MII study was designed as an anonymous monocentric approval study. It was performed at the

Centre of Internal Medicine, Clinic of Nephrology – University of Marburg -, Baldinger Straße, Marburg, Germany. Principal Investigator : Prof. Dr. H. Lange;

Practical measurements were done from Sept. 30th, 1998 to November 28th, 1998; Early data analysis has been performed in 1998, enhanced analysis was terminated in December 2001.

Patient collective and hemodialysis parameters

The MII study was an in-vivo hemodialysis study with 10 patients who gave their informed and written consent. The study included 93 dialysis sessions and 264 evaluated automatic step-function- and 173 bolus-measurements of ionic dialysance. The mean number of step function measurements for each session was 2.8. Those measurements that were interrupted have not been evaluated. This regularly occurred by timeout with the third bolus measurement being interrupted by the end of dialysis. Only in some rare sessions time was sufficient to perform three bolus measurements. Session duration could not be changed due to study demands. Only ESRD patients without severe concomitant diseases participated (see inclusion- and exclusion-criteria). Underlying diseases were chronic glomerulonephritis (4), diabetic nephropathy (3), cystic kidney (2), analgetic nephropathy (1).

The flows were adjusted to Q_b= 213±1.8ml/min, Q_d= 531±23.8 ml/min, Q_f= 534.0±96 ml/h. The dialysers in use were Fresenius F8 (FMC Bad Homburg, Germany), both polysulfone membrane types. The dialysate has been the same as in the MI study.

Technical setup

The procedure, timings and the technical setup for the data acquisition are identical to the MI study except the reshaping of the bolus. The bolus shape is depicted in figure 3.3.a. Computer-linked dialysis machines (4008 series, FMC) with pre- and post- conductivity sensors and a dedicated software were used. All relevant patient data (anamnesis, aetiology, weight, height, medication), blood and dialysate parameters (urea, Na, K, hct, total protein, blood pressure, temperature, recirculation) at the beginning, at the measurements, at the end and 30 minutes later for rebound inclusion were sampled (see table 3.2.1.2.a), and all machine data related to the patient (dialysate flow and temperature, conductivity pre and post, dialysis status, blood flow, UF-rate) were recorded continuously throughout the complete dialysis session. Furthermore, approximately 2% of the spent dialysate was collected using a valve switched by the machine in fixed mark space ratio (1.5s on, 58.5s and during alarms off), see figure 3.2.1.2.a. Thus the urea concentration of this sample represents the mean urea concentration of the total amount of spent dialysate as calculated by balance chamber counts. The volumetric error is confined to 0.1% because the sample volume error itself cancels. The mass quantification error is predominated by 6%(SD) laboratory error for urea in dialysate. The sample was transferred to analysis immediately after termination of dialysis. Time

dependence of urease activity was monitored but was found not to be relevant within the period for laboratory analysis. The analysis system was frequently calibrated by urea-dialysate blind samples to avoid bias of the enzymatic urea sensors in aqueous solvents. The bloodlines originate from a single production batch to ensure best coincidence of the hydrodynamic properties. The blood and dialysate flow meters of the dialysis machine were under frequent volumetric control by a sound velocity meter (Transsonic HD01) to compensate the sliding drift of the peristaltic pump, the pressure sensors and bloodlines, but no relevant drift of the flow measurements was found during the study period. The dialysis machine was modified to generate the conductivity variations automatically. Every sequence consists of a step-profile with 7.5 min increase of inlet conductivity to +10%, a subsequent 7.5 min decrease to –4%, followed by the reference blood sampling procedure and finally the bolus, each with a delay of 10 min (figure 2.4.1.a). Due to the hydraulic properties of the 4008 module for highest possible accuracy and stability the profile duration of 7.5 min has been regarded as being necessary prior to certification of the machine software but was found to be reducible retrospectively. All timings were rigidly controlled by the dialysis machine to avoid inaccurate sample timing. The duration of the boli was 70-90 s (width at half maximum), the height of the boli peaks was ±10% of baseline CD alternatively. Urea blood concentration was measured both at full bloodflow and at the end of a 2 min period of reduced blood flow (Q_b= 50ml/min) for calculation of the recirculation ratio and correction to effective blood water urea clearance. All data were combined in a suitable evaluation software which performed the analysis. The dialysances derived from electrolytical measurements of the machine were compared in the same way as in the MI study, see chapter 3.2.1.2.

Further an analysis for

- Plasma sodium (see chapter 2.7)
- Anthropometric volume measurement (see chapter 2.5.2)
- electrolytically measured sodium distribution volume (see chapter 2.5.3.)

was included. The last point is divided in an in-vivo and an in-vitro part:

In-vitro: E 2.5.3.j was tested by performing a standard dialysis with a Fresenius 4008 machine and a Fresenius HF80 polysulfone filter, but replacing the patient with a container of known volume. The container was filled with dialysate (37^o) of 14.1mS/cm in a sequence of 5l, 7.5l, 10l, 12.5l, 15l, 20l to 70l in steps of 10l, permanently stirred. At each volume 4 measurements were performed using the same automatically generated step-profiles as used in-vivo (figure 2.4.1.a). Dialysate flow has been adjusted at 800ml/min, the ‘bloodflow’ at 350 ml/min. Data recording and analysis were done in the same way as in the in-vivo studies.

In-vivo: A dialysance derived distribution volume according to E 2.5.3.j has been calculated and was compared to the volume V_{DQ} derived from the direct quantification method. It was the goal to investigate the applicability of E 2.5.3.j with respect to its limitations. The dialysance error versus the urea distribution volume from DQ was analysed.

3.3. Refinement of MRII Study

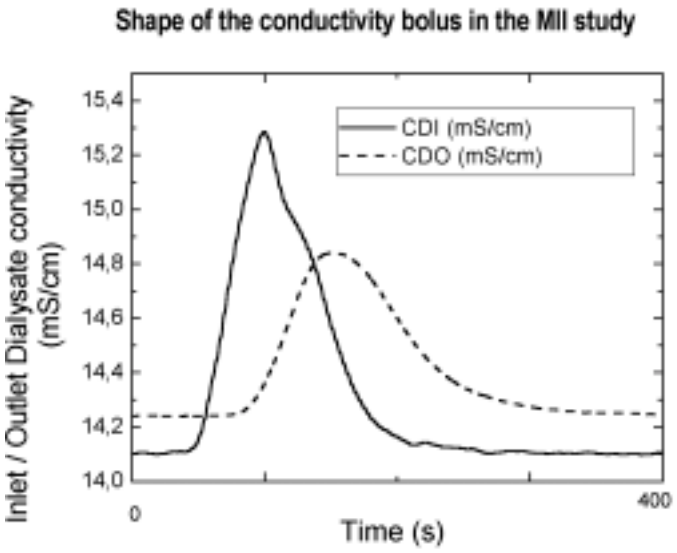


Figure 3.3.a: *The conductivity bolus that was applied in MII study. In comparison to the bolus of MI study (see figure 4.1.1.1.c) the bolus is longer, slightly higher and has a much less noisy baseline. These were necessary conditions to improve the results of the bolus measurements*

Both studies in general were designed identical except the shape of the bolus and the number of patients and recorded dialysis sessions. In the MRI study the focus was on the step profile principle and the bolus was only of side interest. Although the principal idea to investigate the transmission function has influenced also the first study, it was not that clear that the transmission function could be the key to a dynamic principle of measurement in time of designing the study protocol. The bolus was more of scientific than of clinical interest. On the other hand in study MI preparation the additional burden for the patients by applying boli was regarded to be negligible so that also with not being in the focus of interest the ethical committee accepted to apply them for only scientific interest and unclear benefit for the patient. The expected gain of information and understanding of the mass kinetic dynamics of the patient – machine system was reason enough to accept it.

Without anticipating the results that are discussed in chapter 4.1.1.2.2. it became apparent that the understanding of some details of the step profile clearance measurement was not complete. The sodium transfer process has not sufficiently been reflected. This in particular led to the derivation of the sodium distribution volume electrolytic measurement theory and the insight that the bolus is preferable in comparison to the step function. The technical realisation of the bolus in the MI study was found to be not correct. It was too small and to noisy and did not rise enough above or below baseline. On the other hand the bolus results of the MI study were sufficiently encouraging to model a better bolus for the MII study (figure 3.3.a).

4. The experimental results and first discussion

4.1. The MI Study

4.1.1. Comparison of ionic Dialysance to Reference

4.1.1.1. Momentary Dialysance Measurements

In the tables 4.1.1.1.a,b the relations of $KeCn_{I,J}$ to its references KeUB and KeUD are depicted. The reference is regarded to be 100%. The mean error, SD, minimum deviation and maximum deviation is given for each class. Below the identifier of the variable also the absolute range that was covered by the variable is given. The mass balance error was not restricted in table 4.1.1.1.a and restricted to 5% in table 4.1.1.1.b. The mass balance error is defined by E 2.3.a.

It has been found that $KeCn_{12}$ matches best the blood side urea clearance KeUB, independent of a MBE restriction. With unrestricted MBE a result of (error±SD) (-1.46±4.7)% was found, with 5% MBE limitation it was (–0.46±4.65)%. The other permutations differed slightly more: -3.40± 5.81% ($KeCn_{01}$) and 3.51±7.1% ($KeCn_{02}$) versus KeUB with MBE = 5%. It should be denoted that the SD for $KeCn_{02}$ is higher than that of the other permutations.

unrestricted MBE	KeCn01 Range: 105.0 – 192.6 ml /min	KeCn12 107.1 – 179.7 ml /min	KeCn02 55.3 – 226.7 ml /min	KeUB Reference 118.9 – 182.4 ml /min
KeUB Reference Range: 118.9 – 182.4 ml /min	- 4.35 % err 5.56 % SD -24.1 % Min 15.0 % Max n=493 Sign.diff.(p<0.0001) 4 Outlier	- 1.46 % err 4.75 % SD -22.5 % Min 15.6 % Max n=494 Sign.diff.(p<0.0001) 5 Outlier	2.53 % err 7.17 % SD - 29.4 % Min 19.7 % Max n=485 Sign.diff.(p<0.0001) 2 Outlier	-
KeUD Reference 75.4 – 264.1 ml /min	- 2.00 % err 11.17 % SD -42.5 % Min 45.9 % Max n=464 Sign.diff.(p<0.0001) 4 Outlier	0.92 % err 11.33 % SD - 42.3 % Min 50.4 % Max n=466 n.s. (p=0.954) 4 Outlier	5.27 % err 13.23 % SD - 41.1 % Min 60.6 % Max n=458 Sign.diff.(p<0.0001) 3 Outlier	2.57 % err 11.05 % SD -40.8 % Min 49.3 % Max n=482 Sign.diff.(p<0.002) 6 Outlier

Table 4.1.1.1.a: *M1 data: The different methods to determine the electrolytical dialysance KeCn are compared to the referring reference blood- and dialysate samples, including all technically available reference data independent of their accuracy. The reference (100%) is in the left column. The mean error (%) compared to each reference, the standard deviation (%), the minimum and maximum error (%), the number n of measurements, the significance of the difference (p=0.95) and the number of outliers (>4.5SD) that have been erased from the dataset are depicted in the remaining columns..*

Overall 545 step profiles have been performed in the MI study, but not all of them could be related to their respective reference counterpart due to the lack of information: Occasionally laboratory values were missing or non plausible, a technical inconsistency was found or no sufficient stability of the outlet conductivity was achieved. Only those were included which did not obviously seem to lack in some of these issues. The decision was made by the software.

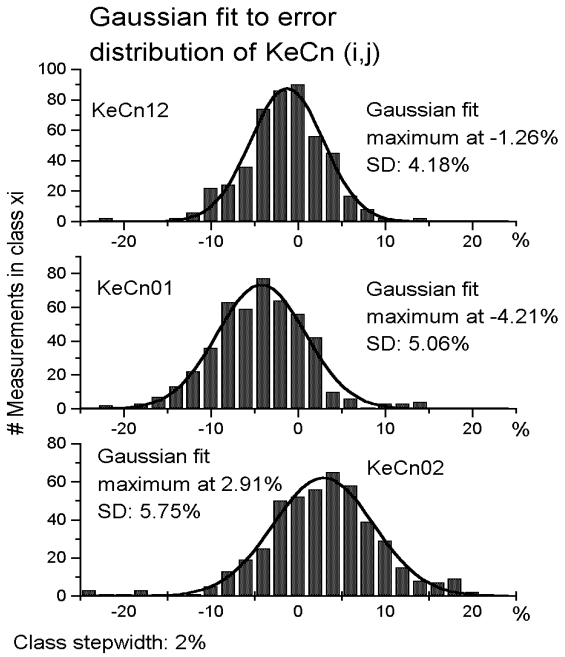


Figure 4.1.1.1.a: *The histograms show the error distribution of M1 study measurements of KeCn12 (top), KeCn12 (middle) and KeCn02 (bottom) in 2% classes. The 100% reference was KeUB from the corresponding blood sample. As an overlay a gaussian fit has been made to each data set. Exactly the data of table [4.1.1.1.a] have been included. The maximum and SD for the gaussian fit – not for the data itself – is shown. At the maximum of the KeCn02 gaussian fit the KeCn01 fit has already decayed to 37% of its own maximum. The distribution clearly shows a difference of the 3 permutations.*

The error distribution of the 3 different types of step profiles is depicted in figure 4.1.1.1.a. It is clearly to be seen there that the result classes are shifted in dependence of the selected evaluation permutation of the step profile (high-low, baseline high, baseline-low). At 529 of 545 measurements all three permutations $KeCn_{01}$, $KeCn_{12}$, $KeCn_{02}$ were available. The rest has been disturbed by alarms, instabilities of conductivity or other interruptions and timeouts. 453 of the 529 measurements (85,6%) showed $KeCn_{01} < KeCn_{12} < KeCn_{02}$, the other 76 measurements (14.4%) either showed only $KeCn_{01} < KeCn_{12}$ or only $KeCn_{12} < KeCn_{02}$ or none of it.

Figure 4.1.1.1.b is a plot of the KeUB vs. $KeCn_{12}$ analysis to visualise the distribution. Because more than 90% of the measured values are within a ±2*SD interval the calculation of a correlation is not very expressive. It has been made as an example in this plot. Typically it is at 0.8 for all results.

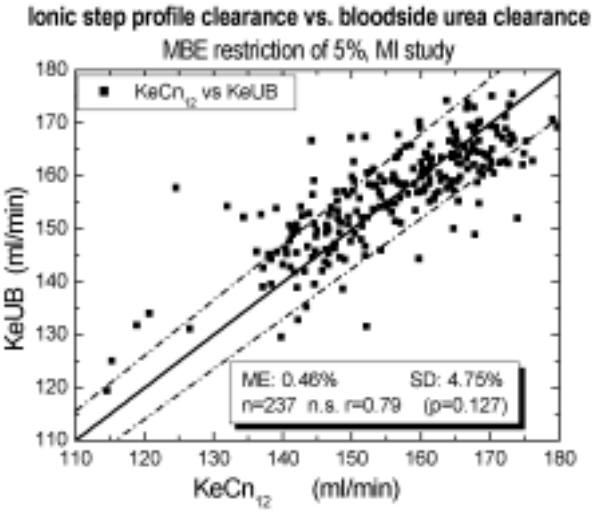


Figure 4.1.1.1.b: *Analysis of KeCn12 vs KeUB using the low high step profile*

Mass balance error < 5%	KeCn01 Range: 105.0 – 192.6 ml /min	KeCn12 107.1 – 179.7 ml /min	KeCn02 55.3 – 226.7 ml /min	KeUB Reference 118.9 – 182.4 ml /min
KeUB Reference Range: 118.9 – 182.4 ml /min	- 3.40 % err 5.81 % SD -24.1 % Min 15.0 % Max n=238 Sign.diff.(p<0.0001) 0 Outlier	- 0.46 % err 4.65 % SD -21.1 % Min 15.6 % Max n=237 n.s. (p=0.127) 1 Outlier	3.52 % err 7.08 % SD - 24.7 % Min 19.7 % Max n=236 Sign.diff.(p<0.0001) 1 Outlier	
KeUD Reference 75.4 – 264.1 ml /min	- 3.14 % err 6.30 % SD -26.8 % Min 15.5 % Max n=238 Sign.diff.(p<0.0001) 0 Outlier	- 0.17 % err 5.50 % SD -20.5 % Min 19.6 % Max n=237 n.s. (p=0.502) 1 Outlier	3.82 % err 7.74 % SD - 27.4 % Min 24.1 % Max n=236 Sign.diff.(p<0.0001) 1 Outlier	0.30 % err 2.75 % SD - 4.8 % Min 4.9 % Max n=249 r=0.91 n.s. (p=0.160) 2 Outlier

Table 4.1.1.1.b: *The different methods to determine the electrolytical dialysance KeCn are compared to the referring reference blood- and dialysate samples. Only the most accurate reference data with mass balance error of blood-side to dialysate-side less than 5% were included. The reference (100%) is in the left column. The mean error (%) compared to each reference, the standard deviation (%), the minimum and maximum error (%), the number n of measurements, the significance of the difference from students t-test for paired data and the number of outliers (>4.5SD) that have been erased from the data set are listed in the remaining columns. A correlation has not been plotted because the variation of the clearance is in the same order as the SD and therefore the data set is a dot cloud (96% of all clearances are within the interval from 140ml/min to 170ml/min.*

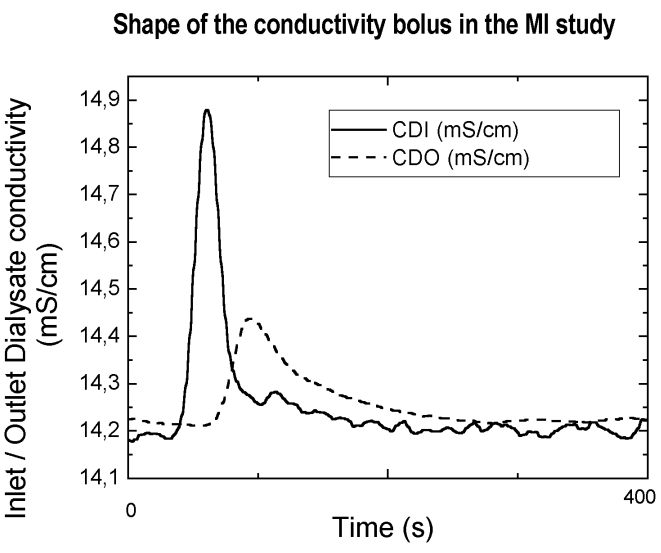


Figure 4.1.1.1.c: *The conductivity as measured at the inlet and outlet of the dialysis filter during application of the conductivity bolus in the MI study. The noisy background of the inlet bolus is explained by a lack of sufficient internal distribution volume within the mixing system of the dialysate preparation system. The 4008 B dialysis machine has not been modified compared to a serial device except the insertion of the downstream conductivity cell. The mixing system in particular has a discontinuous concentrate injection during the flow phase of the balance chamber which in combination with the low internal mixing volume has been identified to be the reason for the noisy background of the inlet. At the outlet the dialysate has passed 2m of plastic tube and the filter and therefore is much more smooth.*

The Bolus principle

The bolus was shaped as depicted in figure 4.1.1.1.c. A signal like the inlet conductivity cdi shown in this figure is not easy to analyse automatically by software. In particular if there is an integration involved to calculate the clearance from this noisy signal it is a problem to find the beginning and the end of the integration range. In the first analysis which was performed on the data of the MI study it was assumed that the integration would have to be started at the point where the signals of inlet and outlet essentially decline or increase from the baseline. To find this point in a shape like figure 4.1.1.1.c is not absolute deterministic and has a jitter. This is reflected in the results that have been derived from the bolus

application in the MI study (table 4.1.1.1.c). The mean error of approximately 10% and the high SD of 11%-16% indicates that the evaluation has been technically difficult.

Mass balance error < 5%	KeCnBolus Range: 105.0 – 225.0ml /min	**Mass balance error unrestricted**	KeCnBolus Range: 90.0 – 225.0ml /min
KeUB Reference Range: 118.9 – 182.4 ml /min	+ 10.0% err 11.4% SD - 27.1% Min 35.0%Max n=156 p<0.001 5 outlier		+ 8.42 % err 11.5 % SD -38.49 % Min 35.17 % Max n = 338 p< 0.001 3 outlier
KeUD Reference 125.0 – 177.1 ml /min	+ 10.1 % err 11.7 % SD - 24.4% Min 39.8 % Max n = 156 p< 0.001 2 outlier		+11.17 % err 16.9 % SD - 42.1 % Min 69.4 % Max n = 314 p< 0.001 7 outlier

Table 4.1.1.1.c:*The result from KeCnBolus analysis are compared to the referring reference blood- and dialysate samples. In column 2 only those were included with the mass balance error less than 5%, in column 4 all boli that could be evaluated by the software are included. In many cases the treatment time was not sufficient to perform all three boli that were scheduled. More than 150 measurements could not be performed due to the timeout condition (end of dialysis prior to end of measurement). Another 50 measurements could not be evaluated by the software due to the noisy background that did not permit to find both the integration start and end point. The reference is regarded as 100% in the left column. The mean error (%) compared to each reference, the standard deviation (%), the minimum and maximum error (%), the number n of measurements, the significance of the difference from students t-test for paired data and the number of outliers (>4.5SD) that have been erased from the dataset are depicted in the remaining columns.*

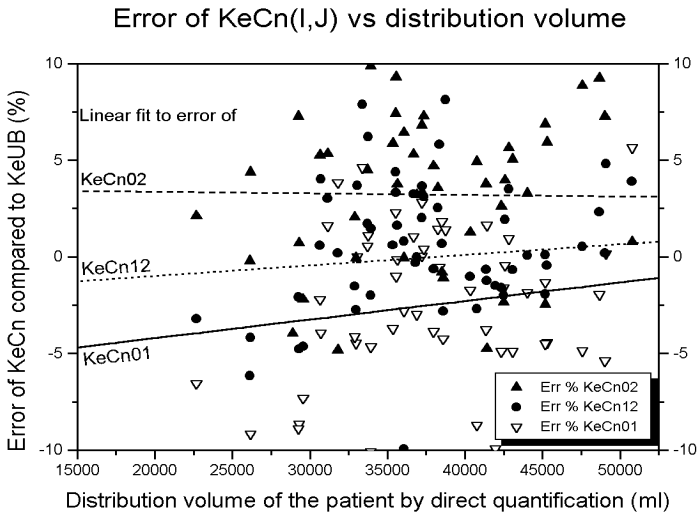


Figure 4.1.1.1.d:*The error of $KeCn_{ij}$ compared to KeUB as reference is plotted versus the patients distribution volume as calculated from direct quantification method, corrected for the amount of ultrafiltration that has elapsed at time of the related ionic clearance measurement. Although the data seem diffusive the trend is emphasised by a linear fit. Only points with MBE <5% are included (further points skipped*

Astonishingly a weak correlation of urea distribution volume and the error of the results from the $KeCn_{IJ}$ clearance values has been found (figure 4.1.1.1.d). This has not been expected. The weak trend is emphasised by plotting the linear regression curve of each subgroup. The difference of the results using the permutations in I,J and the weak KeCn error dependence from the urea distribution volume was the first advice that sodium transfer could play a role in the measurement of the clearance. This was the start point to develop the theory on electrolytical distribution volume measurement described in chapter 2.5.3.

To be able to discuss the relation of the sodium imbalance generated by the step profiles for the clearance measurement in comparison to the total sodium transfer (diffusive and convective) throughout the whole treatment the mean sodium transfer of all treatments has been calculated, see table 4.1.1.1.d.

Mean Sodium transfer quantities (n=545)	Diffusive sodium shift per step profile	Sum of diffusive and convective sodium shift per step profile	Total (diffusive and convective) sodium shift throughout the treatment
Absolute amount: SD:	0.64 mmol 7.3 mmol	-36.4 mmol 11.1 mmol	-244.6 mmol 129.3 mmol

Table 4.1.1.1.d :*It is shown the absolute amount of sodium that has been shifted from (negative) or to (positive) the patient. For the step-profile measurement it is separately shown the fraction of sodium shift from only diffusive transfer and the total transfer including also the convective fraction from ultrafiltration. To have an impression what amounts are shifted throughout a complete dialysis session the total transfer is depicted at the right column. The values are based on conductivity measurements.*

The diffusive sodium shift per step profile was not zero but positive. This means at the studies average the step profile has not been symmetric to the baseline that was prescribed by the physician. At an average the phase of high conductivity was more elevated relative to the baseline than the phase of low conductivity was decreased. A small diffusive sodium transfer has occurred during the step profiles that has weak potential to increase plasma sodium. Because the step profiles were always superimposed by ultrafiltration it never has been observed a net sodium flux to the patient during the step profile.

4.1.1.1.1. Clinical outcome

	baseline*	OCM HD**	sign.	last OCM HD***	sign.	
BP_{sys} start	163±17	160±15	n.s.	161±21	n.s.	mmHg
BP_{dia}	85±10	84±8	n.s.	85±11	n.s.	mmHg
BP_{sys} end	159±14	156±12	n.s.	155±10	n.s.	mmHg
BP_{dia}	82±10	83±6	n.s.	82±9	n.s.	mmHg
Body weight	76.9±9.4	76.7±8.8	n.s.	76.9 ±8.8	n.s.	kg
Heart ratet	75.0±1.92	76.2±1.24	n.s.	76.0 ±5.66	n.s.	1/min
Breathing rate	16.9±0.8	16.4±0.6	n.s.	17.5 ±1.9	n.s.	1/min
pO_2 Start	80±10	81±11	n.s.	77±11	n.s.	mmHg
pCO_2 Start	37±1.9	37.25±1.3	n.s.	38.0±1.4xx	n.s.	mmHg
S-Na Start	139±1.7	138±1.7	n.s.	138±3	n.s.	mmol/l
S-Na before impulse: 138.4±1.4		S-Na after impulse: 138.6±1.4			<0.002	mmol/l

BP: blood pressure S-Na: Serum sodium *Mean of 10 haemodialysis before the study ** Mean of 10 haemodialysis during the study *** Mean of last OCM - haemodialysis

Table 4.1.1.1.1.a: *Physiological parameters related to sodium and water balance that describe the clinical outcome of the patients during the MI study.*

No patient reported of a change in thirst, muscle cramps, headache, nausea and vomiting in comparison with the baseline values. Serum sodium, body weight,

breathing and heart rate at rest, arterial oxygen and carbon dioxide partial pressures pO_2 and pCO_2 at start as well as arterial blood pressure at the start and end of the dialysis remained unaffected in comparison with the baseline values. A slight increase of the average serum sodium concentration (0.2 ±1.5 mmol/l, $p<0.002$) was obtained pre and post a step profile, which might be in accordance with a calculated diffusive sodium shift into the patient of 0.64 ±7.3 mmol per OCM impulse (table 4.1.1.1.d and 4.1.1.1.1.a).

4.1.1.2. Integral Dialysis References

Since the problem of Kt/V dose quantification is not solved just by correctly measuring momentary ionic dialysance values throughout the treatment the results that include a complete dialysis session are reported here. The correct integration about a complete treatment must be approved. This is done by comparison according to the single pool model and the Daugirdas second generation formula.

4.1.1.2.1. Single pool kinetic model, direct quantification and Daugirdas equation

To achieve the integrated results the body of the patient or an external dialysate sampling container works as the volume that integrates the urea concentration effecting clearance during dialysis. The results are calculated according to

1. the single pool model according to E 2.6.1.a
2. the equilibrated single pool variable volume kinetic model (E.2.6.1.g)
3. Daugirdas equation (E 2.6.3.a)
4. direct quantification (E 2.6.2.a)

The results can be found in table 4.1.1.2.1.a.

Determination Method	Kt/V Single Pool	Kt/V eSPVVKM	Kt/V Daugirdas2nd Gen	Kt/V DQ
Kt/V ($KeCn_{12}$)	- 2.01 % err 6.99 %SD 0.917 = r -19.3 % Min 28.2 % Max n=111 Sign. diff. (p=0.004) 1 Outlier	2.88 % err 4.15 % SD 0.983 = r - 8.9 % Min 19.9 % Max n=118 Sign. diff (p<0.0001) 0 Outlier	- 16.1 % err 5.2 % SD 0.937 = r -30.6 % Min -3.29 % Max n=107 Sign. diff. (p<0.0001) 2 Outlier	-3.69 % err 8.1 % SD 0.898 = r -26.9 % Min 29.7 % Max n=128 Sign. diff. (p<0.0001) 2 Outlier
Kt/V ($KeCn_{Bolus}$)	9.51 % err 12.5 % SD 0.822 = r -18.5 % Min 54.7 % Max n=105 Sign. diff. (p<0.0001) 1 Outlier	14.55 % err 10.66 % SD 0.930 = r -11.2 % Min 53.4 % Max n=112 Sign. diff. (p<0.0001) 0 Outlier	-5.68 % err 10.61 % SD 0.826 = r -29.9 % Min 30.4 % Max n=102 Sign. diff. (p<0.0001) 1 Outlier	7.19 % err 14.3 % SD 0.73 = r - 41.6% Min 62.0% Max n=120 Sign. diff (p=0.0003) 0 Outlier

Table 4.1.1.2.1.a: *Ionic Kt/V determination based on $KeCn_{12}$ and $KeCn_{Bolus}$ in comparison to urea based Kt/V from single pool, equilibrated single pool urea kinetic model, Daugirdas second generation equation [Daugd] and direct quantification (DQ) as reference (=100%).*

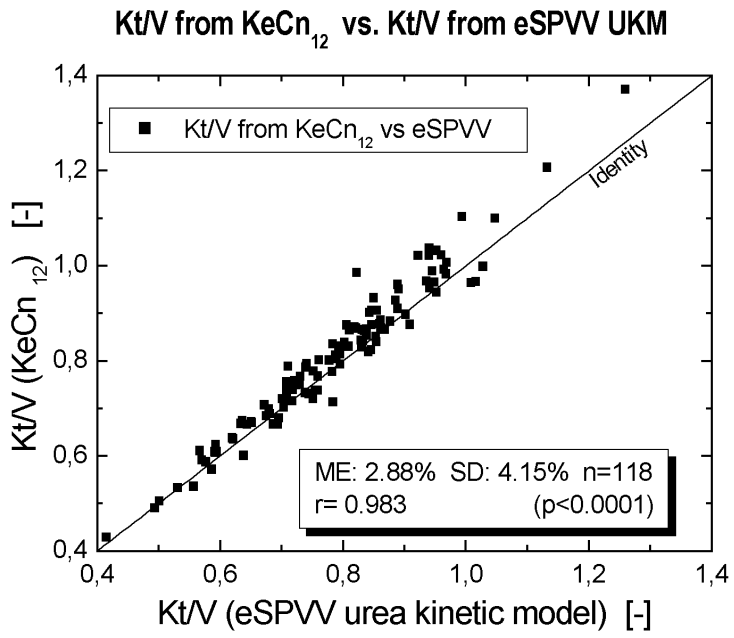


Figure 4.1.1.2.1.a: *The figure shows the Kt/V results from electrolytically measured clearance $KeCn_{12}$ and the clearance as evaluated by the eSPVV urea kinetic model. student's t-test for paired data was applied and analysed the data to be significantly different due to the deviation of the mean and the paired deviations. The t-test for unpaired data regards the data to be not significantly different.*

The results according to single pool and eSPVV volume that have been derived from electrolytical measurement of K, using the high-low level combination of the step-profile ($KeCn_{12}$) showed best coincidence with their reference (error±SD): (-2.01±6.99)% for single pool, (2.88 ± 4.15) % for eSPVV kinetic model. In particular in comparison to eSPVV model the outstanding correlation to electrolytical Kt/V measurement of r=0.983 was found. The affiliated plot is shown in figure 4.1.1.2.1.a

In spite of this high correlation student's t-test for paired data still regards them to be significantly and not statistically different. This may indicate that the mean deviation in combination with the single data pair error is to high to accept it as an measurement principle that has no bias error.

The difference of Daugirdas empirical second generation formula to electrolytical measurements is evident (-16.1±5.2)% and can not be statistical.

With respect to a different subset of the data the comparison of electrolytical Kt/V to direct quantification shows similar results like the single pool methods, as it was expected by theory (-3.69±8.1)%. The higher standard deviation can be attributed to the difficulty of urea concentration measurements in aquatic sodium solutions. It should be emphasised that it is not a simple task for the laboratories to measure urea concentration in spent dialysate with its total different properties than blood or urine (pH, sodium content, protein content, other metabolites).

In the MI study the bolus clearance measurement as is has been performed in 1997 from today's view must be regarded as not very elaborated. It has already been described that compared to the step profile the bolus results have been approximately 10% higher. This is found uniformly again in the Kt/V analysis. The typical result of (14.55±10.7)% in comparison to the eSPVV urea kinetic model is in accordance with the single measurement results. In comparison to all other references a difference of roughly 10% is found. The reason is of technical nature and will be discussed in the next chapter.

4.1.1.2.2. Discussion of the results

Hemodialysis dosage in terms of urea Kt/V has been shown to have a distinct impact on the morbidity and mortality rate in patients on renal dialysis therapy [Go1]. Therefore the adequacy of each dialysis should be guaranteed. Different methods for quantification of Kt/V have been suggested. Some authors expected for non-scientific purposes a single pool model to be sufficient for calculating dialysis dosage, while others suggested the two pool model, which takes the urea rebound into account. However, the practicability of the two-pool-model is limited because the final measurement of serum urea has to be done with a 30min delay to equilibrate the second pool, further a lot of different input data have to be evaluated. Therefore, Daugirdas suggested an empirical formula to estimate equilibrated Kt/V without waiting for the rebound, which is still discussed controversially. Direct quantification by a urea monitor is claimed to be the "gold standard" [Ba] but it necessitates a special device and is up to now limited to special - e.g. scientific - studies. Hence, disadvantages like the inconvenience for the patient and the indispensable sampling of blood and dialysate prevent the regular assessment of Kt/V at each dialysis.

Conductivity cells are ideal sensors for the continuous measurement of the conductivity of the dialysate. The insertion of these cells into the dialyser's in- and outlet line allows a simple and safe continuous measurement of the actual electrolytic clearance. Since sodium and urea diffusion coefficients are almost equal, electrolytic clearance monitoring should give exact figures for the effective total urea clearance. Furthermore, conductivity-based clearance measurements could be performed several times monitoring repeatedly the efficiency of each hemodialysis with consideration of real blood and dialysate flow, dialysis time, recirculation and ultrafiltration without additional burden to the patient and the dialysis staff.

This study was designed to evaluate the accuracy and patient safety of conductivity-based dialysate side online clearance in comparison to established reference methods for clearance and Kt/V. Sodium imbalance was assessed by clinical parameters as change in body weight, in heart and breathing rate or in arterial blood gas analysis as well as by the balance of ionic shift of the OCM step profile.
In order to improve the quality of the urea reference values, the data set in a first step was limited to an error in mass balance of less than 5%. This restriction cut down the data set to less than half (approximately 240/545 measurements). The corresponding OCM single measurement values within this data set has been proven to be of high accuracy compared to urea clearance corrected for recirculation (KeCn12 to KeUB: -0.46±4.75%; KeCn12 to KeUD: -0.17±5.5% error±SD, both not significantly different, n=237). This is in good coincidence to other studies of similar design [DF1, DF2, DF3, DF4, Loc, Manz, Pe1, Pe2, Pe3, Pol, St]. In spite of MBE < 5% screen the references (KeUB/KeUD) were correlated by r=0.91 and not by r=1.0 as you would expect from a perfect reference. The remaining “error” of conductivity-based clearance measurement is assumed to be strongly related to the residual error of the reference and not only to the conductivity measurement itself. The correlation between blood or dialysate clearance of urea and conductivity dialysance confirmed the results obtained with a single conductivity probe operating alternately at the dialysate inlet and outlet line [DF4, Manz, Pe3, Mer]. Dead space, sample mixture effects and the need of temperature compensation, however, rise problems at switching from high to low and from low to high conductivity within one probe. The apprehension of an important drift between the two conductivity probes generated by the presence of urea and other waste products [Mer] seems obviously to be spurious. The use of two probes instead of one permits to obtain a reliable approach to really simultaneous information of dialysate inlet and outlet conductivity without the need of switching from the inlet to the outlet line.
The conductivity-based Kt/V showed good correlation (r=0.92/0.98/0.94/0.90) as well as a reliable coincidence of -1% (weighted mean ±SD, except Daugirdas equation) with the single pool modelling / eSPVV UKM / Daugirdas second generation / direct quantification. The urea generation rate of about 2.5 g/4h dialysis time was not considered by the single pool model, eSPVV UKM and direct quantification. Hence, urea concentration at the end and 30 minutes after hemodialysis treatment appeared higher with than without urea generation and therefore urea clearances calculated by these formulas have been underestimated. The determination of a electrolytic dialysance is independent of urea generation. Therefore, it could be speculated that the remaining error and SD of the conductivity-based Kt/V was partially related to this source of error and not to the principle of the conductivity measurement itself.

Implications for the MII study:

As shown in figure 4.1.1.1.d a slight but non-negligible distribution volume dependence of $KeCn_{IJ}$ was significant. For $KeCn_{01}$ nearly 5% of accuracy difference was found from small to weighty patients. Further a ranking in magnitude of $KeCn_{01}$ < $KeCn_{12}$ < $KeCn_{02}$ was found in more than 85% of the measurements. Only 3 of 529 measurements showed reverse order which might be due to statistical inaccuracy. Further the $KeCn_{02}$ results showed the highest SD. All this can be sodium transfer related. Because the $KeCn_{02}$ clearance calculation uses conductivity measurements with a longer time span between them, the possible period for sodium variations is

the longest. Even if the step profile is assumed to be symmetric in its positive and negative half wave the time averaged value of the plasma sodium during the profile is always higher than the plasma sodium prior to the step profile if the first half wave was positive as in this study. Accordingly the patient will not be in the same constitution as without the step profile when the profile ends. This takes more time. If we consider that the rebound time is typically 30 min and therefore much longer than the duration of the step profile (14 min) as well as it is patient individual it can be assumed that the effect of the step profile has not completely decayed at termination of the negative half wave. This means the step profile will not be able to re-extract all sodium that has been shifted into the patient during the positive half wave. A remaining rest, strongly dependent on the individual patient and his ECF-ICF clearance, will always play a stronger relative role at time t_2 when the second conductivity is measured. This can be the reason of the unexplained, increased SD in comparison to the 0-1 and 1-2 permutations that do not have such a long time span between their related conductivity measurements.

All these considerations are a clear indication of an additional parameter taking affect that has not been reflected during the preparation phase. Primary these results were not understood. A ionic dialysance measurement that is not independent of urea distribution volume or of the time of measurement can not be accepted as an accurate, theoretically integer method. Any side effect must be excluded or integrated. After careful analysis it became clear that it can only be understood in the context of sodium transfer which is generated by the step profiles. Finally this consideration led to the theory of the electrolytical sodium distribution volume measurement as described in chapter 2.5.3 and therefore had a good impact on the theoretical understanding of the results. The conclusion that can be drawn from this theory is the necessity to avoid sodium transfer. This means the conductivity variation should be as low as reasonable possible. This implies a preference for the bolus principle with its much smaller variation but lack of stability.

From this point of view it would be desirable to find a technical approach that produces similar accuracy and SD with the boli as with step profiles. Unfortunately the situation is completely different with the boli results as they were applied in this study. The single measurement deviations compared to KeUB and KeUD are in the range of 8.4% - 11.2% with unrestricted MBE. Accordingly the derived integrated results show similar deviations: 9.51% / 14.55% / 7.19% (single pool / eSPVV UKM / DQ). The SD was always higher than 10% for all evaluations and therefore not acceptable. This is an indicator that theoretical as well as technical errors in the shape of the bolus and in the software evaluation occurred. Detailed analysis of the shapes of the boli and the evaluation algorithm showed that the baseline was too noisy relative to the height of the boli. The integration process that is involved by performing the Laplace transform was not able to find the points where to start and where to end the bolus analysis correctly. On the other hand the results are not so general different from the step profile results so that they seem to be encouraging to improve the technical solution that was chosen. After careful analysis of the mathematical demands for a better bolus function it was decided to launch a second study MII with emphasis to an improved bolus measurement. Based on this results the MII study was designed to find and approve a technical solution to measure the clearance with boli exactly as demonstrated with step profiles in the MI study.

Discussion of the clinical outcome

In this study the mean sodium baseline of the dialysate (14.2 mS/cm) was asymmetric with regard to implemented safety limits of conductivity variation (safety limits: 12.8 mS/cm – 15.7 mS/cm). The resulting diminutive "cut off" of the negative phase of the impulse could account for a small influx of sodium into the patient. Although not taking into account the concentration of other electrolytes the ratio of 1:10 for conductivity (mS/cm) over concentration of sodium (mmol/l) in general is a rough estimate. Based on this simplification a net sodium transfer of 0.64 mmol per measurement was calculated to occur, which could be blamed for the slight increase in serum sodium (table 4.1.1.1.1.a). Therefore the ultrafiltration had to be intensified theoretically by (4.6 ±53)ml per step profile (with S-Na 138 mmol/l) to eliminate the presumed additional sodium. Nevertheless, no significant signs of sodium and fluid overload were observed during the study period of 10 OCM hemodialysis sessions without adjusting ultrafiltration. Presumably an increased sodium efflux by diffusion and convective transmembrane transport had counterbalanced completely the small net sodium influx caused by the sodium impulses of the OCM application.

Conclusion

In conclusion, the OCM based on $KeCn_{12}$ step profile measurements provides a safe and accurate tool to monitor the mean urea clearance of each hemodialysis session with weak but acceptable dependence of urea distribution volume.

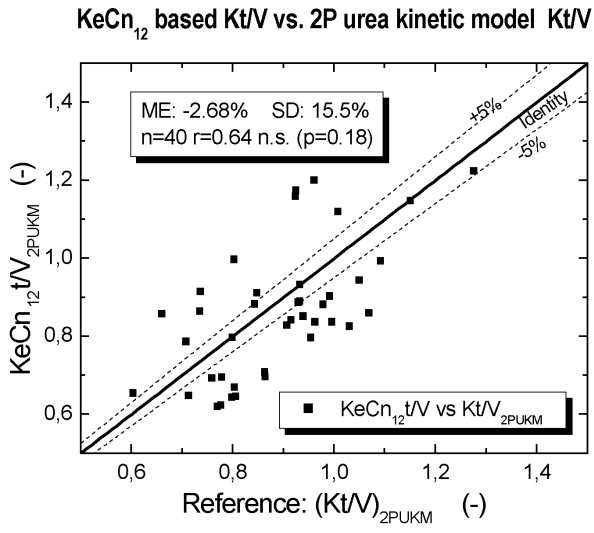


Figure 4.1.1.2.3.a: *The MI study data in comparison to the 2 pool analysis. The Kt/V values are based on the same t and V but on different clearance values. The urea clearance reference as derived from the 2PUKM (abscissa) fits the actual laboratory data best. $KeCn_{12}$ (ordinate) is the value as it has been found by electrolytical measurement using the step-profile (high-low state). $KeCn_{12}$ is corrected for operational parameters according to [E 2.4.4.h]*

4.1.1.2.3. Two pool kinetic model

Neither for the MI nor for the MII study initially it was planned to analyse the data using the two pool model. The reason was the treatment schedule which was not to be altered by the study protocol. It could not be supposed that a particular patient would participate the study for an uninterrupted sequence of 10 dialysis sessions. The patients and the nursing staff were free in their decision for which shift the patient would be scheduled next dialysis. Not always a physician that was member of the study group was attending. For two pool analysis it would be necessary to have complete data for at least two subsequent

dialysis sessions. The evaluation software was programmed to assume a patient has interrupted the sequence if the delay of a study dialysis session exceeds 4 days. This decreased the 206 sessions of the MI study to 40 sessions accessible to evaluation.
The ionic clearance as measured with the high-low states of conductivity step profiles and corrected using E 2.4.4.h was able to match the mean urea clearance from the 2PUKM with a mean error±SD of (–2.68±15.5)%. Students t-test for paired data did not find the data to be significantly different at a significance level of 0.95 although the correlation was only 0.64.

Discussion

A mean error of -2.68% is within the possible resolution of the analysis. The relatively high SD can be explained by the manifold of different variables that are needed to apply the model (see table 2.6.9.a). The high SD further is the explanation that student's t-test for paired data did not find the data to be significantly different. If an analysis shows this SD the deviation in the mean value is not sufficient to assume that a different statistical population has been measured. This means it can be expected that at an average the ionic dialysance measurement using step profiles measures the urea clearance as evaluated by two pool kinetic model.

Weighting both the mean error and the SD it can be concluded that at an average the ionic clearance measurement matches the best available reference principle, the two pool urea kinetic model, but there is only little reliability of the single result. The question is if the SD is caused by the reference or by the measurement. This can be decided looking at figure 4.1.1.2.1.a, where it is to be seen that the same ionic dialysance measurement shows an extreme high correlation of 0.983 and similar error in comparison to the equilibrated single pool. This model is much simpler to apply and has much less input variables. This is a clear indicator that the reference could be involved in the reason of the high SD. The impression remains that the two pool model as it has been applied here seems to be very complicated. The experience during the development of the analysis algorithms shows that it would need further blood samples during dialysis, in particular during the rebound phase, to have a more precise image of what really happens in the compartments of the patient. More blood samples however are contrary to the important interest not to add any burden or risk to the patient that is only due to the study and therefore the care for the patient forbid to do so.

4.2. The MII Study

The evaluation of the MII study data consists of three subdivisions. These are

- Comparison of ionic dialysance to urea clearance including momentary measurements and integrated procedures for Kt/V calculation.
- in-vitro and in-vivo analysis of the measurability of sodium distribution volume
- Plasma sodium measurement by electrolytical methods in comparison to the laboratory reference.

The results and the discussion of these issues are summarised in the following chapters.

4.2.1. Comparison of ionic Dialysance to Reference

4.2.1.1. Step-function momentary dialysance measurements

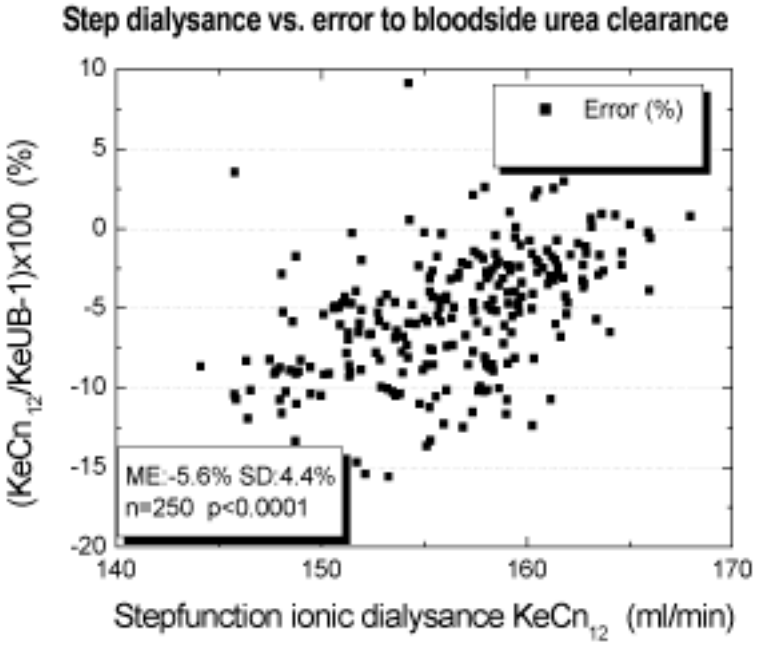


Figure 4.2.1.1.a: *Comparison of the machine side measured total effective ionic dialysance using conductivity step-profiles to the related total effective urea reference clearance as calculated from blood sample laboratory urea analysis. Plotted vs. error. The t-test for paired data was applied. A drift of the error is attributed to a deviation of blood flow measurement*

In the tables 4.2.1.1.a,b the relations of $KeCn_{I,J}$ to its references KeUB and KeUD are depicted. The reference again is regarded to be 100%. The mean error, SD, minimum deviation and maximum deviation is given. The mass balance error was not restricted in table 4.1.1.1.a and restricted to 5% in table 4.1.1.1.b.

Overall 265 step profiles have been performed in the MII study, but not all of them could be related to their respective reference counterpart due to the lack of information, eg. laboratory values missing or non plausible, technical inconsistency or no sufficient achievement of stability of

unrestricted Mass balance error	KeCn01 Range: 88.5 – 168.0 ml /min	KeCn12 90.3 – 170.4 ml /min	KeCn02 91.7 – 208.9 ml /min	KeUB Reference 92.8 – 182.9 ml /min
KeUB Reference Range: 92.8 – 182.9 ml /min	- 9.07 % err 4.81 % SD -23.4 % Min 2.7 % Max n=250 Sign. diff. (p<0.0001) 1 Outlier	- 5.62 % err 4.36 % SD -19.2 % Min 9.1 % Max n=250 Sign. diff. (p<0.0001) 1 Outlier	6.82 % err 7.65 % SD - 18.1 % Min 34.2 % Max n=250 Sign. diff. (p<0.0001) 1 Outlier	-
KeUD Reference 73.4 – 214.2 ml /min	- 2.37 % err 11.79 % SD -32.2 % Min 41.6 % Max n=248 Sign. diff. (p<0.0001) 2 Outlier	1.34 % err 11.97 % SD - 28.0 % Min 51.9 % Max n=248 n.s. (p=0.937) 2 Outlier	14.45 % err 14.02 % SD - 18.1 % Min 75.6 % Max n=247 Sign. diff. (p<0.0001) 3 Outlier	6.71% err 14.30% SD -36.8 % Min 56.9 % Max n=258 Sign. diff. (p<0.0001) 2 Outlier

Table 4.2.1.1.a: *The different methods to determine the electrolytical dialysance KeCn are compared to the referring reference blood- and dialysate samples, including all technically available reference data of the MII study independent of their accuracy. The reference (100%) is in the left column. The mean error (%) compared to each reference, the standard deviation (%), the minimum and maximum error (%), the number n of measurements, the significance of the difference from students t-test for paired data and the number of outliers (>4.5SD) that have been erased from the dataset are depicted in the remaining columns.*

Mass balance error < 5%	KeCn01 Range: 88.5 – 168.0 ml /min	KeCn12 90.3 – 170.4 ml /min	KeCn02 91.7 – 208.9 ml /min	KeUB Reference 92.8 – 182.9 ml /min
KeUB Reference Range: 92.8 – 182.9 ml /min	- 7.73 % err 3.74 % SD -21.9 % Min 0.1 % Max n=115 Sign. diff. (p<0.0001) 0 Outlier	- 4.37 % err 3.19 % SD -17.2 % Min 2.5 % Max n=115 Sign. diff. (p<0.0001) 0 Outlier	7.98 % err 7.16 % SD - 18.1 % Min 23.9 % Max n=115 Sign. diff. (p<0.0001) 0 Outlier	
KeUD Reference 73.4 – 214.2 ml /min	- 7.44 % err 4.37 % SD -22.3 % Min 1.5 % Max n=115 Sign. diff. (p<0.0001) 0 Outlier	- 4.07 % err 3.77 % SD -17.6 % Min 4.3 % Max n=115 Sign. diff. (p<0.0001) 0 Outlier	8.29 % err 7.22 % SD - 18.1 % Min 28.8 % Max n=115 Sign. diff. (p<0.0001) 0 Outlier	0.25 % err 2.79 % SD - 4.8 % Min 5.1 % Max n=118 n.s. (p=0.322) 0 Outlier

Table 4.2.1.1.b:*The different methods to determine the electrolytical dialysance KeCn are compared to the referring reference blood- and dialysate samples. Here only the more accurate reference data with MBE less than 5% were included. The reference (100%) is in the left column. The mean error (%) compared to each reference, the standard deviation (%), the minimum and maximum error (%), the number n of measurements, the significance of the difference and the number of outliers (>4.5SD) that have been erased from the dataset are depicted in the remaining columns.*

the outlet conductivity. Only those were included which did not obviously seem to lack in some of these issues.

Figure 4.2.1.1.a is a plot of the $KeCn_{12}$-KeUB analysis of table 4.2.1.1.a to visualise the distribution. Although only generated by a few points the cloud seems to show an error drift. It was not possible to correct it retrograde. Careful analysis showed the drift was due to the miscalibration of the blood flow measurement. Although the blood flow measurement unit of the dialysis machine was frequently controlled using a Transsonic HD01 this drift was not discovered. The error can be understood if we look at how the Transsonic device works: it measures flow velocities and multiplies it with the tube diameter. Changes of the diameter due to pressure changes do not affect the result, even if it should. Flow dependent diameter changes may occur. If the flow decreases, the diameter grows in the arterial inlet tube and shrinks downstream the arterial blood pump. Nevertheless the data can be used in a mean because an absolute flow calibration at 200ml/min that additionally has been performed using a measured volume of water assures the correctness of the mean blood flow.

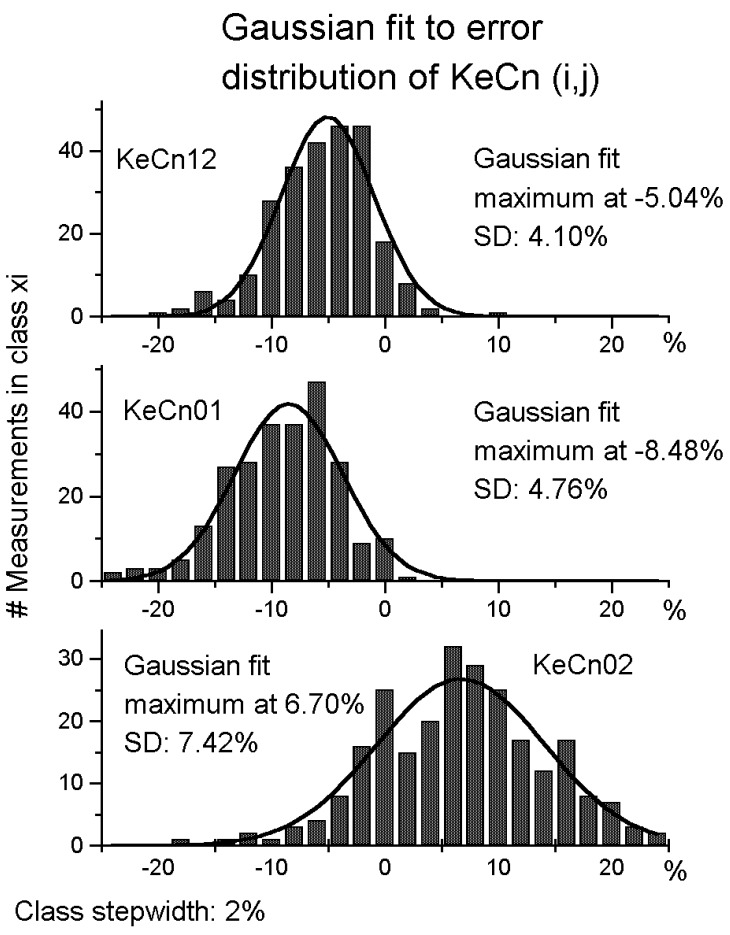


Figure 4.2.1.1.b:*The histograms show the error distribution of M2 study measurements of KeCn12 (top), KeCn12 (middle) and KeCn02 (bottom) in 2% classes. The 100% reference was KeUB from the corresponding blood sample. As an overlay a gaussian fit has been made to each data set. Exactly the data of table [4.2.1.1.a] have been included. The maximum and SD for the gaussian fit – not for the data itself – is shown. At the maximum of the KeCn02 gaussian fit the KeCn01 fit has already decayed to less than 0.01% of its own maximum.*

It has been found even with unrestricted mass balance error that the high-low permutation of the step profile dialysance, $KeCn_{12}$ again matches best the laboratory reference of both the urea clearance KeUB /KeUD (error ± SD): -5.6±4.4% / 1.3±12.0%, but still being significantly different. A relative high SD comparing $KeCn_{12}$ to KeUD seems to be due to an increased laboratory error because it is no longer found when the mass balance error is limited to 5% (table 4.2.1.1.b). The KeUB-KeUD difference of 6.7% in the unlimited MBE screen is a clear indicator of a laboratory error. The error seems to be related to KeUD because after limiting the MBE to 5% the difference of the KeUB-KeUD references vanishes (0.25%) and all results inclusive the KeUD reference are similar to the KeUB results. Now this subgroup of data indicates the same

trend as the unlimited MBE subgroup: KeCn12 differs (-4.4±3.2)% from the KeUB result. Another important observation is that the SD of the $KeCn_{02}$ values is the highest, independent of the MBE limitation. This is in accordance with the MI study. The correlations are not reported here in detail by the same reasons as mentioned: The clearance value distribution is of comparable magnitude than the SD and does not sufficiently cover the measurement range. They are all in the range of 0.75-0.9. No patient was dialysed with lower or higher clearance for study purposes than prescribed.

The error distribution of the 3 different types of step profiles is depicted in figure 4.2.1.1.b. Again it can clearly be seen that the result classes are shifted relative to each other in dependence of the selected evaluation permutation of the step profile (high-low, baseline high, baseline-low). Further it can be observed that the KeCn02 values have the broadest distribution. At 264 of 265 measurements all three permutations $KeCn_{01}$, $KeCn_{12}$, $KeCn_{02}$ were available. 253 of the 264 measurements (95.8%) showed $KeCn_{01} < KeCn_{12} < KeCn_{02}$, the other 11 measurements (14.4%) either showed only $KeCn_{01} < KeCn_{12}$ or only $KeCn_{12} < KeCn_{02}$.

Volume dependence

The analysis of volume dependence of the dialysance results has been made (figure 4.2.1.1.c). It has been found a similar result as before in the MI study:

Although the data points seem very diffusive the linear fits show that the different permutations 01, 12 and 02 have the same order as found previously. It was found to be impossible to simply scale the error of $KeCn_{I,J}$ to the reference because the error is dependent on the patients urea distribution volume which a priori is not known, although this dependency for $KeCn_{12}$ and $KeCn_{02}$ is possibly acceptable with respect to the resolution applied. The strongest distribution volume dependency was found for the positive step-function $KeCn_{01}$, which covered an error from -12.5% for small patients (Vdist = 20l) to –5% for large patients (Vdist = 45l). This is vice versa for the KeCn02 measurements that have positive deviations for small volumes (9% / 20l, 7% / 45l), but the dependence is

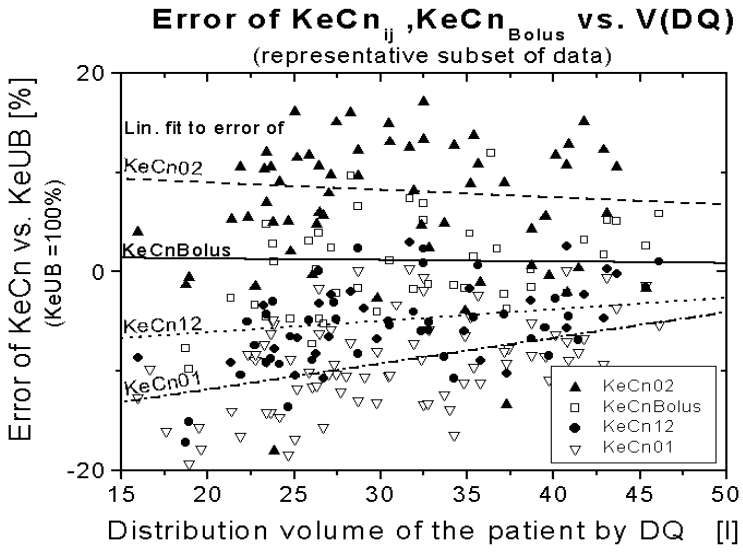


Figure 4.2.1.1.c: *- The error of $KeCn_{ij}$ and $KeCn_{Bolus}$ compared to KeUB as a reference is plotted versus the patients distribution volume ("dry volume") as calculated from direct quantification method, corrected for the amount of ultrafiltration that has elapsed at time of measurement. Although the data seem diffuse the trend is emphasised by linear fit curves. MBE limited to 5%.*

weaker. Also KeCn12 shows a weak dependence of the urea distribution volume. The bolus ionic dialysance measurement principle appeared to be nearly independent of the distribution volume (1.5% at Vdist=20l; 1.0% at Vdist=45l). In summary the errors diminish with increasing distribution volumes for all principles.

To be able to discuss the relation of the sodium imbalance generated by the step profiles for the clearance measurement in comparison to the total sodium transfer (diffusive and convective) throughout the whole treatment the mean sodium transfer of all treatments of the MII study has been calculated, see table 4.2.1.1.c.

Mean Sodium transfer quantities (n=264)	**Diffusive sodium shift per step profile**	**Sum of diffusive and convective sodium shift per step profile**	**Total (diffusive and convective) sodium shift throughout complete treatment**
Absolute amount:	3.04 mmol	-33.7 mmol	-237.1 mmol
Standard dev.:	6.3 mmol	11.1 mmol	84.9 mmol

Table 4.2.1.1.c: *The table shows the absolute amount of sodium in the MII study that has been shifted from (negative) or to (positive) the patient. For the step profile measurement it is separately shown the fraction of sodium shift due to only diffusive transfer and the total transfer including also the convective contribution by the ultrafiltration. To have an impression what amounts are shifted throughout a complete dialysis session the total transfer is depicted in the column at the right margin.*

The diffusive sodium shift per step profile was not zero but positive. This means at the studies average the step profile has not been symmetric to the baseline that was prescribed by the physician. At an average the phase of high conductivity was more elevated relative to the baseline than the phase of low conductivity was decreased. A small diffusive sodium transfer has occurred during the step profiles that has weak potential to increase plasma sodium. Because the step profiles were always superimposed by ultrafiltration it never has been observed a net sodium flux to the patient during the step profile.

4.2.1.2. Bolus momentary dialysance measurements

The reshaped bolus for the MII study as depicted in figure 3.3.a did not show the disadvantage of poor analysability with the larger area under the curve and the low noise baseline. It was no problem for the mathematical algorithms to find all relevant points to do the Laplace transform. The technical improvement directly condensed to better results: The mean error versus KeUB reference was found to be only 0.06±4.8% (error±SD) in the unrestricted MBE data group and 1.3±3.3% with the MBE limited to 5% (table 4.2.1.2.a). Both are regarded to be not significantly different by student's t-test for paired data. The data distribution is shown in figure 4.2.1.2.a. The error drift that is to be seen in this figure is due to the same reasons as explained for the step function in the last chapter. The SD that has been more than 10% in the MI study now has shrunk to 3-5% depending on the reference. This is of comparable magnitude as could be achieved using step profiles.

Ionic dialysance and its reference	KeCnBolus	KeUB	Referer
KeUB Reference	0.06 % err 4.76 % SD n=162* n.s. (p=0.87) *1.28 % err* *3.33 % SD* *n=79*		
KeUD Reference	6.72 % err 10.90 % SD n=163* (p<0.0001) *2.18 % err* *4.13 % SD* *n=79*	6.71% err 14.30% SD n=258 (p<0.0001) *0.25 % err* *2.79 % SD* *n=118*	

Table 4.2.1.2.a: *Error±SD of ionic bolus dialysance determination in comparison to urea clearance from blood (KeUB) and dialysate (KeUD) as reference (=100%). The mean error (%) compared to each reference, the standard deviation (%), the minimum and maximum error (%), the number n of measurements, the significance of the difference and the number of outliers (>4.5SD) that have been erased from the dataset are depicted.Typed in italics: MBE <5%;Typed* normal*: unrestricted MBE. * Within 4h session no time remained for a third bolus according to fig.2.4.1.a.*

Discussion

The comparison of the momentary measurements completely confirms the assumption that using electrolytical methods it is possible to measure the urea clearance with an accuracy significantly below 5%. The large number of single measurements - overall 808 step profile measurements and 510 bolus measurements in both studies – allows to confess that the difference of urea clearance and ionic dialysance is less than the resolution of the evaluation, which is at about 3% of clearance in a statistical average. The contribution of the laboratory reference errors is significant and the difference that still remains can not clearly be attributed to the method of measurement or to the reference.

The sodium transfer that is applied by asymmetric step profiles can be found in a weak deviation of the patients plasma sodium (MI result) and therefore must exist. The transfer is sufficient to clearly establish a ranking $KeCn_{01}<KeCn_{12}<KeCn_{02}$ in accordance with theory. Further the step profile is dependent of the urea distribution volume of the patient. It must be concluded that the sodium transfer is responsible for measurement inaccuracy and

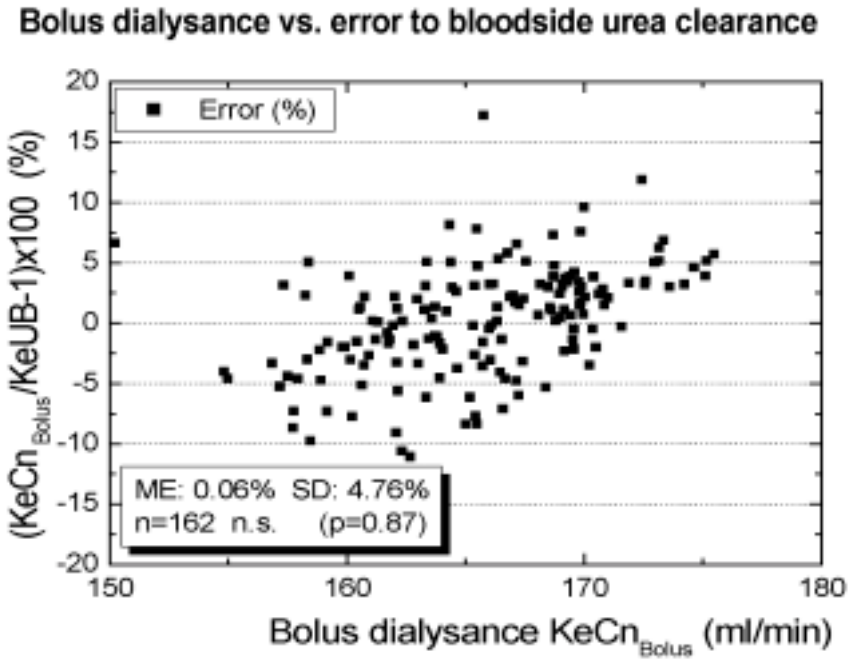


Figure 4.2.1.2.a: *Bland-Altman plot : Comparison of the machine side measured total effective ionic dialysance using conductivity boli to the related total effective urea reference clearance as calculated from blood sample laboratory urea analysis. It is plotted vs. the error. The t-test for paired data was applied. The number of bolus measurements is only 2/3 of the step profile number due to time restrictions: Within a 4h session no time remained for the application of the last bolus. A drift of the error is attributed to blood flow measurement deviations (see chapter 4.2.1.1)*

should be minimised not only by medical but also by technical reasons. This insight resulting from the MI study has been correctly converted to a new technical solution to measure the ionic dialysance, the bolus principle. This principle is purely dynamic and is not dependent on any static conductivity state that continuously transfers sodium. The lower limit of necessary sodium transfer is predominated by technical considerations and the resolution of the conductivity measurement system. If this is implemented correctly it has been found that the bolus principle can achieve comparable or even better results than a step profile as it is introduced in the literature since years. The improvement of the bolus consists in its better independence of the urea distribution volume. The urea distribution volume of the patient a priori is not known and can not be an input variable for a clearance measurement. It can be stated that one of the major goals of the MII study has been reached.

4.2.1.3. Integral Dialysis References

In this chapter no more single results of particular blood samples are compared to their individual reference. In the sense of a dialysis dosage calculation in terms of Kt/V it is necessary to derive the accumulative value for K and V from the data according to the different models. This is done exactly according to the theory described in the related theory chapters. In the first part the equilibrated single pool variable volume model is applied to the data (chapter 4.2.1.3.1). The second part describes the application of the two pool kinetic model. In the third part a brief comparison of the two pool model to the Daugirdas second generation formula [Daugd] is made.

4.2.1.3.1. Single pool urea kinetic model

Due to the study protocol the single pool analysis has only been done on single dialysis sessions. It was not enhanced to a period of more than one treatment like the two pool analysis. Data that were not available within a single session have not been included to the analysis. The schedule of dialysis treatments did not regularly permit to have continuous data access from subsequent sessions because the patients frequently were dialysed on another shift. Subsequent sessions were recorded only occasionally.

Each single ionic dialysance $KeCn_{12}$ and $KeCn_{Bolus}$ and their related urea references have been integrated over treatment time to achieve Kt/V. Urea generation has been neglected. This will artificially increase the V and therefore decrease the reference. A rough estimate for this deviation can be achieved if it is considered that the patient being on a 4h dialysis/48h schedule will generate 8-12% of his urea within the dialysis session. If this leads to a too high V - as it stands in the denominator - Kt/V will be too low. The comparison of the results are shown in table 4.2.1.3.1a with the reference set to 100%. The mean errors±SD varied from –21.1±6.0% ($KeCn_{12}$ versus

Daugirdas second generation equation) to 5.32±3.9% ($KeCn_{Bolus}$ versus equilibrated single pool variable volume Kt/V). For all principles, correlations above 0.89 could be found, and an outstanding correlation of r=0.98 and lowest SD of 3.9% was found comparing $KeCn_{Bolus}$ versus eSPVVKM based Kt/V (figure 4.2.1.3.1.a) .

Determination Method	Kt/V Single Pool	Kt/V eSPVVKM	Kt/V Daugirdas2ndGen	‹t/V ›Q
Kt/V ($KeCn_{12}$)	- 7.54 % err 7.6 %SD 0.895 = r -25.2 % Min 12.1 % Max n=45 Sign. diff. (p<0.0001) 0 Outlier	0.05 % err 5.0 % SD 0.956 = r -12.2 % Min 19.2 % Max n=45 n.s. (p=0.993) 0 Outlier	-21.1 % err 6.0 % SD 0.91 = r -36.8 % Min -6.4 % Max n=45 Sign. diff. (p<0.0001) 0 Outlier	-1.97 % err 6.4 % SD 0.948 = r -12.6 % Min 20.4 % Max n=68 Sign. diff. (p=0.036) 2 Outlier
Kt/V ($KeCn_{Bolus}$)	- 2.75 % err 6.6 % SD 0.934 = r -18.7 % Min 11.2 % Max n=44 Sign. diff. (p=0.008) 0 Outlier	5.32 % err 3.9% SD 0.979 = r -4.6 % Min 15.5 % Max n=44 Sign. diff. (p<0.0001) 0 Outlier	-17.2 % err 5.2 % SD 0.94 = r -31.3 % Min -6.7 % Max n=43 Sign. diff. (p<0.0001) 1 Outlier	3.15 % err 6.3 % SD 0.953 = r - 12.4 % Min 19.1 % Max n=66 Sign. diff (p<0.0001) 0 Outlier

Table 4.2.1.3.1.a: *Ionic Kt/V determination based on $KeCn_{12}$ and $KeCn_{Bolus}$ in comparison to urea based Kt/V from single pool, equilibrated single pool urea kinetic model, Daugirdas second generation equation [Daugd] and direct quantification (DQ) as reference (=100%).*

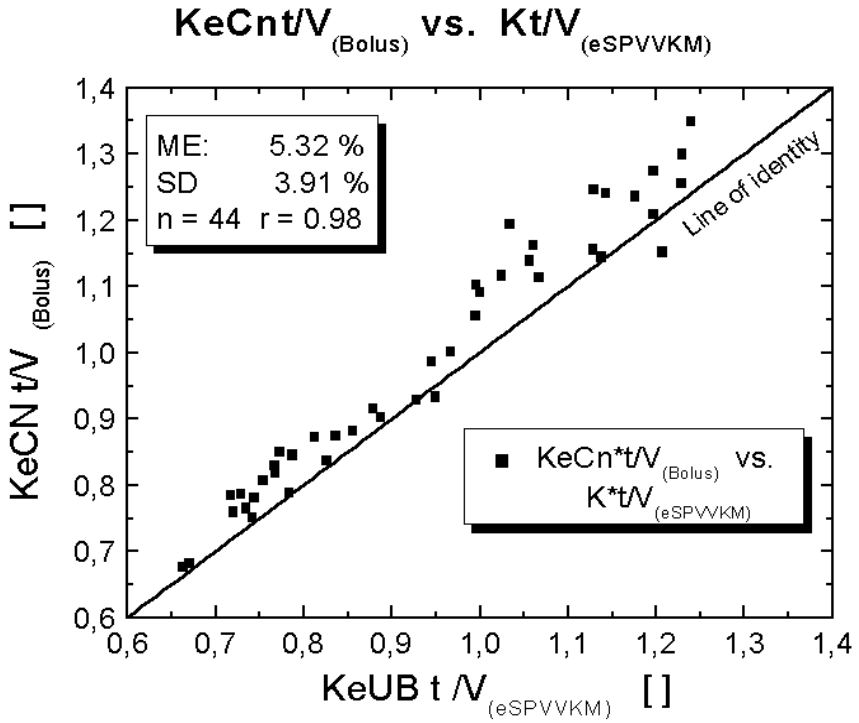


Figure 4.2.1.3.1.a: *Kt/V as derived from electrolytical $KeCn_{Bolus}$ by the dialysis machine is compared to Kt/V as calculated using the equilibrated single pool variable volume kinetic model. The rebound was included by stopping the dialysate flow 30 min prior to end of ultrafiltration and drawing of the final blood samples. A two-pool model was not used and the urea generation rate was neglected, therefore the reference is expected to be too low for some amount because the final plasma urea concentration at t_{30} was achieved even with urea generation.*

4.2.1.3.2. Two pool kinetic model

Preface

To introduce the results of a two pool urea kinetic model it is helpful to initially present some typical curves the model has calculated for a single dialysis session. To understand the curves the considerations of chapter 2.6.5 and 2.6.6 should be helpful. In particular it is important to know that only those sessions have participated in the 2PUKM that have an ancestor session that is less or equal four days ago. If the time span is longer the software automatically assumes that the patient underwent dialysis somewhere else or has not participated in the study for one or more sessions - even if this could be wrong in particular. Not every shift a physician that was member of the study group was scheduled to supervise the treatments and to draw all the necessary blood-samples. These 'lost' sessions are the missing link to a continuous 2PUKM of a full study period of 10 sessions every patient participated the study. The number of sessions that could be evaluated is shown in table 4.2.1.3.2.a.

Sessions *(total MR II)*	*93*
Sessions *(participating 2PUKM)*	44

Table 4.2.1.3.2.a: *The total number of dialysis sessions that could be evaluated in MR II study and the number that was accessible to 2PUKM (47.3%).*

The analysis has been performed in two steps:

- Step A: The variation is performed on ECF, ICF and on KeCNBolus.
- Step B: The variation is performed on ECF, ICF, KeCNBolus and G'.

Is it sufficient to calculate according to step A in clinical practice ?

Of course in clinical practice 2PUKM is not usual because it is impractical, cost intensive and needs additional blood samples and care. In particular if the analysis is based on a continuous, real time data stream that is recorded online from the dialysis machine like in this study. Nevertheless using a medium class PC as it is available in many facilities in place it is not impossible to perform such a modelling for a single dialysis session. Having a suitable software installed on both the PC and the machine could permit to immediately get a two pool result to make a decision if the patient has been dialysed sufficiently according to 2PUKM. If he was not dialysed enough the session could be prolonged. Not all patients would agree to the prolongation but from a quality aspect this should be desirable. It may be speculated if the dialysis facilities would agree because the prolongation interferes with the schedule of the dialysis shifts and commits staff. The considerations if step A is sufficient are made on the background that it is a useful idea to implement the 2PUKM as a compact solution in a dialysis machine, working on the base of an non-invasive electrolytical measurement principle without samples or additional attendance of nursing staff.

During the data analysis and the program development the Step B variation has found to be of high computational costs and not practical for calculation on a PC. A more sophisticated linear optimisation using the Levenberg-Marquardt algorithm or

Broyden update matrices and their improvements [Lev, Brow, Broy1, Broy 2] in the algorithm to fit the data could accelerate it to not more than square convergence. This would not be sufficient and less reliable to find the result within a few minutes. Reduction of the degrees of freedom brings much more calculation saving than accelerating the algorithm and so it was of interest if it is sufficient to perform only step A in a clinical environment without loss of information. The two steps are not equivalent and omitting the variation of G' could be a simplification that makes the results less worthy. Because a dialysis machine has restricted calculation resources it would be desirable nevertheless. A comparison of step A and B is anticipated before the results are represented.

A typical 48h urea concentration time curve originating from the 2PUKM is depicted in figure 4.2.1.3.2.a. The calculation starts at the end of the rebound phase of the previous dialysis and terminates after passing of the rebound of the present dialysis session.

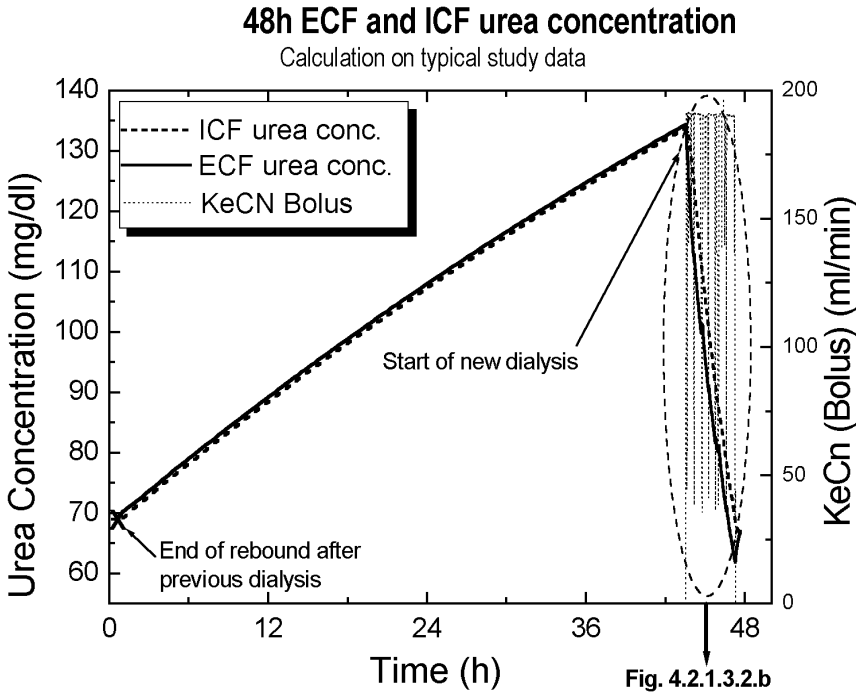


Figure 4.2.1.3.2.a: *Outline of the urea concentration curves of a 48h interval from dialysis end to follow up dialysis end plus rebound time as calculated by the 2PUKM. The dialysance applied by dialysis is depicted as KeCn Bolus. A magnification of the range with KeCn Bolus <>0 (dialysis session) is shown in the next figure. The "X" indicates the systemic plasma urea reference concentration which has been used to minimise the difference of the calculated curve and the reference. The generation rate G' was not fitted (Step A).*

The generation G' in step A was not fitted but calculated according to E.2.6.5.e inserting for (V_i + V_e) the mean of V_{DQ} (direct quantification) and V_{eSPVV} =V_0 (initial volume from single pool model with variable volume, E 2.6.1.e'):

$$V_{DQ} = \frac{M_{Urea} - Qf\ t_{Dial} U_{PW,t30}}{U_{PW,t0} - U_{PW,t30}} \qquad V_{eSPVV} = \frac{Qf\ t_{Dial}}{1 - \left(\frac{U_{PW,t30}}{U_{PW,t0}} \right)^{\frac{Qf}{Qf - KeUB}}}$$

It is apparent that due to the generation of urea into the extracellular pool the model clearly reflects the ECF urea concentration being slightly higher than the ICF concentration during the interdialytic phase. This process endures until the onset of clearance that is provided by dialysis. From the very start of dialysis ECF immediately decreases much faster on an exponential curve which obeys purely to the single pool model in the very first minutes. The details of the right encircled part enclosing dialysis are better visible in their magnification of figure 4.2.1.3.2.b. Here it becomes apparent that the urea concentration of ICF does hardly react in the very first time of dialysis. As soon as the ICF-ECF urea concentration gradient rises by dialysis taking effect the inter-compartment clearance $K_{i,D} + K_{i,c}$ shifts an substantial amount of urea from ICF to ECF and therefore delays the decrease of ECF urea concentration.

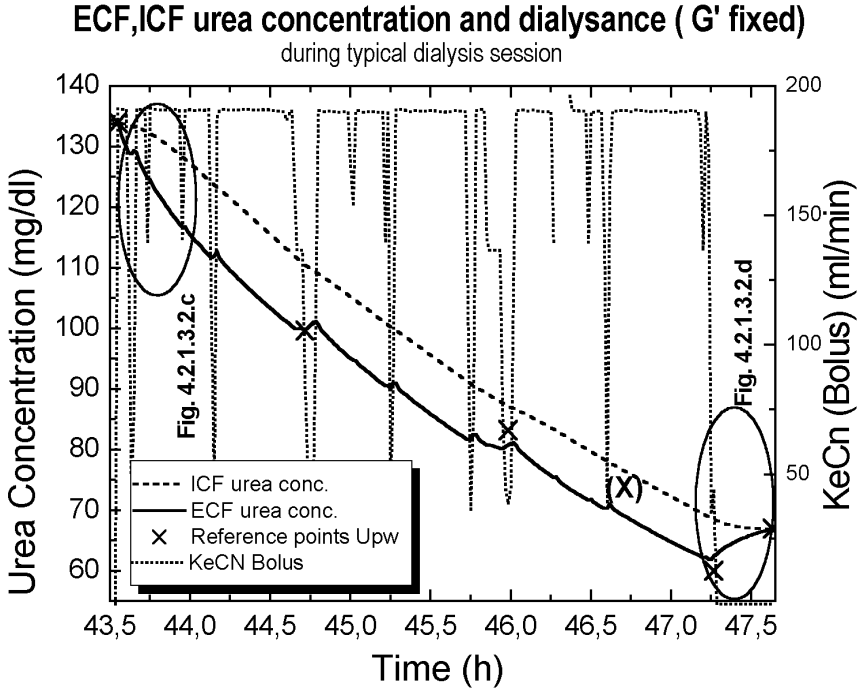


Figure 4.2.1.3.2.b: *Magnification from Fig. 4.2.1.3.2.a.:Typical outline of the urea concentration curves during dialysis as calculated by the 2PUKM. The dialysance applied by the dialysis machine has not been constant due to alarms, blood samples and flow variations. In particular the flow reductions performed to measure the recirculation, during bypass of the dialysis machine and for some of the blood samples appear as a deep dip. The "X" indicate the position of the laboratory reference values which have been used to minimise the difference between the calculated curve and the reference. The embraced X indicates that this point was not always available because in some sessions the prescribed session time was not sufficient to draw the third sample. The generation rate G' was not fitted (Step A).*

This effect can be seen clearly in particular at those times the clearance is paused or decayed. The interruption of urea unload from ECF instantaneously increases urea concentration in ECF, which is fed from ICF urea resources. From the view of the ICF pool there is a large buffer between ICF and the dialysis filter which smoothes any sharp urea variations in the ICF pool so that it will decrease more continuously than ECF in case of dialysis interruptions. Further it has a delay until it reaches the same urea concentration like the ECF. This concentration gradient is maintained throughout the whole dialysis –sometimes with severe discomfort for the patient - but in absolute values usually is slightly decreasing towards the end of dialysis. As soon as dialysis terminates the rebound sets on and establishes urea concentration equilibrium of ICF and ECF again. At the end of the rebound the ECF urea

concentration curve returns to be determined by urea generation, it overcomes the ICF concentration and the interdialytic phase is beginning.

Both figures, 4.2.1.3.2. a and b stem from a Step A calculation. The corresponding calculation of the same session using the Step B calculation including the variation of G is depicted in figure 4.2.1.3.2.c,d by overlaying the concentration curves of both methods for the initial (c) and rebound (d) phase. The difference of Step A and B for the extracellular pool is negligible because the laboratory references are the fixed points. This is not the case for the intracellular pool. By the lack of accessibility the ICF pool can not be compared to a reference.

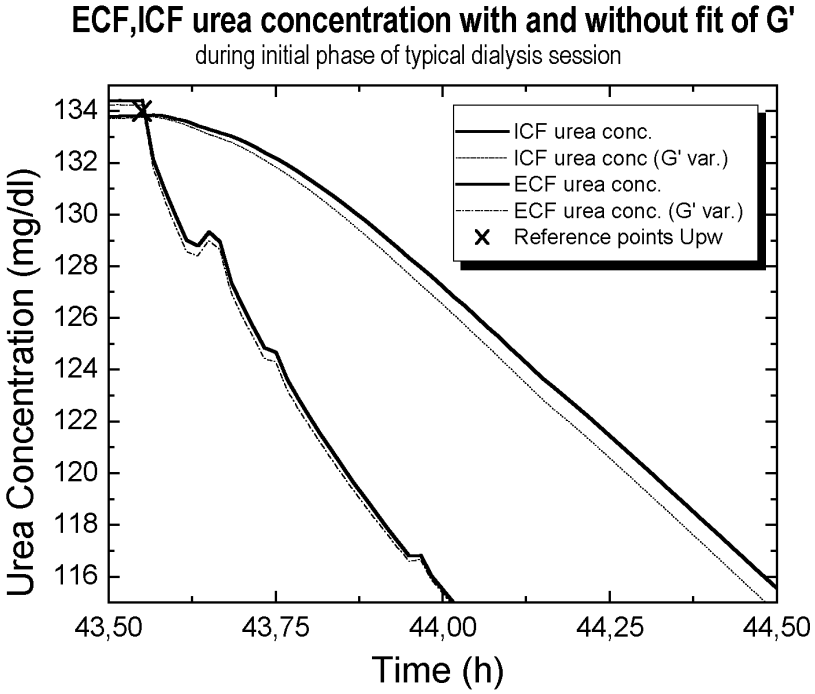


Figure 4.2.1.3.2.c: *Magnification from Fig. 4.2.1.3.2.b.Outline of the urea concentration curves during initial phase of dialysis as calculated by the 2PUKM. The "X" indicates the position of the laboratory reference value which has been used to minimise the difference between the calculated curve and the reference. The generation rate G' was not fitted (Step A, thick continuous line) and fitted by the model (Step B, thin dotted line). The difference is clearly apparent only in the ICF, not in the ECF. The generation rate in Step A has been slightly higher, which corresponds with the ECF urea concentration curve of Step A being slightly above that of step B. It is apparent that the difference of the step A-step B calculation is rising only within dialysis, it is not existing before dialysis.*

Therefore it can not be controlled directly if the model has fitted ICF correctly. The only absolute knowledge is that the expression E 2.6.8.a is better minimised in step B, thus all variables V_i, V_e, KeCnBolus and G' are better selected to fit the ECF urea concentration data. To understand the difference of step A - step B it is necessary to know the absolute data shown in tab. 4.2.1.3.2.b of this typical data set. It is apparent that the model (B) has fitted less total volume and less G' with having G' free for variation. The reason is that in step A G' has been fixed using a volume that originates from a mean of direct quantification and equilibrated single pool model results.

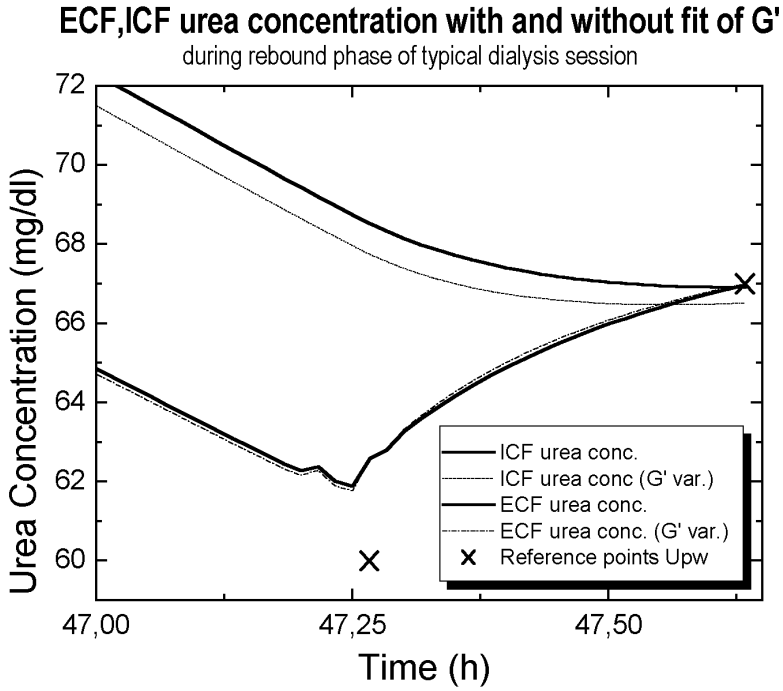


Figure 4.2.1.3.2.d: *Magnification from fig. 4.2.1.3.2.b.Outline of the urea concentration curves during rebound phase as calculated by the 2PUKM. The "X" indicate the position of the laboratory reference values which have been used to minimise the difference between the calculated curve and the reference. The generation rate G' was not fitted (Step A, thick continuous line) and fitted by the model (Step B, thin dotted line). The difference is clearly apparent only in the ICF, not in the ECF. The generation rate in Step A has been slightly higher, which corresponds with the ECF urea concentration curve of Step A being slightly above that of step B.*

Both have been calculated with neglect the urea generation, therefore the value of G' has been selected erroneously with the same relative error than V. Only their ratio G' / V is more or less constant. Because it is known that V_{DQ} and V_{eSPVV} are too high the step B calculation has corrected this once G' is free for selection by the model. It must be concluded that their erroneous measurement by single pool methods (if G is not measured) introduces the same error into the two pool model as if prior to the calculation V was not corrected for urea generation. The difference can be in the range of –8 to –12%. In table 4.2.1.3.2.b V from the single pool calculation is corrected. The correction is shown in brackets and it is clear that the single pool considerations without taking urea generation into account are not sufficient for a precise modelling. It is the same for the calculation according to step A.

	Step A	***Step B***	***eSPVV /Direct Quantification***
G' (mg/min)	17,29	15,79	
$V_{i,t0}$ (ml)	23428	21228	
$V_{e,t0}$ (ml)	19314	15614	
Total V (ml)	42742	36842	43175* (38494)
Mean urea clearance (ml/min)	167,95	146,91	149,31

Table 4.2.1.3.2.b: *Comparison of the values of those variables that have been varied in step A and B to the single pool model and V_{DQ} (*) of direct quantification. V_{DQ} can be regarded as too high because the urea generation has not been considered. Assuming the G' value of step A is correct, V_{DQ} would have the value in brackets. All values are selected to be typical for the data set.*

From table 4.2.1.3.2.b can also be seen that the dialysance KeCnBolus of step B has been found to be substantially lower than in step A calculation. This is a consequence from the lower urea distribution volume that has been modelled now and in consistency the previous considerations. The same decrease of urea in a smaller volume can be

achieved with a smaller clearance. The coincidence with the urea clearance reference value from the single pool model is significantly better. This is a second important reason to decide to use step B.

In summary it must be concluded that all parameters including the generation rate G' are necessary to establish a valid 2PUKM. All data shown here therefore are derived from a step B calculation.

4.2.1.3.2.1. 2PUKM Kt/V versus electrolytic Kt/V

Due to the lack of a follow up session the two pool analysis was only possible in 44 of 93 dialysis sessions (tab. 4.2.1.3.2.a). 7 of the 44 dialysis sessions were not accessible to analysis because according to E 2.6.8.a no unique minimum or no minimum at all was found within the range of plausible values. These sessions have been excluded from the evaluation. On the data base of the remaining 37 dialysis sessions Kt/V was compared, see figure 4.2.1.3.2.1a.

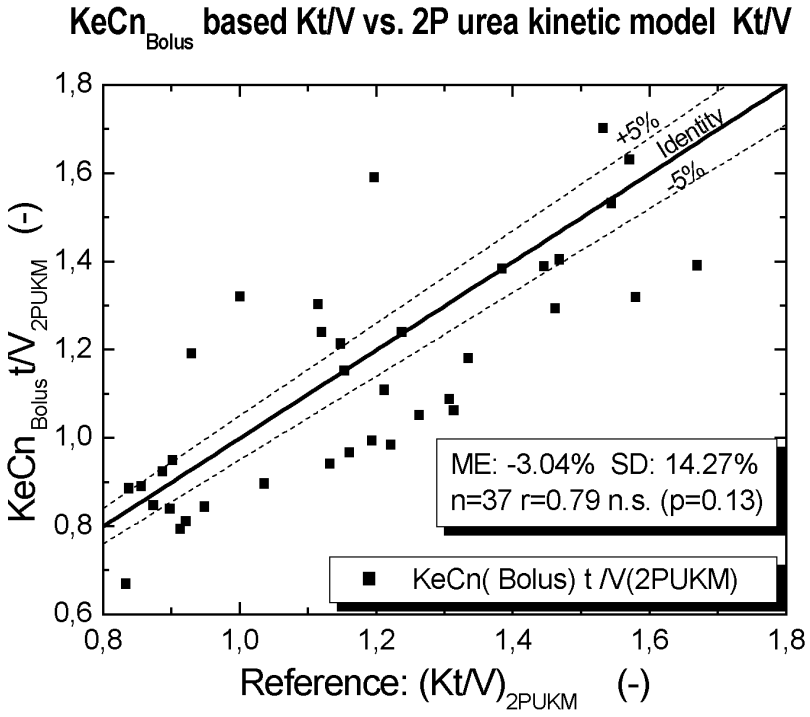


Fig. 4.2.1.3.2.1.a:*Comparison of the Kt/V as calculated purely on conductivity based K to the K from two pool kinetic model. V in both cases originates from two pool kinetic model and equals the sum V_i+V_e .7 of 44 measurements could not be evaluated because the fit algorithm did not find a minimum or the minimum was ambiguous*

Ordinate: Kt/V with K= $KeCn_{Bolus}$ as measured from conductivity by the 4008 dialysis machine and calculated according to E 2.4.4.h;V is fitted as V_i+V_e from 2PUKM step B.

Abscissa: Kt/V with K= KeUB as found from two pool modelling, step B, as to minimise E 2.6.8.a on basis of 6 known ECF urea concentrations (c_{t30} previous dialysis, c_{t0}, 2-3 concentrations during dialysis by blood samples, corrected for recirculation, c_{tend} and c_{t30} of present dialysis). V is fitted as V_i+V_e from 2PUKM step B and therefore is identical to V of the ordinate.

This means figure 4.2.1.3.2.1.a essentially is only a K-comparison according to the two pool model, but t and V are maintained to present comprehensible values.

The integral bolus clearance throughout a complete session at an average matches the urea clearance from the two pool urea kinetic model with (error±SD) –3.04±14.3% of difference. The difference was found to be not significantly different by student's t-test (p=0.13) although the SD again is in the same order as with the MI study.

4.2.2. In vitro volume measurements

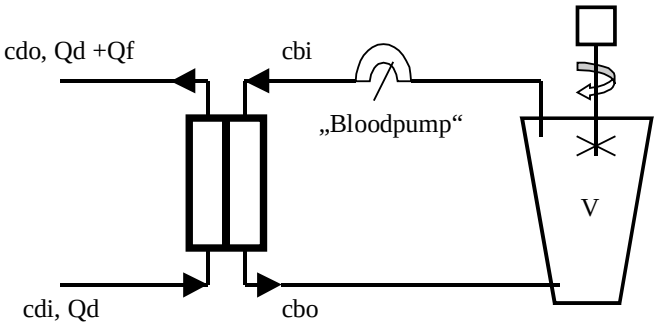


Figure 4.2.2.a: *The setup to analyse the deviation of the clearance values using different time index permutations. All data have been automatically recorded to a computer for analysis with the evaluation software. It is a standard dialysis configuration except that the blood has been replaced by dialysate and the artificial distribution volume was connected at opposite sides and stirred with a electric mixer. V was variated from 5L to 70 L. The dialyser was a HF 80 polysulfone type (Fresenius Medical Care). No ultrafiltration has been performed.*

Some introducing remarks about this analysis are necessary: On one hand there is doubt that urea and sodium distribution volume are equal under dialysis conditions because the distribution volume for sodium is not total body water as it is for urea. Contrary to urea the majority of sodium is confined to the ECF volume. On the other hand water can shift freely from ICF to ECF and vice versa. If a sodium bolus is applied to the vascular system via the dialysis filter the osmotic force is still effective at the ECF-ICF boundary. While sodium is confined to ECF by the sodium pump an equivalent of water may pass the membrane from ICF to ECF without restriction. This could be an argument that the virtual osmotic space that is visible to external dilutive analysis could be the same as for urea. This can not be decided on the poor data base of only this study, therefore it will not be subject of this investigation if urea and virtual sodium distribution volume are equal. These considerations should not prevent the analysis at all but it clearly focuses to its limitations. Nevertheless it would be a great improvement to dialysis quantification technique to also measure V

	KeCn01	KeCn02
KeCn12 used as reference (100%) **Qb=350ml/min** **Qd=800ml/min** Range: 261.8 – 335.2 ml /min	- 6.84 % ME 5.95 % SD -21.9 % Min 2.1 % Max n=38 Sign. diff. (p<0.0001) 0 Outlier 207.6 – 337.6 ml/min	16.93 % ME 14.45 % SD -4.7 % Min 53.7 % Max n=37 Sign. diff. (p<0.0001) 0 Outlier 291.1 – 472.0 ml/min

Table 4.2.2.a: *The electrolytical dialysances $KeCn_{01}$ and $KeCn_{02}$ are compared to the related $KeCn_{12}$ from the same step profile which has been used as the reference (=100%) because no reference from the "blood"-side of the filter is available. The mean error (ME), SD, the minimum and maximum error, the number n of measurements, the significance of the difference (students paired t-test), the range over which measurement values occurred are depicted in the right columns.*

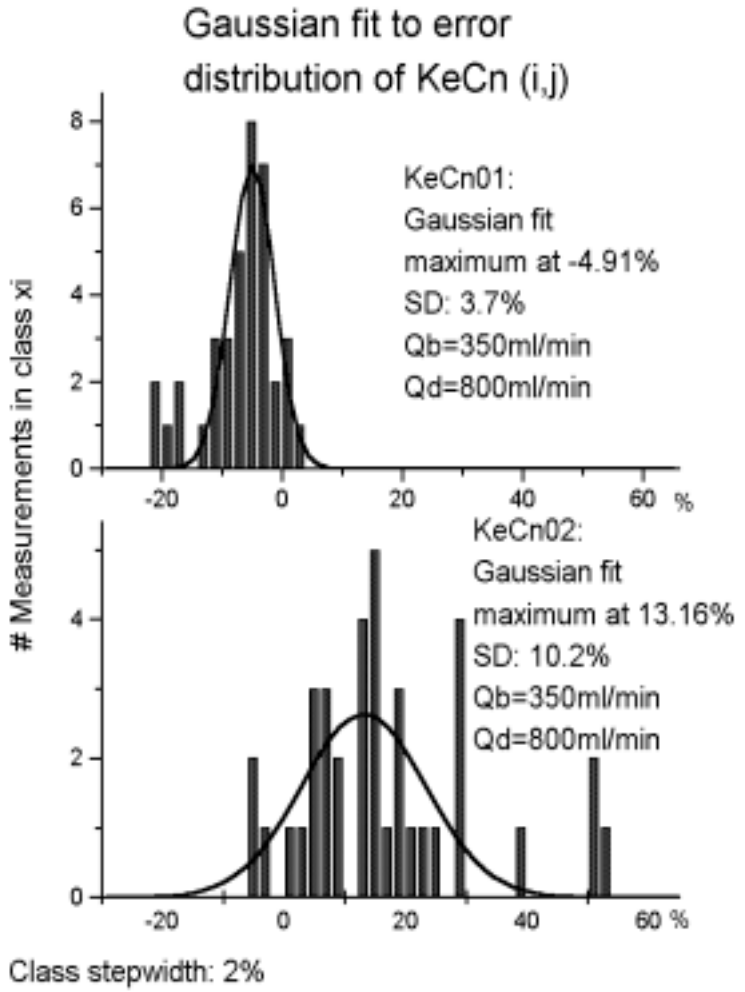


Figure 4.2.2.b: *The histograms show the error distribution of the in-vitro $KeCn_{01}$ (top) and and $KeCn_{02}$ (bottom) measurements in 2% classes with operation parameters of Qb=350ml/min, Qd=800ml/min. The 100% reference was the $KeCn_{12}$ from the same step profile. As an overlay a gaussian fit has been made to each data set. Exactly the data of table [4.2.2.a] have been included. At the maximum of the $KeCn_{02}$ gaussian fit the $KeCn_{01}$ fit has already decayed to less than 1% of its own maximum.*

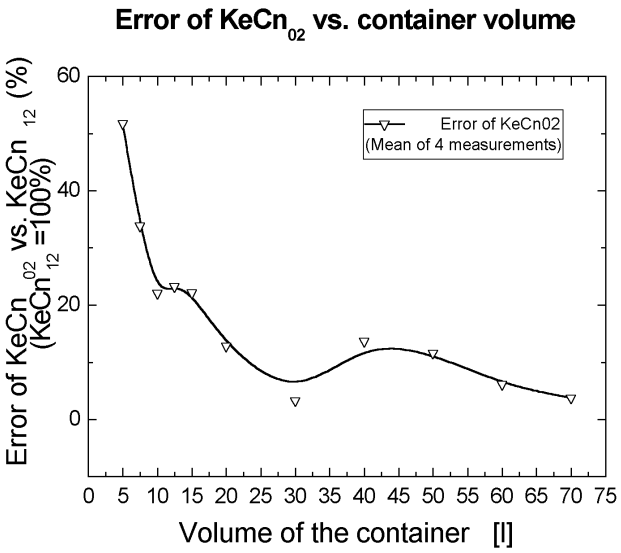


Figure 4.2.2.c: *The error (%) of $KeCn_{02}$ vs. KeCn12 (=100%) from the in-vitro measurements is plotted versus the volume of the container. The data points are connected by a spline function.*

with simple means like conductivity cells. Doing only some first steps even if at the end there is not a functioning principle would also be a challenge.

The in-vitro setup is depicted in figure 4.2.2.a. A standard dialysis configuration was used which was connected to a container with known volume of dialysate and a mixer to stir the container. The temperature of the fluid was maintained at 37°C.

The step profiles and the data recording has been performed exactly as in-vivo. The measurements have been automatically released by software using identical timing.

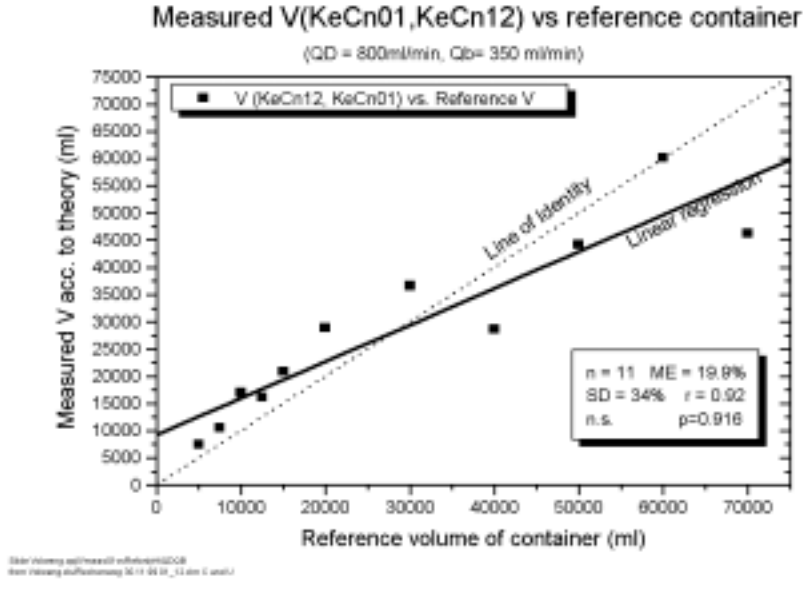


Figure 4.2.2.d:*The measured V according to E 2.5.3.j of all in-vitro dialysis sessions (n=11) with QD=800ml/min, "Qb"=350ml/min is compared to the distribution volume in the container (see figure 4.2.2.a) and therefore the reference does not essentially contribute to the error. Each measured value is a mean of four single measurements. The mean error ME of all errors of the measured V in comparison to its related reference, the standard deviation SD of the error and the correlation coefficient r of the two data sets is given in the graph. Student's t-test for paired data found p=0.916, the confidence interval for mean measured V was [76.3; 121.5]% of the mean reference.*

The data analysis software was identical and the same algorithms as used for the in-vivo clearance calculations have calculated the ionic dialysance permutations $KeCn_{01}$, $KeCn_{12}$, $KeCn_{02}$ in-vitro.

A total of 44 step profile dialysance measurements has been made. The volume of the container has been changed in 11 steps. It was subsequently filled with 5l, 7.5l, 10l, 12.5l, 15l, 20l – 70l in steps of 10l. To improve the resolution the absolute sodium transfer was increased in

comparison to normal dialysis by adjusting the operating parameters to 350ml/min 'blood flow', the dialysate flow at 800ml/min. The conductivity baseline of the step profiles were adjusted to the conductivity of the dialysate in the container (14.2 mS/cm). By lack of a reference it was decided to use $KeCn_{12}$, the high-low permutation ionic dialysance as the 100% reference, because it matches the urea clearance best in the in-vivo measurements. The difference to the related $KeCn_{01}$ and $KeCn_{02}$ are shown in table 4.2.2.a. The $KeCn_{01}$ dialysance values differ –6.8±6% (error±SD) from the related $KeCn_{12}$ values, the $KeCn_{02}$ results differ 16.9±14.5%. Again a higher SD for the $KeCn_{02}$ values is found. The highest error is found for small container volumes and the error is decreasing for higher values (figure 4.2.2.c). The high $KeCn_{02}$ error for small values is the explanation for the high SD. In particular the 1/x character of E 2.5.3 k is shown by the experiment here clearly if we look at the spline fit that has been overlaid.

At 40 of 44 measurements all three permutations $KeCn_{01}$, $KeCn_{12}$, $KeCn_{02}$ were available. 38 of the 40 measurements (95%) showed $KeCn_{01}<KeCn_{12}<KeCn_{02}$, the other 2 measurements (5%) showed only $KeCn_{01} < KeCn_{12}$. The distribution of the measurements relative to $KeCn_{12}$ is shown in figure 4.2.2.b. The $KeCn_{02}$ distribution again is broader like it has been found in the in-vivo measurements.

The clearance differences have been used to calculate the volume of the container according to E 2.5.3.j . The result is shown in figure 4.2.2.d. The mean error±SD of the ionic dialysance volume measurement was found to be 19.9±34%, the correlation was 0.92 and the data are not significantly different by student's t-test for paired data (p=0.916), thus with a 91% probability the hypothesis that the principle does not measure the volume of the container is wrong according to this test.

4.2.3. Urea Distribution Volume

The urea distribution volume that is an important part of the quantification parameter Kt/V has been analysed from the different aspects that have been described in chapter 2.5 and 2.6. The analysis should help to clarify the relation of the results from the different methods.
There are on one side the analytic methods to measure V: The comparison of the V values from direct quantification, equilibrated single pool model and two pool model can be understood as an analysis that is based on urea dilutive measurements, has commonly accepted theoretical basements and therefore is free of doubt about the functionality of the evaluation itself. The deviations of the results are due to measurement errors and systematic errors related to the simplifications that characterise the models.
This is different with the anthropometric methods and the conductivity based V measurement. The previous are purely empirical and therefore individual deviations may occur showing a statistical distribution due to a different population. The result of anthropometric formulas therefore can only be an indicator for the true urea distribution volume, not a measurement. The latter is a new, unpublished method that

has been found within this work. So on the one hand of course it can not yet be accepted by the nephrologist's community and must be subject of further investigation and improvement, on the other hand –even with its simplifications – it is based on a consistent theory and therefore has potential to become an accurate method for individual sodium distribution volume measurement without any empirical component. The results from all the methods will be compared here.

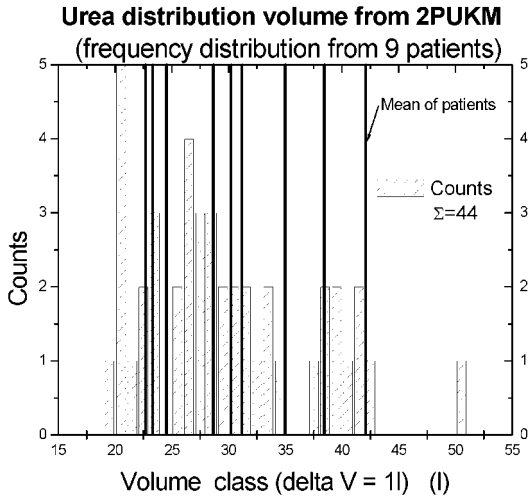
4.2.3.1. V from direct quantification, eSPVV and 2PUKM

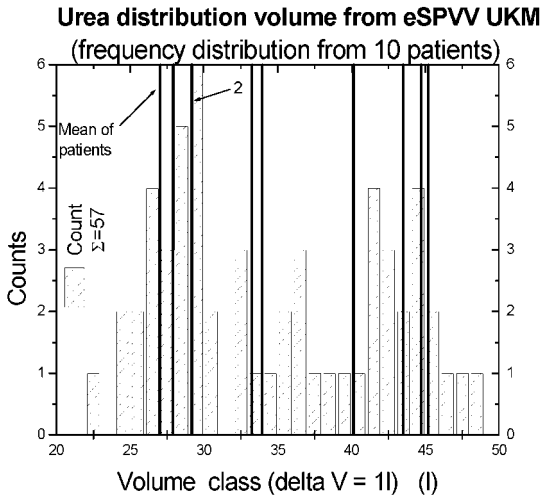
First the analytical methods are compared. They all are accumulative methods, thus they all can be characterised by the analysis of the urea concentration of a fluid pool measured at least at two different times. The pool is compared to a known amount of urea that has been transferred. In this sense it is a dilutive method. This allows to conclude what volume must be accessible to dilution. In an overview the number of patients and measurements that have been evaluated are depicted in table 4.2.3.1a:

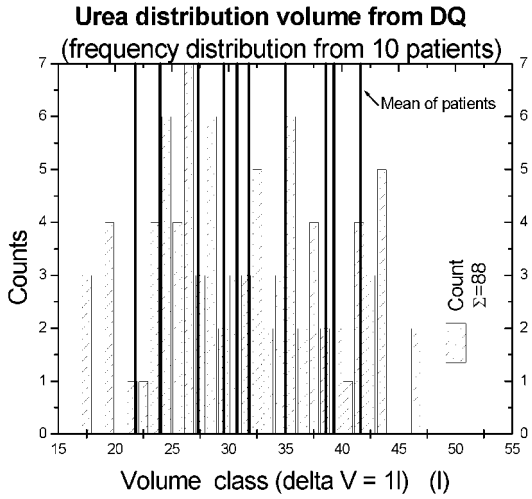
	V_{DQ}	V_{eSPVV}	V_{2PUKM}
# of patients	10	10	9
# of measurements	88	57	44
Mean value (ml)	31519	34838	29824
Mean single patient SD	3690	2599	4575
Total SD on all data	7516	7373	7440
Equation	E 2.5.1.a	E 2.6.1.d'	numerical fit

Tab. 4.2.3.1a: *The table gives an overview on number of participating patients, the number of measurements and the mean results that have been found using the different methods of urea distribution volume determination. The SD first has been calculated for every patient separately and averaged afterwards assuming that their urea distribution volume should be fairly constant during the study period. The total SD of the complete data of all patients is shown in the next line.*

As pointed out in chapters 2.5 and 2.6 both V_{DQ} and V_{eSPVV} tend to be too high in this analysis for reasons of urea generation neglect. The urea generation inclusion would reduce the mean urea distribution volume. Only in the two pool model the urea generation is correctly integrated.







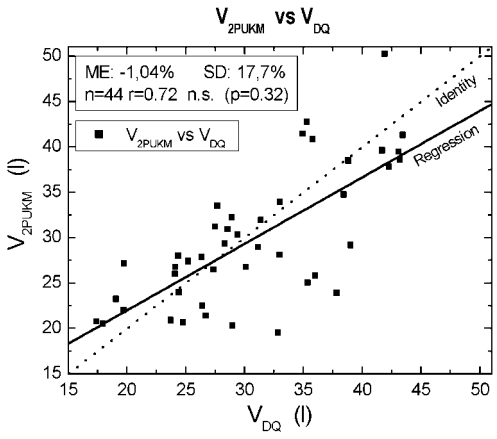
Figures 4.2.3.1.a-c: *The figures show the frequency distribution of the measured volume in classes of ⊿V=1l as calculated from 2PUKM (a), equilibrated single pool variable volume kinetic model (b) and direct quantification from the whole data set available. The vertical bars indicate the mean result from all measurements of a single patient.*

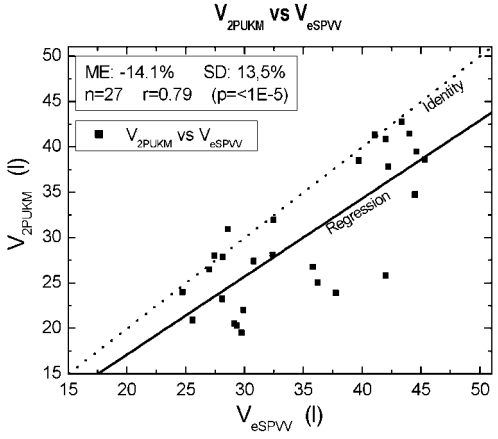
The frequency distribution of the results from the different methods are plotted in figures 4.2.3.1.a-c. In determining the absolute volume the congruence of 2PUKM and DQ visually appears to be good. Also student's t-test does not regard them to be significantly different (p=0.32). After calculation of the correlations (see table 4.2.3.1.b) DQ and eSPVV UKM however show better congruence, but the correlation tests only proportionality, not absolute values. It is apparent that the intra-patient SD is too high to resolve all individual patients from the entire data set because the patients do not differ sufficiently in their urea distribution volume and the standard deviations overlap. This is not only due to the measurement principle itself but also to the variation of the initial weight of patients when they start dialysis. The ideal condition to analyse the SD of a method would be to perform the measurement multiple times on the same subject under identical conditions. This can not be realised because the measurement takes the whole dialysis session and therefore can not be performed twice under the same conditions. Nevertheless it is possible to compare the three methods directly because they are based on the same dialysis sessions. This is shown in table 4.2.3.1.b which lists the direct comparison of the 10 patients using the three methods.

Pat	Mean of all (ml)	$V_{2PUKM} \pm$ SD; (ml)	Error to mean (ml)	%	$V_{eSPVV} \pm$ SD (ml)	Error to mean (ml)	%	$V_{DQ} \pm$ SD (ml)	Error to mean (ml)	%
1	24867	22716	2264	-8,6	27916	5433	12,3	23967	2676	-3,6
2	24062	23325	2987	-3,1	27053	1417	12,4	21810	3478	-9,4
3	42213	42118	940	-0,2	45212	1670	7,1	39309	4609	-6,9
4	41196	38456	6800	-6,6	43493	1708	5,6	41638	2823	1,1
5	29515	24529	3724	-16,9	33256	3377	12,7	30760	3734	4,2
6	29679	30235	2347	1,9	29202	2172	-1,6	29602	3141	-0,3
7	38261	35010	8242	-8,5	44731	3358	16,9	35041	6317	-8,4
8	28391	28652	3555	0,9	29169	1980	2,7	27352	2027	-3,7
9	36654	31200	(10313)	-14,9	40154	1619	9,6	38606	4174	5,3
10	32874				33958	3253	3,3	31790	3920	-3,3
corr.		- 0,87	- with	V_{eSPVV}	0,92	with	V_{DQ}	0,87	with	V_{2PUKM}
Mean			(4575) 3857	**Mean err: -6,2**		2599	**Mean err: 8,1**		3690	**Mean err: -2,5**

Table 4.2.3.1.b: *The table shows some details of the volume measurements using the 2PUKM, eSPVV and direct quantification. The data are listed for every individual patient who participated in the study. A three-method mean urea distribution volume has been calculated and the error of each method has been related to the mean urea distribution volume due to the lack of an absolute urea distribution volume reference. The outstanding SD value in brackets (column 4) seems to originate from an erroneous measurement and the related mean SD value including 10313ml has also been set in brackets.The correlation has been calculated from the mean volumes of the patients as shown in the columns 3,6,9 and not from the single data points as shown in figures 4.2.3.1.d-f.*

To give a further impression of the relation of the three methods a data pair comparison has been made. Every two methods have been compared directly, value by value to show the correlation.





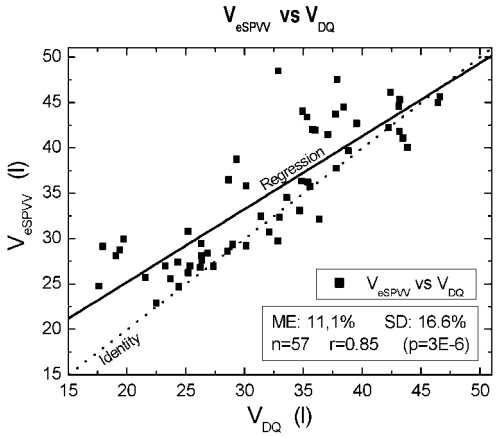


Fig 4.2.3.1.d-f
The figures show the comparison of the urea distribution volume data pairs as calculated by two pool urea kinetic model vs. direct quantification (d), 2PUKM vs equilibrated single pool variable volume urea kinetic model (e) and eSPVV vs DQ (f). The number of data points in (e) is reduced because only data pairs are plotted, thus both of the methods had to show a result for a particular dialysis session.

Discussion

The absolute differences of the methods are due to the principles itself: The single pool method, even though urea rebound is included, shows the highest values for the urea distribution volume because urea generation is neglected and the body itself is used as integration volume. This of course will hide some amount of urea from the observer because rebound effects from different internal compartments like fat in extremities, low vascularised tissue areas of diabetic patients and similar circumstances could delay the urea transfer for more than 30 min. 30 min is defined within this study as the time of observation for all 'after rebound' measurements. Without doubt rebound is not a well defined period that could be regarded to have terminated after a specific period in a particular patient. This may influence the result without knowledge of the observer and in dependence of the time the after-rebound sample has been drawn V is influenced to higher or lower values and virtually a function of time.

Using DQ the integrating volume is not the patient's body but an external container. This excludes the hidden presence of urea in compartments not accessible but still involves the body because the initial and final urea plasma water concentrations for the calculation according to E 2.5.1.a are based on blood samples. Therefore the same arguments as for the single pool model apply. Nevertheless this principle commonly is accepted as 'gold standard'. From a theoretical point of view the 2PUKM should be the most general attempt to measure V. The inclusion of G decreases the mean result compared to the previous methods.

Obviously the precision of the two pool urea kinetic model is slightly lower as found in the single pool or direct quantification method. The two pool method – although more elaborated in principle - is based on more different uncertain values in this analysis because the reference data for the urea concentration during dialysis have been measured with the blood-pump running and therefore have been corrected for recirculation (see chapter 2.6.8, E 2.2.1.b). In common with the general higher complexity of the model overall this may lead to a higher standard deviation compared to DQ or single pool models. Further the additional inclusion of urea generation is subject to errors and potentially can increase the standard deviation, on the other hand the inclusion of G is an indicator for higher mean absolute accuracy.

The fundamental problem of the in-vivo volume quantification is the lack of an absolute reference. To circumvent this problem the mean of all three methods has been adopted as reference, assuming that the measurement of a quantity with different methods should bring a more precise result than using only one. Using this 'reference' the method of direct quantification seems to reflect the mean value best, but without respect to urea generation. Concerning the lack of G inclusion the value from 2PUKM seems to better reflect the distribution volume. The volume from the single pool method appears to bee too high. In spite of the absolute deviations the SD qualifies the single pool method to be the most precise of them, at an average being only two thirds of that of the DQ or 2PUKM method. This further indicates that an essential part of the standard deviation of the DQ and 2PUKM method will be due to result variation of the method itself and not due to the patients weight variation, because other principles like the single pool method are able to decrease the SD to significantly lower values.

Also in the single data point comparison (figures 4.2.3.1.d-f) it appears that due to the higher number of involved variables and the complexity of the algorithm the 2PUKM shows the highest SD although it is not significantly higher (17.7% compared to 16.6%) than with a eSPVV / DQ comparison. This is also reflected by the t-test analysis that does not indicate a significant difference for the DQ and 2PUKM method. For none of the principles compared to each other the correlations in general are extreme high, even if the best data pair correlation of 0.85 is between eSPVV and DQ. This shows in general that the complete path to the result could be too complicated and that the model is subject to many input variations. In the case of 2PUKM further the numerical fit analysis that is sensitive to small statistical variations with finding a minimum increases the SD. The single pool model with urea generation neglect again tends to show a too high distribution volume.

A detailed analysis would need much more information not only on the initial and dry weight but also on the bodily condition of the patients like grade of obesity and fat-muscle relation. Further the principles 2PUKM and DQ are not very handy in the clinical situation. It depends on the intention what principle should be preferred. In terms of accuracy on base of this data a decision which principle is the best finally can not be made. DQ and 2PUKM seem to show a slightly better congruence in the absolute values but not in the correlation, but the difference to eSPVV does not really seem to be systematic. The problem remains: All the references appear to scatter in their results and a reliable V_{urea} measurement is still not found. Only a small advantage of the eSPVV model can be stated due to its simplicity.

4.2.3.2. V from anthropometric methods

In clinical practice anthropometric methods like described in chapter 2.5.2 are commonly used because they give an instant but empirical result on base of available data and a simple calculation. This could be advantageous to determine an individual Kt/V. Therefore a comparison of the dilutive and empirical anthropometric methods could be of interest to help the clinician with finding an subjective feeling how accurate the methods may work in clinical practice.

If anthropometric formulas are used at all predominantly the Watson formula E 2.5.2.a is used. Figures 4.2.3.2 a and b demonstrate the relation of the Watson formula results to the two pool modelling and direct quantification results.

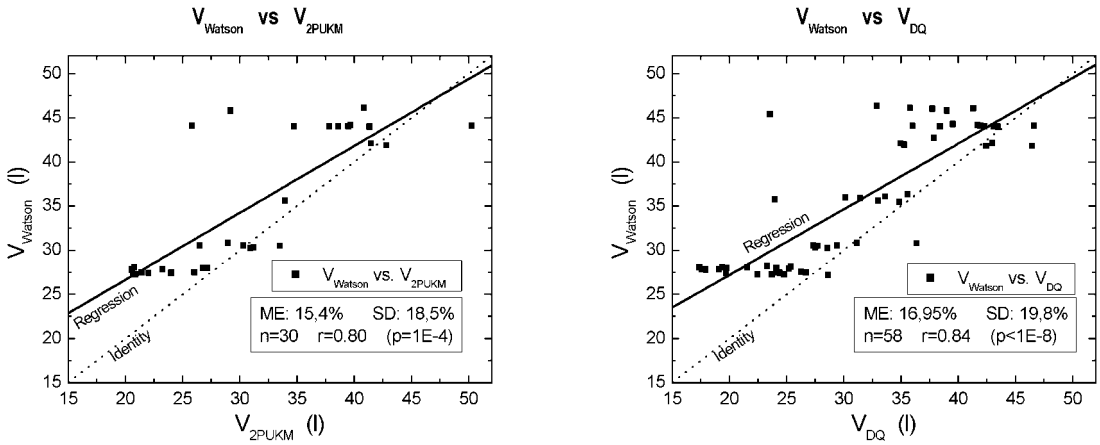


Fig 4.2.3.2.a,b:*The figures show the urea distribution volume calculated using the Watson formula E 2.5.2.a compared to the volume from 2PUKM (a) and direct quantification (b).*

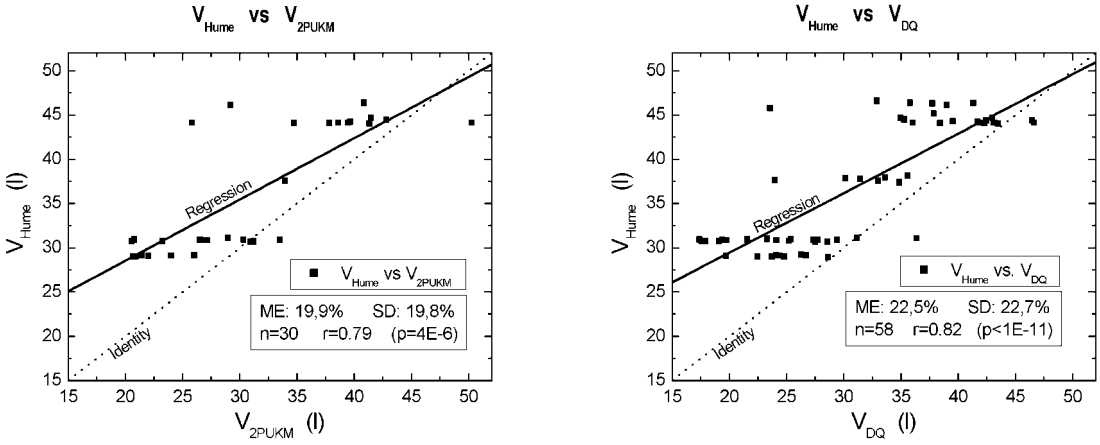


Fig 4.2.3.2.c,d:*The figures show the urea distribution volume calculated using the Hume formula E 2.5.2.b compared to the volume from 2PUKM (c) and direct quantification (d).*

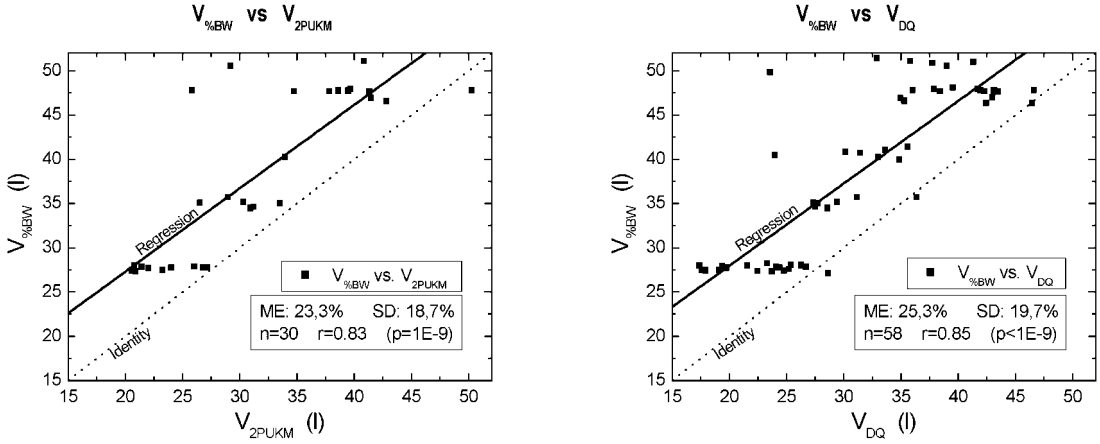


Fig 4.2.3.2.e,f :*The figures show the urea distribution volume calculated using the %body weight formula E 2.5.2.c compared to the volume from 2PUKM (e) and direct quantification (f).*

The same comparison is depicted in figures 4.2.3.2.c,d for the Hume formula and in figures 4.2.3.2.e,f for the % body weight formula.

The highest difference of all dilutive measurements shown in the figures above is 25,3% ($V_{\%BW}$ vs V_{DQ}). 10% but not more than 15% of this difference may be due to the neglect of urea generation.

The remaining error can only be attributed to

- the empirical character of the anthropometric formulas and patient individual deviations of the study collective from a typical population from which the formulas have been derived. It is noticeable that the empirical methods among themselves show a difference of approximately 8%.
- a wrong assumption on the urea generation rate. The 2PUKM assumes the urea generation rate constant during dialysis and equal to the inter-dialytic phase. This can be erroneous up to the factor 1,5 because urea generation may be increased to this rate during dialysis [Sar2]. This in consequence must lead to an significant error of the two pool model and the DQ.

As mentioned the dilutive methods in general show a high standard deviation. This seems to be their general property: Even if their mean result should be reliable due to the stringent method of quantifying mass it is not possible to derive a reliable result with less than 3-5% error from only a single measurement. Beside of their handling effort this makes both methods, direct urea quantification and 2PUKM not very useful in clinical practice. It seems these methods are more suitable for the scientific domain with interest in detailed questions.

Another indication that the DQ and 2PUKM methods themselves have an increased SD is that it is not found in a V_{eSPVV} comparison to the anthropometric methods. The knowledge of urea generation neglect is rising doubts in the absolute value of the V_{eSPVV} result, but an outstanding correlation of 0.94 in a paired data analysis is found, see figures 4.2.3.2.g,h.

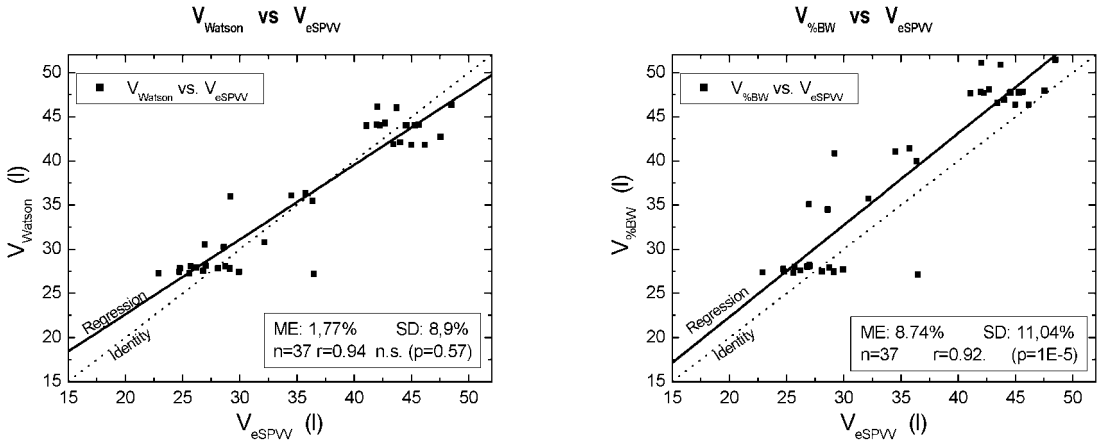


Fig 4.2.3.2.g,h: *The figures show the urea distribution volume calculated using the Watson formula (g) and the %body weight formula E 2.5.2.c (h) compared to the volume from equilibrated variable volume urea kinetic model. Urea generation has been neglected.*

In conclusion it seems appropriate to use the V_{eSPVV} value for analysis if urea generation is included. This is not possible in those cases when no data of a longer

period than only one dialysis session are available. If in the clinical situation it is desired to achieve a value for V without the need of blood samples it is recommended to use the Watson formula, but correcting it for some amount of approximately -12% to -14% due to urea generation neglect.

4.2.3.3. Conductivity based V measurements

With an identical setup and method as in-vitro chapter 4.2.2 the patient's urea distribution volume has been analysed. The data of all 10 patients of the MII study have been taken into account. An average of 8.5 dialysis sessions per patient have been analysed and averaged to find the mean result of the study period. The data shown for one patient is based on 85 single measurements (figure 4.2.3.3.a).

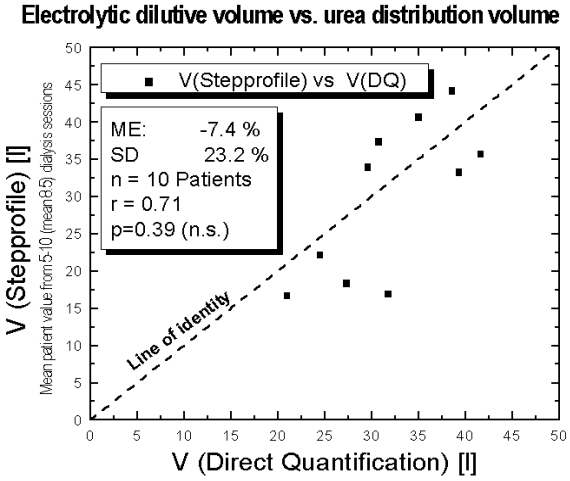


Fig. 4.2.3.3.a: *The in-vivo measured V using $KeCn_{01}$ and $KeCn_{12}$ according to E 2.5.3.j is compared to the distribution volume for urea as evaluated by the direct quantification method. Each measured value is a mean of all sessions per patient (mean 8.5). Both methods contribute significantly to the error. The mean error ME of all measurements, its standard deviation SD and the correlation coefficient r is given. Student's t-test for paired data did not find the data to be significantly different. The mean dialysate flow was 526ml/min, the mean blood flow was 213ml/min, the mean ultrafiltrate flow was 530ml/h.*

The mean error of the volume found by the electrolytical volume measurement was –7.4% with a SD of 23.2% compared to direct quantification. The correlation was 0.71 and the data were not significantly different. It is to remind here that the reference volume as measured using the direct quantification has been calculated under neglect of the urea generation rate. It should be some percent lower if the urea generation was included. This further decreases the mean difference.

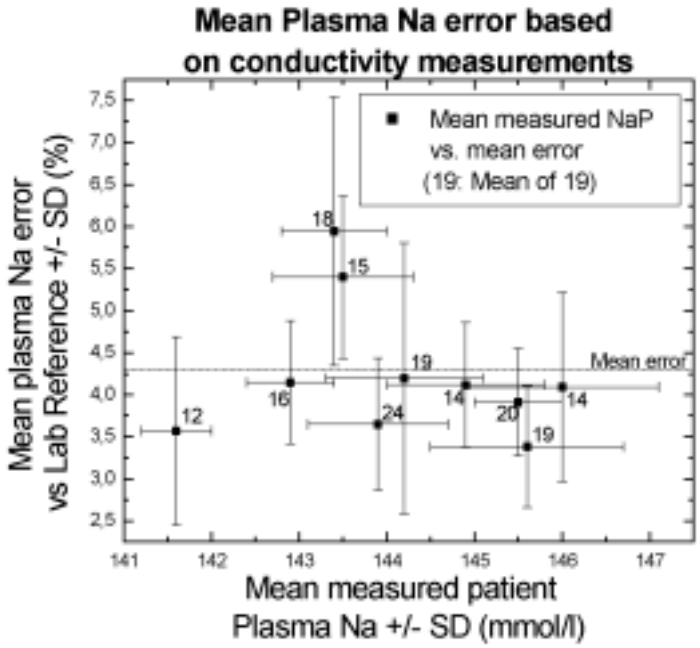


Figure 4.2.4.a: *The ionic dialysance $KeCn_{Bolus}$ has been used to calculate the plasma sodium according to [] E 2.7.a,b. The abscissa shows the absolute values as measured by the dialysis machine and their absolute SD. The centre of the crosses mark the average of n (number near centre) single measurements. The ordinate shows the relative error and SD (%) of the mean value vs. the laboratory reference. Each cross represents one patient. The mean of mean deviation of all measurements is 4.3%.*

4.2.4. Plasma Sodium

For analysis of plasma sodium measurements E 2.7.a,b has been used. The result is presented in figure 4.2.4.a. From every single bolus dialysance measurement a plasma sodium value has been derived. The plasma sodium values have been averaged patient by patient they originate from. The mean result and its SD is depicted in fig. 4.2.4.a, abscissa. Every cross represents one patient. The number near the cross centre indicates the number of the contributing single values to the mean. It is to be seen that some of the patients could clearly be distinguished from one another. Every single measurement has been related to its laboratory plasma sodium reference and the error (%) has been calculated. In the same patient related groups the errors have been averaged and their SD has been calculated. It is plotted along the ordinate of figure 4.2.4.a. This is the reason why the length of the cross bars of a single cross are not proportional: The x-bar indicates the intra patient deviation of the pure electrolytical principle as it can be performed on the dialysis machine alone without laboratory facilities. It is a measure for the repetitive accuracy and resolution, because it can be assumed that the patients tend to be stable in their plasma sodium concentration because their thirst mechanism still works. The y-bar indicates the variation of the error relative to laboratory result and therefore also includes the laboratories inaccuracy which is approximately 6%. This means the laboratory itself contributes substantially to the error and its SD. The mean of the mean errors is found to be (4.3±1.2SD)%.

It is encouraging to see it supported by these data that it is possible to measure the plasma sodium correctly with only 1.2% SD. In absolute numbers this is still 1.7mmol/l and therefore not totally satisfactory. The algorithm further has to be empirically corrected for the error of 4.3% to match the laboratories mean value. The formula E 2.7.a,b itself is empirical, so it is acceptable to apply the correction and adapt the algorithm to the technical environment is has been used in. Further the validity of the results should be confined to the dialysate composition as depicted in table 2.7.a.

Discussion

The manifold of different dialysis situations that may occur in the clinical practice can not be covered by these relative small number of plasma sodium measurements. In using a plasma sodium value that has been determined by conductivity measurements it is recommended to bear in mind that the measurement is not a measurement in the analytical sense. There is an empirical component involved that can be erroneous in the particular situation.

Before the principle can be accepted as a proven measurement many further investigations under different conditions are necessary. This should be clarified to a potential user. Other laboratories further may have other bias than the one involved in this study. All this may be responsible for a larger error and SD. But the result may encourage the clinician to use this principle and find a subjective relation of his own to the electrolytic result, the conventional laboratory result and the subjective feeling of the patients.

5. General discussion of the results

Ionic dialysance and urea clearance

It could be proven for the bolus principle that the differences between ionic dialysance and urea clearance are less than 3% and a further resolution with the methods of this study is not possible. The analysis of the data has proven the high correlation of urea and electrolytical dialysance similar to that already demonstrated by other authors. Most of these studies were dated in the second half of the 90's. Although the total number of published ionic clearance measurements meanwhile is far above 1000, these studies always lacked in comparability. Some authors did not clearly report about the mathematical expressions or the methods they have used. Others did, but the expressions were not identical to those of comparable studies. Further a two pool modelling on numerous data with substantial statistical relevance has not been reported. To the knowledge of the author until autumn 2001 no other survey that overviews more than 1300 electrolytical measurements (step profile and bolus) that all have been recorded under identical conditions, with identical methodology and strict timing has been published.

The major goal of the MI study was the approval of the step profile. This approval was not completely possible due to urea distribution volume dependence of the step profiles and because the three possible permutations of baseline-high, baseline-low and high-low state did not give identical results. Although it was possible to find excellent results using the step profiles in MI study – the mean error of KeCn12 compared to KeUB was found to be only –0.46± 4.65% and the integral mean error of ionic dialysance versus eSPVV UKM was 2.88±4.15% - the inconsistency is not satisfactory. Further in the second study the result of the step function was –4.4±3.19% and therefore not totally congruent. The difference seems to be more than purely statistical and can be due to small differences in the balance the profiles of MI and MII had. This was possible because the machines were not the identical ones, only the software for step function control was identical. As it can be seen from tables 4.1.1.1.d and 4.2.1.1.c the net diffusive sodium transfer was not identical in the studies. It was higher in MII study. Some improvements to stabilise the baseline can have a weak influence on the balance. This emphasises the impression that the step profiles are sensitive to sodium transfer and the machines must be very carefully calibrated to achieve accurate results. In this sense it must be accepted that the central goal of the first study has not completely been reached. This is the reason the second study has been launched. On the other hand it intensified understanding and finally resulted in a better principle:

The bolus principle

The sodium load effect intensified the search for a dynamic principle which would work with a smaller amount of sodium transfer. This led the eye to the tools engineers use for system analysis and opened the door to a more theoretical approach using transient spikes. A natural caution will always prevent the medical

physicist from transferring technical solutions that work in machines or electronic circuitry directly to humans.

Therefore it is very encouraging that it could be demonstrated that after application of an arbitrary dynamic signal like a composite sinuous or bolus shape, a mathematical algorithm (e.g. Laplace's transform) that determines the transfer function g(t) of a system can supply all necessary information for calculating the ionic dialysance without requiring stability. This allows to confine measurement related sodium transfer to the usually high technical resolution of a sensor system and not to the recommendations of stability. The information of dialysance is provided long before the system becomes stable at a new dialysate concentration and additional duration of the profile does nothing more than vary the patient's plasma sodium and therefore falsifies the result. Moreover the use of g(t) may allow to measure the dialysance truly continuous since transfer functions can be calculated permanently and online during arbitrary conductivity variations, although this is a high burden for a dialysis machine's central processing unit.

The direct comparison of momentary ionic dialysance measurements using short, non-stable boli with subsequent calculation of the transfer function shows lower absolute errors in comparison to step profiles with stable states, once the boli are performed technically correct. Furthermore, the accuracy of stable step profiles shows stronger dependence of the unknown urea distribution volume than boli type profiles: The smaller the patient the less accurate the result when using step profiles. Only a symmetric combination of full positive and negative step profiles with averaging of the result may cancel the error partially due to inverse loading. Conductivity boli hardly showed this dependence. The direct comparison of momentary measurements in the second study when the boli have been performed in a technically correct way showed a nearly vanishing difference of 0.06±4.76% without limitation of MBE. Expressed in terms of Kt/V after integration on the whole treatment the boli measurements seem to have a mean difference of +5.3% (table 4.2.1.3.1.a) compared to the urea reference from eSPVVUKM, which includes rebound. The reason is the neglect of the urea generation rate which plays no role in single measurement comparisons but in integrated comparisons: it reduces the reference Kt/V and it was expected that the remaining inconsistency of boli measurements compared to integrated references will diminish on taking intra dialysis urea generation into account. This expectation has been confirmed by both study data sets using the 2PUKM, where the urea generation rate is comprised. The integrated bolus based Kt/V in a mean differed –2.68% from the 2PUKM, in MII the difference was -3.04%. Both results are within or at the limit of the resolution of the analysis. The remaining difference can not regarded to be systematic.

Two pool urea kinetic modelling

Nevertheless the usually high standard deviations the two pool urea kinetic modelling shows in this study reflects its complexity. The complexity is both due to the model structure and due to the effort in handling. In general it can be assumed that two pool modelling describes the real situation more precise, because it is sure that there are at least two main pools present in the patient. Further not all tissue areas are equally supplied with blood and a local disequilibrium may be formed that acts as a further

urea pool. This in particular could be the case in patients with severe vascular dysfunction. Diabetes patients with decreased blood flow rates in the distal legs are an example, where the additional pool may not be negligible. These considerations can make it appear appropriate to add further small pools to the model. The complexity would further be increased and the model would loose practicability completely. This is only one side of the coin. The other side is the handling. Even if the model includes only two pools it is a high effort to establish the urea reference concentration for the ECF and ICF. To image the time course of the ECF concentration during the period from the previous to the end of the present dialysis it takes at least 6 blood samples and more would even be better. The rebound phase would further take 2-3 additional blood samples because the shape of the rebound is most important to model the inter-compartment clearance and therefore find a value for ICF urea concentration, which is not accessible from outside directly. To correctly integrate the residual clearance also all of the patients urine from the interdialytic phase must be sampled.

This overall would burden the patient to an unacceptable extend. It is clear that two pool urea kinetic modelling – in particular in combination with permanent data recording during dialysis like in this study - is only of interest for single investigation purposes of scientific character.

Sodium shift and electrolytic volume measurement

The primary goal of these studies was not to find a principle to measure V, and even if this is an important result it remains a side result. The difference of 2-5% for momentary measurements that is reported in several studies in the past has mainly been attributed to differences in the diffusion coefficients of urea and sodium, to different membrane effects or to inadequate correction of ultrafiltration. Also CPR has been made responsible (see chapter 2.2.1 and 2.4.1). The effect of sodium load or unload to the whole body water when performing the conductivity variations has erroneously been neglected. It could be demonstrated that sodium transfer via the membrane is the predominant reason for the inaccuracy of the electrolytical dialysance measurement and even a dilutive distribution volume measurement is considerable.

Further it was demonstrated that different types of conductivity variations - each with specific sodium loads – differ in their results under the same conditions. A major criterion achieving accurate results is to keep the transferred amount of sodium comparably small in relation to the sodium present in the total sodium distribution volume of the patient. The sodium load to measure the ionic dialysance should not exceed approximately 0.05mmol/10l of urea distribution volume. If this is sufficiently fulfilled, the contribution of all other effects that could be responsible for inaccuracy are of remarkably smaller magnitude. Some authors may have partially violated this condition in applying step-shaped variation of inlet conductivity lasting until the patient-machine system has become stable. As the data clearly show in-vitro and also indicate in-vivo the sodium transfer during this period seems to be diluted in at least large fractions of body water: The transfer is sufficient to allow a distribution volume measurement (r=0.92) in-vitro and even a good estimation in-vivo. A correlation of r= 0.71 to the patients urea distribution volume was found at an

average of 8.5 dialysis sessions. Although the correlation only tests for linearity, the absolute difference of –7.1% is striking. This had not been expected following the simplified linear mathematical model which does not take into account the inequality of sodium and urea distribution spaces and the exponential behaviour of the system. Moreover recirculation was not introduced as well as urea generation and the physiological sodium transfer properties of living cells. Using E 2.5.3.j based on a linear model does not seem to be sufficient to measure V with low SD precisely but seems to describe correctly the quality and roughly the quantity of the underlying sodium transfer effect. A more sophisticated model including the correct differential equation with respect to recirculation should be more appropriate but is still under development and was not applied within this work. On this background the in-vivo correlation as well as the absolute error is remarkable low and indicates that cardiopulmonary recirculation can not be responsible for the dialysance measurement error alone, because CPR is not proportional to urea distribution volume. The result shows that the whole fluid fraction of the patient seems to be involved in sodium transfer and in that sense it could be understood as a kind of ‘whole body recirculation’. Intracellular water also seems to partially participate in spite of the majority of the sodium ions being confined to ECF, because the concentration variations generated by the measurement can not be explained by ECF alone. This could be an argument for a sodium load/unload equivalent osmotic transmembrane water shift, so that the virtual, dilutively visible sodium distribution volume would match urea distribution volume or at least be proportional. If this is true, larger molecules should also be removed from the cells by osmotic convection initiated by an oscillating sodium load/unload sequence and a convenient push-pull combination of sodium and ultrafiltration profiles. Further this would allow a true measurement of the urea distribution volume, thus not only K but actually Kt/V could be measured electrolytically.

6. Conclusions

Within the three percent resolution of this investigation conductivity based measurements of dialysance and Kt/V equal the related urea based values if the measurement is performed in a technically correct way. Within this limits it can be assumed that urea clearance and ionic dialysance are equivalent under typical dialysis conditions and can be regarded as a substitute for each other. If no absolute urea mass transfer information is desired, there is no need to involve a laboratory to measure Kt/V.

According to this study the information on V can be acquired with sufficient accuracy using Watson's formula but decreasing the result for 12-14% because the urea generation rate seems not to be included correctly in Watson's formula. This should be done with great care because a V reduction increases Kt/V and bears the risk of under-dialysis if it is used for dose prescription. The careful clinician will frequently control the prescription using laboratory values. Only after a longer period of own experience it can be recommended to use conductivity based results for prescription purposes, but never exclusively.

The same recommendation is made for plasma sodium measurements. In this case the measurement also comprises an empirical component, the translation from measured conductivities to sodium concentrations. This does not mean that the values should not be included in own considerations but it means they should be used very critical. A patient that is strongly deviating from his usual plasma sodium condition when starting dialysis will clearly be detected. Plasma sodium variations of only 1.5 mmol/l will not clearly be identified, the method will show valid results only at derivations above 2 mmol/l .

The predominating reason for inaccuracy of clearance measurements is sodium transfer into large fractions of body water during the measurement. This conclusion is based on these findings:

- a relation $KeCn_{01} < KeCn_{12} < KeCn_{02}$ in accordance with theory exists.
- the theory based on the assumption that sodium is transferred is able to measure quite accurate in-vitro the volume of a container whose volume is absolutely known. This also shows the simplifications are acceptable and the theory is not over-stressed.
- in-vivo with a real patient whom we hesitate to simply regard as a container we have also found a noteworthy correlation of 0.71 with measuring the urea distribution volume by means of sodium step profiles (figure 4.2.3.3.a). This is found versus a reference which itself is not perfect (direct quantification).
- the error of sodium step profiles is volume dependent. Higher sodium transfer relative to V_{DQ} reduces accuracy .

The conclusion is to confine the sodium transfer necessary to measure the dialysance to the technical limits of the system resolution and to avoid prolonged periods of sodium transfer like those generated by steady state profiles. This can be

achieved especially using small conductivity variations such as boli or continuous composite sinuous shapes instead of sustaining stepwise variations. If it is necessary to use stepwise variations, a mean dialysance result of symmetric up and down step-profiles should be used to better cancel their inverse errors. A violation of the principle of sodium transfer reduction may result in urea distribution volume related errors of the dialysance measurement.

Electrolytic sodium distribution volume analysis clearly proves the influence of sodium transfer during step-profiles by showing plausible results according to the theory of dilution. It encourages further development of the principle of dilutive volume measurements to a valid method to determine sodium distribution volume. It is beyond the scope of this work to discuss if the virtual sodium distribution volume nearly equals urea distribution volume or at least is proportional, as the data might indicate. The question if and why we see urea distribution volume diluting with sodium must be addressed in the future and can not be decided by only the few data of a single study. A true conductivity based Kt/V(Urea) measurement including V must be the technical goal.

7. Prospect

The findings of this study have been processed to develop an algorithm that is working within the software of Fresenius Medical Care 4008 and 4008B dialysis machines. This algorithm is suited to measure $KeCn_{Bolus}$, t and plasma sodium according to the principles that are explained in the theoretical part of this work and to the same methods as used in the practical part. Also other companies have made strong efforts to implement a similar solution comprising the use of conductivity step functions ($KeCn_{12}$) into their dialysis machines in the last decade. This may demonstrate that there is a strong demand for automated, reliable dialysis quantification. Too many patients have suffered from poor dialysis outcome. A striking argument for better dialysis is found in the USRDS annual data report 1999 that still has reported for 1997 US ESRD patients a remaining life time expectancy of only 5-7 years if they are in the age of 50-54 years compared to 23-31 years averaged for the US population of the same age. Even if the situation is different in Europe this is a number that can not be accepted and overcoming this situation is a challenge to all who are involved in dialysis care.

The discussion about the value of urea based Kt/V is still controversy. In spite of all controversies and different schedules of dialysis there is no doubt that Kt/V in an intermittent 3x4h dialysis should not fall below approximately 1.2 - 1.3 [Go1, Hak, Low2, Par, DOQI] per session. If it does the consequences for the patients morbidity and their quality of life are evident.

In the past it was not possible to control the ‘dose’ of dialysis continuously. The burden to the patients and the additional costs for laboratory and staff prevents to draw blood samples for urea or creatinine analysis every dialysis treatment. Only in intensive care and hospitalised environment it is usual to control the efficiency of dialysis day by day or week by week. In all other dialysis facilities the regular control usually is performed in longer periods. But every single dialysis that has not completely been successful is accumulated and must be avoided to guarantee an optimum of care and quality of life to the patients. The conductivity based ‘dose’ control now gives a tool to the hand of the physician that does not take any additional effort or cost and therefore allows to continuously supervise the efficiency of dialysis. If this tool is consequently used the failure rate of dialysis due to often reused, wrong type or occluded filters, to increased recirculation, wrong access positioning or operation parameters like blood flow are past now. The ability of the system to measure the plasma sodium of the patients is a support for the clinician to determine if a patient is in a different fluid or osmotic balance and needs additional care or different prescription. These improvements indeed can be regarded as a step to better dialysis.

These humanitarian considerations are one aspect. Another aspect is that dialysis nowadays not at least is a growing matter of commerce since approximately $0.5\,^{o}/_{oo}$ of humans in the industrialised nations are ESRD patients. This means the monetary and social costs of dialysis must be kept under control in times the health insurance systems are charged to their limits. A cost neutral, permanent quality control of dialysis -which is possible with the technique described in this work- is a small but not

negligible contribution to a general good clinical outcome of numerous patients. Used correctly it can help to reduce ESRD related hospitalisation and follow up costs.

The results of this work meanwhile are implemented into commonly available dialysis machines and the experience of the first time shows that the principle works accurate and is well accepted by the clinicians, even if there is still enough space for further developments in this field.

In this sense the author wishes that many clinicians consider the advantages of the electrolytical dialysance measurement and are open to integrate it into their therapy if they are convinced it is useful for their patients.

8. Equations and Abbreviations

Dialysance and direct quantification:

$$MBE = \frac{\text{Dialysate side urea quantity - Blood side urea quantity}}{\text{(Dialysate side urea quantity + Blood side urea quantity)/2}}[\%]$$

$$KeCn_{Bolus} = \left(1 - \int g(t)dt'\right)\left(Qd_t + Qf_t\right)$$

$$KeCn_{ij} = \left(1 - \frac{cdo_i - cdo_j}{cdi_i - cdi_j}\right)\left(Qd_t + Qf_t\right)$$

$$KeUB_{ti} = \frac{(1-R)(U_{PWA,ti}\ Qe_{ti} - U_{PWV,ti}(Qe_{ti} - Qf_{ti}))}{U_{PWA,ti} - RU_{PWV,ti}}$$

with $Qe = Qb\left[FP - hct/100(FP - PWF)\right]$ and $FP = 1 - 0.0107\,TP$

$$KeUD_{ti} = \frac{(1-R)U_{DO,ti}(Qd_{ti} + Qf_{ti})}{U_{PWA,ti} - RU_{PWV,ti}}$$

$$V_{DQ} = \frac{M_{Urea} - Qf\ t_{Dial}U_{PW,t30}}{U_{PW,t0} - U_{PW,t30}}$$

$$K_{DQ} = \frac{M_{Urea}\ln\frac{U_{PW,t30}}{U_{PW,t0}}}{f_{corr}(U_{PW,t30} - U_{PW,t0})t_{Dial}} \quad \text{with } f_{corr} = 1\text{-}\frac{t_{Bypass}}{t_{Dial}}$$

$$\left[\frac{Kt}{V}\right]_{DQ} = \frac{K_{DQ}\,f_{corr}\,t_{Dial}}{V_{DQ}}$$

Kinetic model:

$$V_{eSPVV} = \frac{Qf\ t_{Dial}}{1 - \left(\frac{U_{PW,t30}}{U_{PW,t0}}\right)^{\frac{Qf}{Qf - KeUB}}}$$

$$KeUB_{eSPVV} = \frac{V_{eSPVV}U_{PW,t0} - (V_{eSPVV} - Qf\ t_{Dial})U_{PW,t30}}{(U_{PW,t30} - U_{PW,t0})t_{Dial}}\ \ln\frac{U_{PW,t30}}{U_{PW,t0}}$$

$$\left[\frac{Kt}{V}\right]_{SP} = \ln\left(\frac{U_{t0}}{U_{tEnd}}\right)$$

$$\left[\frac{Kt}{V}\right]_{eSPVV} = \frac{KeUB_{eSPVV}\ t_{Dial}}{V_{eSPVV}}$$

$$\left[\frac{Kt}{V}\right]_{\text{Daugirdas 2nd gen}} = -\ln\left(\frac{U_{t0}}{U_{tEnd}} - 0.008\,f_{corr}t_{Dial}\right) + \left(4 - 3.5\frac{U_{t0}}{U_{tEnd}}\right)\frac{\text{total Ultrafiltrate}}{\text{initial patient weight}}$$

Electrolytic Kt/V:

$$Kt = \int_{t0}^{tend} KeCn(t)dt$$

with $KeCn(t) = 0$ during bypass, alarm;
blood - and dialysate flow constant

α_D	Donnan-Factor: concentration ratio of ions at equilibrium at both sides of the filter membrane
cbi, cbo	Blood inlet (i) concentration, Blood outlet (o) concentration
cb, cd	General : concentration in blood, concentration in dialysate. Occasionally also conductivity in blood, dialysate.
Cdi_I	Conductivity of inlet dialysate (with index: at time I). Sometimes also more general used not for conductivity but for concentration of any agent.
Cdo_J	Conductivity of outlet dialysate (with index: at time J) Sometimes also more general used not for conductivity but for concentration of any agent.
D	Diffusive dialysance (without ultrafiltration)
DQ	Refers to direct quantification of urea by dialysate sampling
eSPVV	Refers to equilibrated **S**ingle **P**ool **V**ariable **V**olume urea kinetic model
eSPVVUKM	
FP	Fraction of protein
g, g(t)	(time dependent) conductivity transfer-function of patient / machine system
G, G(t), G'(t)	Generation rate, Generation rate as a function of time, (virtual) generation rate reduced for residual clearance
K	Clearance / dialysance in general
$KeUB_{ti}$	Bloodside effective urea clearance at time i
$KeCN_{I,J}$	Ionic dialysance using cdi_I, cdi_J, cdo_I,cdo_J
$KeCn_{Bolus}$	Ionic dialysance using the bolus principle
J_B, J_D	Bloodside and Dialysate side mass flux at the filter
MBE	Mass balance error
ME	Mean error
M_{Urea}	Total mass of urea in drain dialysate
OCM	Online clearance measurement
PWF	Plasma water fraction, set to 0.8 in this study
Q_d	Dialysate flow rate
Q_b,Q_e	Total, effective bloodflow rate
Q_f	Ultrafiltration rate
R	Recirculation ratio
S	Skewness of a distribution or population
SD	Standard deviation
SP	Refers to **S**ingle **P**ool urea kinetic model
systemic	Used as an index that indicates that a variable (concentration, flow...) refers to systemic blood
t_0	Time at start of dialysis
t_{30}	Time 30 min after termination of dialysis
t, t_{Dial}, t_{Bypass}	Time in general, time of dialysis duration, time of alarms or filter in bypass
t_{End}	Time at the end of dialysis
t_f	Mean flow time of dialysate from inlet to outlet conductivity cell
t_i	Refers to a particular time i
TP	Total protein
$U_{DO,ti}$	Urea concentration of filter outlet dialysate
$U_{PWA,ti}$	Plasma water urea concentration at arterial inlet of dialyser at time i
$U_{PW,t0}$, $U_{PW,tEnd}$, $U_{PW,t30}$	Urea concentration of plasma water at the beginning / at the end / 30 min after termination of dialysis.
$U_{PWV,ti}$	Plasma water urea concentration at venous outlet of dialyser at time i
V	Urea distribution volume in general or
V_{DQ}	according to Direct Quantification
V_{eSPVV}	according to equilibrated single pool variable volume urea kinetic model
V_{2PUKM}	according to Two pool urea kinetic model

9. Literature

[Abram] Abramowitz M, Stegun IA. Handbook of mathematical functions. Dover Publications Inc., New York 9th ed. (1970), p.1020

[Ba] Barth RH: Urea modeling and Kt/V: A critical appraisal. Kidney Int 43 (1993) suppl 41: 252-260

[Bell] Bell RL, Curtis FK, Babb AL. Analog simulation of the patient- artificial kidney system. Trans Am Soc Artif Intern Organs (1965) 11:183

[Bla] Blake P, Daugirdas J. Quantification and prescription, general principles. In: Jacobs C, Kjellstrand KM, Koch KM, Winchester JF (eds.). Replacement of renal function by dialysis. Kluver Academic Publishers, Dordrecht/Boston/London: (1996) : 619-659

[Bo1] Borah MF, Schoenfeld PY, Gotch FA, Sargent JA, Wolfson M, Humphreys MH. Nitrogen banlance during intermittent dialysis therapy of uremia. Kidney Int (1978)14: 491

[Bron] Bronstein Semendjajew, Taschenbuch der Mathematik. B.G.Teubner Verlagsgesellschaft, Leipzig, Moscow (1979)

[Brow] Brown KM, Dennis JE. Derivative-free analogues of the Levenberg-Marquardt and Gauss Algorithms for nonlinear squares approximations. Numer. Math. (1972)18 :289-97

[Broy1] Broyden CG. A class of methods for solving non-linear simultaneous equations. Math. Comp.(1965) 19: 577-93

[Broy2] Broyden CG. The convergence of a class of double-rank minimization algorithms Parts I and II. J.I.M.A. (1970) 6 : 76-90

[Church] Churchill RV. Operational Mathematics. Mc Graw Hill (1958), 2nd ed.

[Coh] Cohen H. Mathematics for scientists and engineers. Chpt. 4.3. Prentice Hall (1992)

[Daugd] Daugirdas JT. Second generation logarithmic estimates of single pool variable volume Kt/V: An analysis of error. J.Am.Soc.Nephrol. (1993) 4: 1205-13

[Dep1] Depner TA, Keshaviah PR et al. Multicenter clinical validation of an on-line monitor of dialysis adequacy. J Am Soc Neprol (1996) 7: 464-471

[Dep2] Depner TA. Prescribing hemodialysis: A guide to urea modelling. Kluwer Academic Publishers; Boston, Dordrecht, London (1994), p265 , G5

[Dep3] Depner TA, Cheer AY. Modelling urea kinetics with two vs three BUN measurements: A critical comparison. Trans Am Soc Artif Intern Organs (1989) 35: 499-502

[DF1] Di Filippo S, Goldau R, Manzoni C, Spickermann R, Marcelli D, Pontoriero G, Orlandini G, Locatelli F. Effective urea clearance and ionic dialysance by conductivity: What is the relationship ? JASN (1998) 9: 299A (abstract)

[DF2] Di Filippo S et al. How to determine ionic dialysance for the online assessment of delivered dialysis dose. Kidney Int (2001) 59:774-782

[DF3] Di Filippo, Goldau R, Manzoni C, Spickermann R, Marcelli D, Pontoriero G, Orlandini G, Locatelli F. Accurate and inexpensive on-line monitoring of hemodialysis performance. JASN (1998); 9: 299A

[DF4] Di Filippo S, Andrulli S, Manzoni C, Corti M, Locatelli F. On-line assessment of delivered dialysis. Kidney Int (1998) 54:263-267

[Diet] Dieter K. Medizinische und verfahrenstechnische Aspekte beim Bau und Betrieb künstlicher Nieren. Chemie-Ing.-Techn. (1972) 24: 1333-376

[DOQI] NKF-DOQI clinical practice guidelines for hemodialysis adequacy. National Kidney Foundation. Am J Kidney Dis (1997) 30 (2): 15-66

[Fa1] Farrel PC, Hone PW. Dialysis induced catabolism. Am J Clin Nutr(1980) 33: 1417

[Fi] Fisher RA, Yates F. Statistical tables, Oliver & Boyd, London

[Fro] Frost TH, Kerr D. Kinetics of hemodialysis: A theoretical study of the removal of solutes in chronic renal failure compared to normal health. Kidney Int (1980) 18:2-10.

[Gil] Gilles R. Mechanisms of volume regulation and control of the level of intracellular osmotic effectors. J Parent Ent Nutr (1980) 4:121-130.

[Gior1] Pathobiology of patients treated for 10 years or more. Eds.: Giordano C, Friedman EA, Milan, Wichig Editore, (1981)

[Go1] Gotch F, Sargent JA. A mechanistic analysis of the National Cooperative Dialysis Study (NCDS). Kidney Int (1985) 28:526-534

[Go2] Gotch F, Krueger K. Adequacy of dialysis. Kidney Int (1975) 7 (suppl. 2):1-263

[Go3] Gotch F. Kt/V is the best dialysis dose parameter. Blood Purif (2000) 18: 276 - 285

[Go4] Gotch FA, Farrell PC and Sargent JA: Theoretical considerations of molecular transport in dialysis and sorbent therapy for uremia. J Dial (1976) 1: 105

[Go5] Gotch FA, Levin NW, Port FK, Wolfe RA, Uehlinger DE. Clinical outcome relative to the dose of dialysis is not what you think: the fallacy of the mean. Am J Kidney Dis (1997) 30: 1-15

[Go6] Gotch FA. Kinetic modelling in hemodialysis, in Clinical Dialysis, Nissensen AR, Gentile DE, Fine RN (editors). Norwalk CT, USA, Appleton & Lange (1989):118pp

[Gra] Grassmann A, Uhlenbusch-Körwer I, Bonnie-Schorn E, Vienken J Composition and management of hemodialysis fluids Ed. Vienken J Pabst Science Publishers, Lengerich Berlin (2000)

[Ha1] Haas G; Versuche der Blutauswaschung am Lebenden mit Hilfe der Dialyse, Klin Wochenschr (1925) 4:13

[Hak] Hakim RM, Breyer J, Ismail N,Schulman G. Effects of dose of dialysis on morbidity and mortality. Am J Kidney Dis (1994); 23: 661-9

[Hei] Heineken FG, Evans MC, Keen ML, Gotch GA: Intercompartmental fluid shifts in hemodialysis patients. Biotechnol Progr (1987) 3: 2

[Hum] Hume R Weyers E: Relationship between total body water and surface area in normal and obese subjects. J Clin Pathol (1971) 24: 234-38

[Klein] Klein E, Holland FF, Eberle K. Comparison of experimental and calculated permeability and rejection coefficients for heodialysis membranes. J Membr Sci (1979) 5 p173-188

[Klein2] Klein E, Holland F, Lebeouf A, Donnaud A, Smith JK. Transport and mechanical properties of hemodialysis hollow fibers. J Mebr Sci (1976) 1: 371-396

[Klop] Kloppenburg WD, Stegeman CA, De Jong PE, Huisman RM Anthropometry based equations overestimate the urea distribution volume in hemodialysis patients. Kidney Int (2001) 59 :3 p1165-74

[Ko1] Kolff WJ: De Kunstmatige Nier, MD Thesis, University of Groningen, The Netherlands, Kampen, JH Kok NV, (1946)

[Kop]Kopple JD et al: A proposed glossary for dialysis kinetics . Am J Kidney Dis (1995) 26:963-981

[Ku] Kuhlmann U, Goldau R, Samadi N, Graf T, Orlandini G, Lange H. Online clearance monitoring of high flux hemodialysis based on conductivity variation. JASN (1998); 9: 298A

[Ku2] Kuhlmann U, Goldau R, Samadi N, Graf T, Orlandini G, Lange H. Accuracy and safety of online clearance monitoring based on conductivity variation. Abstracts XXXVI Congress of the ERA EDTA (1999); 249

[Ku3] Kuhlmann U, Goldau R, Samadi N, Graf T, Gross M, Orlandini G, Lange H. Accuracy and safety of online clearance monitoring based on conductivity variation. Nephrol Dial Transplant (2001) 16 : 1053-58

[Lev] Levenberg K. A method for the solution of certain nonlinear problems in least squares. Quart. Appl. Math. (1944) 2: 164-168

[Ley] Leypoldt JK et al. Effect of dialysis membranes and middle molecule removal on chronic hemodialysis patient survival. Am J Kidney Dis (1999) 33 (2):349-355

[Lind] Lindsay RM, Bene B, Goux N, Heidenheim AP, Landgren C, Sternby J. Relationship between effective ionic dialysance and in vovo urea clearance during hemodialysis. Am J Kid Diseas (2001) 38, 3: 565 - 74

[Lin1] Linholm DD; Experience in the treatment of chronic uremia in an outpatient community hemodialysis center. Trans Am Soc Artif Intern Organs 9: 3, (1963)

[Lo1] Lowrie E, Laird N. Cooperative dialysis study. Kidney Int (1983) 23 (suppl. 13): S1-122

[Lo2] Lowrie E. The normalized Treatment Ratio (Kt/V) is not the best dialysis dose parameter. Blood Purif (2000) 18: 286 - 294

[Loc] Locatelli F, Di Filippo S, Manzoni C, Corti M, Andrulli S, Pontoriero G. Monitoring sodium removal and delivered dialysis by conductivity. Int J Art Org (1995); 11: 716-21

[Low] Lowrie EG. The normalized treatment ratio (Kt/V) is not the best dialysis dose parameter. Blood Purif (2000) 18: 286-294

[Low2] Lowrie EG, Laird N. Protocol for the National Cooperative Dialysis Study. Kidney Int (1983) 23 (suppl. 13) :1-122

[Manz] Manzoni C, Di Filippo S, Corti M, Locatelli F.Ionic dialysance as a method for the on-line monitoring of delivered dialysis without blood sampling. Nephrol Dial Transplant (1996); 11: 2023-2030

[Mer] Mercadal L, Petitclerc T, Jaudon MC, Bene B, Goux N, Jacobs C. Is ionic dialysance a valid parameter for quantification of dialysis efficiency? Artif Organs (1998): 1005-1009.

[Mur1] Murray JS et al: A community hemodialysis center for the treatment of chronic uremia. Trans Am Soc Artif Intern Organs (1962) 8: 315

[Neu] Neumann U, Ziegenhorn J. Kinetic enzymatic method for the determination of urea in serum with the LKB 8600reaction rate analyzer . Scan J Lab Invest (1977) 37 : 90

[Ow] Owen WF, Lew NL, Liu Y, Lowrie EG, Lazarus JM. The urea reduction ratio and serum albumin concentration as predictors of mortality in patients undergoing hemodialysis. New England J Med (1993) 329, 14: 1001-6

[Par] Parker TF, Husni L, Huang W, Lew N,Lowrie EG et al. Survival of hemodialysis patients in the United States is improved with a greater quantity of dialysis. Am J Kidney Dis (1994) 23 (5): 670-680

[Pe1] Petitclerc T, Goux N, Reynier AL, Béné B. A model for non invasive estimation of in vivo dialyzer performances and patients conductivity during hemodialysis. Int J Arg Org (1993) 8 (16): 585-591

[Pe2] Petitclerc T, Béné B, Jacobs C, Jaudon MC, Goux N. Non-invasive monitoring of effective dialysis dose delivered to the haemodialysis patient. Nephrol Dial Transplant (1995) 10: 212-6

[Pe3] Petitclerc T. Recent developments in conductivity monitoring of haemodialysis session. Nephrol Dial Transplant (1999); 14: 2607 – 2613

[Pet] Petersen H. Grundlagen der deskriptiven und mathmatischen Statistik, Bd1: Grundlagen der Statistik und der statistischen Versuchsplanung. Ecomed, Landsberg/Lech (1991)

[Pol] Polaschegg HD. Automatic, noninvasive intradialytic clearance measurement. Int J Art Org (1993) 16, No. 4: 185-91

[Pol2] Polaschegg HD. European patent EP 0097366 (1982/1984) and EP 0428927 (1989/1991)

[RG1] Graf T, Gross M, Goldau R, Bardorz C; Verfahren zur Bestimmung der Leistungsfähigkeit eines Dialysators, einer Dialysevorrichtung und Dialysevorrrichtung zur Durchführung des Verfahrens. European patent application EP 1 062 960 A2

[RG2] Goldau R, Kuhlmann U, Samadi N, Gross M, Graf T, Orlandini G, Marcelli D, Lange H. Ionic dialysance measurement is urea distribution volume dependent: a new approach to better results. Art Org (2002) (in press)

[RG3] Goldau R. Patent application PCT/EP98/00326 (WO 98/32476).

[Sar1] Sargent JA, Gotch FA. Principles and Biophysics of Dialysis; in Replacement of renal function by dialysis, Jacobs, Kjellstrand eds. Kluwer Academic Publishers (1996)

[Sar2] Sargent JA, Gotch FA. Principles and Biophysics of Dialysis; in Replacement of renal function by dialysis, Maher ed. Kluwer Academic Publishers (1989),119pp

[Sar3] Sargent JA, Gotch FA. The analysis of concentration dependence of uraemic lesions in clinical studies. Kidney Int (1975) 7 (suppl. 2):35-44

[Sar4] Sargent JA. Control of dialysis by a single-pool urea model: The National Cooperative Dialysis Study. Kiney Int (1983) 23 (suppl 13):19-26

[Sar5] Sargent JA, Lowrie EG. Which mathematical model to study uremic toxicity – National Cooperative Dialysis Study. Clin Nephrol (1982) 17: 303-314

[See] Seeling WD Ahnefeld FW. Störungen des Wasser-, Elektrolyt- und Säuren-Basen-Status. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart (1988)

[SemDial] Opinion: Is Kt/V Urea a satisfactory measure for dosing the newer dialysis regimes ?Seminars in Dialysis (2001) 14 No1: 8-21

[Sha1] Shackman R, Chisholm GD, Holden AJ, Pigott RW. Urea distribution in the body after hemodialysis. Br Med J (1962) 2:355

[Si] Silbernagl S, Agamemnon D. Taschenatlas der Physiologie . Thieme Verlag (1991). Engl. Version: Agamemnon D. Color Atlas of physiology.

[Sig] Sigdell JE. Calculation of combined diffusive and convective mass transfer. Int J Art Org (1982) 5 (6): 361 - 372

[Spi] Spiegel MR, Laplace Transforms, Schaum Outline Series, McGraw-Hill, New York (1964)

[Steg] Stegeman CA, Huisman RM, De Rouw B et al. Determination of protein catabolic rate in patients on chronic intermittent hemodialysis : Urea output measurements compared with dietary protein intake and with calculation of urea generation rate . Am J Kidney Dis (1995) 25: 887-95

[St] Steil H, Kaufmann AM, Morris AT, Levin NW, Polaschegg HD. In vivo verification of an automatic noninvasive system for real time Kt evaluation. ASAIO Journal (1993) 39,3: M348-52

[USRDS] United States Renal Data System, Annual data reports since 1992. US Department of Health and Human Services. Available at http://www.med.umich.edu/usrds/

[Van] Vanholder R Dhont AM van Bisen W. Is Kt/V Urea a satisfactory measure for dosing the newer dialysis regimes ? Seminars in Dialysis (2001) 14 No1:12-14

[Wat] Watson PE Watson ID Batt RD. Total body water volumes for adult males and females estimated from simple anthropometric measurements. Am J Clin Nutr (1980) 33: 27.39

[Wo] Wolfe RA, Ashby VB, Daugirdas JT, Agodoa LYC, Jones CA, Port FK. Body size, dose of hemodialysis, and mortality. Am J Kidney Dis (2000) 35 (1): 80-8

10. Appendix

10.1. The source code of the two pool calculation loop.

```
void CFileDoc::TwoPoolCalcer(double t, double *SetOfParams, double *Y_at_t, double *dY_dParams, int NrOfParams)
{


 //First we do the calculation during the session: from index InbetwStepnumber to SumOfSteps-1 to get V:
//See theory 99 Aug. 17, sheet 4 in UWü directory

// The analysis theory: GStr, the generation rate, in a first aproach is calculated from direct quantification volume.
// With this fixed rate we calculate a volume with respect only to the start and t30 urea concentration using the clearance
// from array KeCn_of_t. That means we get a volume including generation rate and rebound. This is better than that of
// direct quantification. This new volume again now is used to calculate the generation rate again. And so on.
// The end is found if both values become stable within a particular variation epsG and epsV which can be specified.
// V is meant as the sum of intra- and extracellular volume. The inner distribution of both values is found by first fitting
// the extracellular concentration to the measurements we have from the 2 or 3 clearance references and further to the
// tend concentration. So the variation of the concentration of the outer pool over time is well known. (Always done with a
// least square fit.

// Starting values for the sums:
  TotalUF_of_T = 0;
  IBTotalUF_of_T = 0;
  Total_Generation = 0;
  IBTotal_Generation = 0;
  Heavi_Ce_Ci = 0;
  Heavi_Ci_Ce = 0;


  S1[0]._1 = 0;S1[0]._2 = 0;S1[0]._3 = 0;S1[0]._4 = 0;
  S2[0]._1 = 0;S2[0]._2 = 0;S2[0]._3 = 0;S2[0]._4 = 0;
  S3[0]._1 = 0;S3[0]._2 = 0;S3[0]._3 = 0;S3[0]._4 = 0;
  S4[0]._1 = 0;S4[0]._2 = 0;S4[0]._3 = 0;S4[0]._4 = 0;
  S5[0]._1 = 0;S5[0]._2 = 0;S5[0]._3 = 0;S5[0]._4 = 0;


  Vl[0]._1 =                    SetOfParams[0];
  VE[0]._1 =                    SetOfParams[1];
  GStr     =                    SetOfParams[2];
  ClearanceCorrFct =        SetOfParams[3];



// The inbetween fit:

for (int runner = 0; runner <= InbetwStepnumber ; runner++)
{
        //first assignments of the last turn:
        if (runner > 0)
        {
                // preparing the Sx:
                S1[runner]._1 = S1[runner-1]._1 + kr*(CE[runner-1]-Cl[runner-1].First)*InbetwStepwidth/60*100; // in ml
                S2[runner]._1 = S2[runner-1]._1 + K1D/100*(Cl[runner-1].First-CE[runner-1])*InbetwStepwidth/60; // in
                                                                                                                //mg
                S3[runner]._1 = S3[runner-1]._1 + kr*(CE[runner-1]-Cl[runner-1].First)*CE[runner-
                1]*Heavi_Ci_Ce*InbetwStepwidth/60;// in mg
                S4[runner]._1 = S4[runner-1]._1 + kr*(CE[runner-1]-Cl[runner-1].First)*Cl[runner-
                1].First*Heavi_Ce_Ci*InbetwStepwidth/60;//mg
                S5[runner]._1 = S5[runner-1]._1 + ResClr/100*CE[runner-1]*InbetwStepwidth/60;

                IBTotalUF_of_T  -= TotalUF/InbetwStepnumber;
```

```
                IBTotal_Generation += SetOfParams[2] * InbetwStepwidth/60;

                //assume the next Ci,Ce:

                CI[runner].First  = CiBinoms[runner-1].Root1;
                CI[runner].Second = CiBinoms[runner-1].Root2;
                CE[runner]  = CeBinoms[runner-1].Root1;
                //CE[runner]._2  = CeBinoms[runner-1].Root2;

                //and calc the next volumes:
                // if kr == 0 we automatically distribute the fluid transfer 2/3 to ICF and 1/3 to ECF
                // (its the standard approach if you dont know about internal fluid shifts

                if (kr ==0)
                {
                        VI[runner]._1  = SetOfParams[0] - 2*IBTotalUF_of_T/3;
                        VE[runner]._1  = SetOfParams[1] - IBTotalUF_of_T/3;
                }
                else
                {
                        VI[runner]._1  = SetOfParams[0] -S1[runner]._1;
                        VE[runner]._1  = SetOfParams[1] +S1[runner]._1-
                                IBTotalUF_of_T;
                }
        }

        // First CI:
        Heavi_Ce_Ci = (((CE[runner]-CI[runner].First)>0)?1:0);
        Heavi_Ci_Ce = (((CI[runner].First-CE[runner])>0)?1:0);

        CiBinoms[runner].m =(kr-kr*Heavi_Ce_Ci)*InbetwStepwidth/60;                     //kr in dl/(mg/dl*min) ; .m unit is
                                                                                        //dl/(mg/dl)
        CiBinoms[runner].n =SetOfParams[0]/100 -     0.01 * S1[runner]._1   +
                        (K1D/100-kr*CE[runner]-
       kr*CE[runner]*Heavi_Ci_Ce+kr*CE[runner]*Heavi_Ce_Ci)*InbetwStepwidth/60;

                                                //.n unit is dl
        CiBinoms[runner].q =S2[runner]._1 + S3[runner]._1 - SetOfParams[0]/100 * CI[0].First + S4[runner]._1
                +(kr*CE[runner]*CE[runner]*Heavi_Ci_Ce - K1D/100*CE[runner])*InbetwStepwidth/60;

                                                //.q unit is mg
        CiBinoms[runner] = SolveSqrEqn(CiBinoms[runner]);

        // and than CE:
        CeBinoms[runner].m =(kr-kr*Heavi_Ci_Ce)*InbetwStepwidth/60;
        CeBinoms[runner].n =SetOfParams[1]/100  -   IBTotalUF_of_T/100
                + 0.01 * S1[runner]._1   +
                (K1D/100-kr*CI[runner].First+kr*CI[runner].First*Heavi_Ci_Ce-kr*CI[runner].First*Heavi_Ce_Ci)      //to run
                                                                                                                  //also the first
                        *InbetwStepwidth/60;
        CeBinoms[runner].q =S5[runner]._1- SetOfParams[1]/100 * CE[0] - S2[runner]._1
                                                -S3[runner]._1 -  S4[runner]._1 - IBTotal_Generation
                                                +(kr*CI[runner].First*CI[runner].First*Heavi_Ce_Ci –
                                               K1D/100*CI[runner].First)*InbetwStepwidth/60;

        CeBinoms[runner] = SolveSqrEqn(CeBinoms[runner]);

}//for (int runner = 0; runner <= InbetwStepnumber ; runner++)

runner--;

// The session fit:
for (runner; runner < SumOfSteps ; runner++)
{
        //first assignments of the last turn:
        if (runner > InbetwStepnumber)
```

```
{
        // preparing the Sx:
        S1[runner]._1 = S1[runner-1]._1 + kr*(CE[runner-1]-CI[runner-1].First)*SessnStepwidth/60*100; // in ml
        S2[runner]._1 = S2[runner-1]._1 + K1D/100*(CI[runner-1].First-CE[runner-1])*SessnStepwidth/60; // in mg
        S3[runner]._1 = S3[runner-1]._1 + kr*(CE[runner-1]-CI[runner-1].First)*CE[runner-
                1]*Heavi_Ci_Ce*SessnStepwidth/60;// in mg
        S4[runner]._1 = S4[runner-1]._1 + kr*(CE[runner-1]-CI[runner-1].First)*CI[runner-
                1].First*Heavi_Ce_Ci*SessnStepwidth/60;//mg
        S5[runner]._1 = S5[runner-1]._1 + (ResClr+SetOfParams[3]*KeCN_of_t.Mean((runner-
                InbetwStepnumber-1)*SessnStepwidth,
                (runner-InbetwStepnumber)*SessnStepwidth))/100
                *CE[runner-1]*SessnStepwidth/60;                                                    //mg
        TotalUF_of_T  += D_RealUfRate.Mean((runner-InbetwStepnumber-1)*SessnStepwidth,
                    (runner-InbetwStepnumber)*SessnStepwidth)
                        /60*SessnStepwidth/60;
        Total_Generation += SetOfParams[2] * SessnStepwidth/60;

        //assume the next Ci,Ce:

                                                        //mg
        CI[runner].First  = CiBinoms[runner-1].Root1;
        CI[runner].Second = CiBinoms[runner-1].Root2;
        CE[runner]  = CeBinoms[runner-1].Root1;
        //CE[runner]._2  = CeBinoms[runner-1].Root2;

        //and calc the next volumes:
        // if kr == 0 we automatically distribute the fluid transfer 2/3 to ICF and 1/3 to ECF
        // (its the standard approach if you dont know about internal fluid shifts

         if (kr == 0)
                {
                  VI[runner]._1  = VI[InbetwStepnumber]._1 -2*TotalUF_of_T/3;
                  VE[runner]._1  = VE[InbetwStepnumber]._1 -TotalUF_of_T/3;
                }
         else       // allns blivt bi'n ohln
                {
                  VI[runner]._1  = VI[InbetwStepnumber]._1 -S1[runner]._1;
                  VE[runner]._1  = VE[InbetwStepnumber]._1 +S1[runner]._1-
                                TotalUF_of_T;
                }
}
else
{
 // Starting values for the sums:

        S1[runner]._1 = 0;S1[runner]._2 = 0;S1[runner]._3 = 0;S1[runner]._4 = 0;
        S2[runner]._1 = 0;S2[runner]._2 = 0;S2[runner]._3 = 0;S2[runner]._4 = 0;
        S3[runner]._1 = 0;S3[runner]._2 = 0;S3[runner]._3 = 0;S3[runner]._4 = 0;
        S4[runner]._1 = 0;S4[runner]._2 = 0;S4[runner]._3 = 0;S4[runner]._4 = 0;
        S5[runner]._1 = 0;S5[runner]._2 = 0;S5[runner]._3 = 0;S5[runner]._4 = 0;


}

// First CI:
Heavi_Ce_Ci = (((CE[runner-CI[runner].First)>0)?1:0);
Heavi_Ci_Ce = (((CI[runner].First-CE[runner])>0)?1:0);

CiBinoms[runner].m =(kr-kr*Heavi_Ce_Ci)*SessnStepwidth/60;                  //kr in dl/(mg/dl*min) ; .m unit is
                                                                            dl/(mg/dl)
CiBinoms[runner].n =VI[InbetwStepnumber]._1/100 -     0.01 * S1[runner]._1   +
            (K1D/100-kr*CE[runner]-kr*CE[runner]*Heavi_Ci_Ce+kr*CE[runner]*Heavi_Ce_Ci)*SessnStepwidth/60;

                                        //.n unit is dl
CiBinoms[runner].q =S2[runner]._1 + S3[runner]._1 - VI[InbetwStepnumber]._1/100 * CI[InbetwStepnumber].First +
                    S4[runner]._1
                   +(kr*CE[runner]*CE[runner]*Heavi_Ci_Ce - K1D/100*CE[runner])*SessnStepwidth/60;
```

```
                                                        //.q unit is mg
        CiBinoms[runner] = SolveSqrEqn(CiBinoms[runner]);

        // and than CE:
        CeBinoms[runner].m =(kr-kr*Heavi_Ci_Ce)*SessnStepwidth/60;
        CeBinoms[runner].n =VE[InbetwStepnumber]._1/100  -   TotalUF_of_T/100
                        + 0.01 * S1[runner]._1   +
                        (K1D/100-kr*CI[runner].First+kr*CI[runner].First*Heavi_Ci_Ce-kr*CI[runner].First*Heavi_Ce_Ci+
                          SetOfParams[3]*KeCN_of_t.Mean(((runner-InbetwStepnumber-1 < 0)? 0 :runner-InbetwStepnumber-
                                1)*SessnStepwidth,
                                  ((runner-InbetwStepnumber<=0)?1:runner-InbetwStepnumber)*SessnStepwidth)/100)       //to run
                                                                                                                        //also the first
                                *SessnStepwidth/60;
        CeBinoms[runner].q =S5[runner]._1- VE[InbetwStepnumber]._1/100 * CE[InbetwStepnumber] - S2[runner]._1
                                                                -S3[runner]._1 -  S4[runner]._1 - Total_Generation
                                                                +(kr*CI[runner].First*CI[runner].First*Heavi_Ce_Ci –
                                                               K1D/100*CI[runner].First)*SessnStepwidth/60;

        CeBinoms[runner] = SolveSqrEqn(CeBinoms[runner]);

}//for (int runner = InbetwStepnumber; runner <= SumOfSteps ; runner++)


// Filling in the return values in dependence of t:


// t = 3: The returnvalue is Ce_t30 of the current treatment, ...
//if (t > CE.GetSize()-1)
if (t > 3)
{
        Y_at_t[0] = 0;
        //Y_at_t[1] = -1;                   //error flag
}
else
{

        switch  ((int) t)
        {
         case 1:
                        {
                        Y_at_t[0] = CE[(long double)Xindex1]; // return CE at t
           //Y_at_t[1] = 0;                  //error flag
                        break;
                        }
         case 2:
                        {
                        Y_at_t[0] = CE[(long double)Xindex2]; // return CE at t
           //Y_at_t[1] = 0;                  //error flag
                        break;
                        }
         case 3:
                        {
                        Y_at_t[0] = CE[(long double)Xindex3]; // return CE at t
           //Y_at_t[1] = 0;                  //error flag
                        break;
                        }
         default:
                        {
                        Y_at_t[0] = 0;
                        break;
                        }
        }

        if (dY_dParams == NULL)                         // see if we have to perform derivatives:

        {// we dont have to !
```

```
                        //Y_at_t[2] = -1;       // error flag in first derivative
                }
                else
                {// we have to derive the derivatives
                        double SetOfParamsDerv[4];                      // 4 fit parameters 2pKM : Vi, Ve, GStr    and KeCN(Bolus)
                        double CE_ThisTurnDerv[4];


                        // dY_at_t[0]/dSetOfParams[0] at t:

SetOfParamsDerv[0]=SetOfParams[0]+1;SetOfParamsDerv[1]=SetOfParams[1];SetOfParamsDerv[2]=SetOfParams[2];
                        TwoPoolCalcer(t,SetOfParamsDerv,CE_ThisTurnDerv,NULL, Nr_OfParams);
        dY_dParams[0] = CE_ThisTurnDerv[0]-Y_at_t[0];
                        //Y_at_t[2] = CE_ThisTurnDerv[1];

                        // dY_at_t[0]/dSetOfParams[1] at t:

SetOfParamsDerv[0]=SetOfParams[0];SetOfParamsDerv[1]=SetOfParams[1]+1;SetOfParamsDerv[2]=SetOfParams[2];
                        TwoPoolCalcer(t,SetOfParamsDerv,CE_ThisTurnDerv,NULL, Nr_OfParams);
        dY_dParams[1] = CE_ThisTurnDerv[0]-Y_at_t[0];
                        //Y_at_t[2] += CE_ThisTurnDerv[1];

                        // dY_at_t[0]/dSetOfParams[2] at t:

SetOfParamsDerv[0]=SetOfParams[0];SetOfParamsDerv[1]=SetOfParams[1];SetOfParamsDerv[2]=SetOfParams[2]+1;
                        TwoPoolCalcer(t,SetOfParamsDerv,CE_ThisTurnDerv,NULL, Nr_OfParams);
        dY_dParams[2] = CE_ThisTurnDerv[0]-Y_at_t[0];
                        //Y_at_t[2] += CE_ThisTurnDerv[1];



                        //if (Y_at_t[2] < 0) Y_at_t[2] = -1;            // error flag in first derivative


                }
}
```

10.2. MI Study: The patient and treatment parameter details

Patient related parameters

Parameter Patient	*Age Years*	*Gender*	*Height cm*	*'Dry' weight kg*	*Mean weight at dialysis start kg*	*Heart rate 1/min*	*init. blood pressure syst mm Hg*	*init. blood pressure diast mm Hg*	*residual creatinine clearance ml/min*	*mean Hemato-crit %*	*mean recircu-lation %*
1	22	male	181	73,0	73,0	76	143	74	1,8	35	5,0
2	63	female	160	76,0	76,7	74	171	85	3,3	31	5,3
3	82	male	168	63,0	63,2	72	152	77	0,0	35	5,0
4	58	male	182	85,0	87,4	77	174	83	7,0	35	4,0
5	26	male	188	78,0	80,4	76	156	89	10,7	35	4,7
6	47	male	184	79,6	79,0	74	129	76	0,0	34	4,6
7	77	male	172	80,0	81,7	76	155	81	0,0	28	5,1
8	73	female	168	45,0	45,3	69	173	89	0,5	30	6,1
9	65	male	170	70,0	72,3	74	145	81	6,3	32	14,2
10	74	female	165	73,0	73,6	76	165	81	4,8	30	6,8
11	72	female	168	62,0	67,4	77	164	78	0,0	30	6,2
12	58	female	168	61,7	60,8	75	153	89	4,0	30	7,0
13	70	male	170	78,0	78,9	76	179	88	0,0	32	5,4
14	51	male	184	84,0	83,8	75	161	90	10,7	32	4,3
15	67	male	175	86,5	86,3	76	135	73	7,5	27	4,7
16	42	male	182	93,0	89,0	78	192	102	4,5	34	4,5
17	47	male	173	82,0	82,6	80	166	89	0,0	31	5,5
18	52	female	168	73,5	73,6	72	141	81	7,2	30	5,1
19	60	male	174	65,7	66,0	73	145	82	3,3	30	5,1
20	68	male	183	68,4	69,2	78	147	73	7,8	29	4,3
Mean	58,7	30%f;70%m	174,15	73,9	74,5	75	157	83	4,0	32	5,6
SD	16,1		7,8	11,0	10,6	2,5	15,9	7,1	3,7	2,5	2,2

Table 10.2.a: *The table shows anthropometric and patient related parameters of the collective participating the MI study.*

Treatment related parameter

Parameter Patient	*Dialysate conductivity mS/cm*	*Blood flow ml/min*	*Dialysate flow ml/min*	*Ultrafiltrate flow ml/min*	*Treatment duration min*	*Filtertype*
1	14,08	189	539	8,1	277	Fresenius F8
2	14,09	183	548	8,0	206	Fresenius F8
3	14,16	186	547	9,1	206	Fresenius F8
4	14,29	191	541	9,0	201	Fresenius F8
5	14,24	190	540	8,7	224	Fresenius F8
6	14,20	187	547	9,5	214	Fresenius F8
7	14,17	202	544	7,3	212	Fresenius F8
8	14,27	197	552	11,7	215	Fresenius F8
9	14,15	205	538	6,1	219	Fresenius F8
10	14,19	208	540	9,2	201	Fresenius F8
11	14,15	202	542	6,0	203	Fresenius F8
12	14,09	204	554	6,2	209	Fresenius F8
13	14,15	207	535	7,7	205	Fresenius F8
14	14,12	214	576	9,3	202	Fresenius F8
15	14,07	213	580	10,3	217	Fresenius F8
16	14,21	201	547	11,3	203	Fresenius F8
17	14,20	208	549	11,8	215	Fresenius F8
18	14,07	209	586	7,7	225	Fresenius F8
19	14,16	211	566	8,0	204	Fresenius F8
20	14,13	207	587	7,7	226	Fresenius F8
Mean	14,16	201	553	8,6	214	

Table 10.2.b: *The table shows treatment related parameters of the collective participating the MI study. The blood flow values are the prescribed whole blood flow values that have been adjusted at the blood pump. Due to the correction of arterial inlet pressure the actual blood flows are lower.*

10.3. MII Study: The patient and treatment parameter details

Patient related parameters

Parameter Patient	*Age Years*	*Gender*	*Height cm*	*'Dry' weight kg*	*Mean weight at dialysis start kg*	*Heart rate 1/min*	*init. blood pressure syst mm Hg*	*init. blood pressure diast mm Hg*	*residual creatinine clearance ml/min*	*mean Hemato-crit %*	*mean recircu-lation %*
1	67	female	160	51,2	51,9	74	143	81	7,4	30	5,0
2	58	female	164	51,9	52,3	75	149	79	5,6	31	6,4
3	70	male	178	80,0	83,4	77	132	74	0,0	32	5,0
4	49	male	173	82,0	84,9	74	132	75	5,4	29	4,9
5	98	female	162	65,0	65,6	81	165	97	5,6	28	4,9
6	63	female	158	62,5	66,6	74	139	66	0,0	30	5,2
7	51	male	176	87,9	90,3	76	156	84	6,3	34	4,7
8	78	male		64,0	64,7	77	127	68	0,0	32	5,7
9	72	male		80,6	80,6	75	135	75	9,1	32	5,0
10	76	male	159	70,0	72,0	84	111	70	5,3	32	5,4
Mean	65	40%f, 60%m	166	70,1	71,2	77	139	77	4,5	31	5,2
SD	14,4		8,1	12,8	13,4	3,3	15,2	8,9	3,3	1,8	0,5

Table 10.3.a: *The table shows anthropometric and patient related parameters of the collective participating the MII study.*

Treatment related parameter

Parameter Patient	*Dialysate conductivity mS/cm*	*Blood flow ml/min*	*Dialysate flow ml/min*	*Ultrafiltrate flow ml/min*	*Treatment duration min*	*Filtertype*
1	14,12	212	520	7,7	210,12	Fresenius F8
2	14,12	217	516	7,0	224,55	Fresenius F8
3	14,12	212	517	10,2	235,35	Fresenius F8
4	14,08	213	512	10,1	223,40	Fresenius F8
5	14,07	212	520	9,4	222,88	Fresenius F8
6	14,09	213	567	11,1	221,71	Fresenius F8
7	14,07	212	515	10,8	223,86	Fresenius F8
8	14,13	215	521	7,1	207,91	Fresenius F8
9	14,13	211	549	7,9	220,36	Fresenius F8
10	14,09	213	577	8,0	213,59	Fresenius F8
Mean	14,10	213	531	8,9	220,37	

Table 10.3.b: *The table shows treatment related parameters of the collective participating the MII study. The blood flow values are the prescribed whole blood flow values that have been adjusted at the blood pump. Due to the correction of arterial inlet pressure the actual blood flows are lower.*

10.4. Declaration of Helsinki

Recommendations guiding physicians in biomedical research involving human subjects.

Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, amended by the 29th World Medical Assembly, Tokio, Japan, October 1975, the 35th World Medical Assembly, Venice, Italy, October 1983 and the 41st World Medical Assembly, Hong Kong, September 1989.

INTRODUCTION

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfilment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words: "The health of my patient will be my first consideration", and the International Code of Medical Ethics declares that: "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient".

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognised between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected. Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. Basic principles

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory -and animal experimentation and on a thorough knowledge of the scientific literature.
2. The design and performance of each experimental procedure involving human subjects should be dearly formulated in an experimental protocol which should be transmitted to a specially appointed committee independent for consideration, comment and guidance.
3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.
4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.
5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subjects or to others. Concern for the interests of the subject must always prevail over the interests of science and society.
6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimise the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.
8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.
9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail-. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.
10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.
11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the minor child is in fact able to

give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. Medical research combined with professional care (clinical research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, re-establishing health or alleviating suffering.
2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.
3. In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method.
4. The refusal of the patient to participate in the study must never interfere with the physician-patient relationship.
5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I.2).
6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. Non-therapeutic biomedical research involving human subjects (non-clinical biomedical research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.
2. The subjects should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.
3. The investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.
4. In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

10.5. Statistics

The statistical methods that have been used in this work are concise and clear but will be added here to complete the description.

Mean values, SD:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} \qquad SD = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}}$$

Correlation:

In all cases the data were presented such that the dependence can expected to be linear. This usually allows to calculate the linear correlation coefficient r:

$$r = \frac{\sum_{i=1}^{n} (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n} (x_i - \bar{x})^2 \sum_{i=1}^{n} (y_i - \bar{y})^2}}$$

In some cases – in particular in momentary clearance comparisons – the value cloud is so dense and located to only a small region so that the SD is comparable to the mean error. In this cases even an excellent measurement principle gives poor correlations. It makes no sense to calculate r in this cases.

Significance evaluation:

Significance statements are based on a significance level of α=0.95. In general student's t-test for paired data has been used because the t-distribution can be applied also for small numbers of samples if they are normal distributed. It approaches the Gaussian normal distribution for large sample numbers. The test has been used for paired data because the comparison was always made for a data pair that was won by measuring the identical physical state with two different principles. This means the data have an inherent context. So it was not only to test if the distribution and the average of the two principles are equal but also if two related measurements from both principles match each other sufficiently. Only if this is the case the t-test for paired data regards the data to be not significantly different. Therefore it is more critical than the standard t-test.

$$S = \frac{1}{(n-1)(n-2)\frac{SD^3}{n}} \sum_{i=1}^{n} (x_i - \bar{x})$$

E 10.5.a

Although student's t-test for paired data is not very sensitive to deviations from the normal distribution all populations have been visually inspected for normal distribution. In doubt the skewness S has been calculated using E 10.5.a [Fi, Pet].

n is the number of samples, x the value of a single sample, $\bar{x}$ the average of all x_i.

According to table 4 in [Pet] the test variable S/SD^3 has been compared to the g-value of this table. If S/SD3 $\leq$ g, the population has been regarded to be normal distributed. There was no population involved in this study that was found to be not normal distributed in this sense. The values for g are given in table 10.5 a. This comparison is the justification to use student' t-test.

# samples	*g*
50	0.55
75	0.454
100	0.395
125	0.354
150	0.324
175	0.301
200	0.282
250	0.253
300	0.231
350	0.214
400	0.201
450	0.189
500	0.180

Table 10.5.a: *g values for skewness analysis [Pet]. α=0.95.*

The t(x) probability density is tabled but can also be calculated using the expression [Bron]

$$t(x) = \frac{\Gamma\left(\frac{n+1}{2}\right)}{\sqrt{\pi n}\,\Gamma\left(\frac{n}{2}\right)}\left(1+\frac{x^2}{n}\right)^{-\frac{n+1}{2}}$$

whereas n is the number of degrees of freedom and Γ is the Gamma function, which can only be calculated by a computer and is defined for all x>0 as [Bron]:

$$\Gamma(x) \underset{m\to\infty}{=} \frac{m^{x-1}m!}{x(x+1)(x+2).....(x+m-1)}$$

with m being a positive integer approaching infinity.

11. Acknowledgements

First of all the author wishes to express his appreciation to the patients for their consent to participate in this study. Though they were suffering from a serious disease they were willing to give from their time and their patience to all their companions in distress who may benefit from their attitude.

In recognition of the enormous amount of extra work, availability and long nights with the computer to type in all the data, for all the scientific consult and deep discussions I want to emphasise my very special regards to Dr. Nader Samadi and Dr. Uwe Kuhlmann and in particular to Professor Dr. Harald Lange, Head of the Center of Internal Medicine, Clinic of Nephrology of the University of Marburg, Germany, who as the principal investigator of these studies has always held his experienced hand upon our group of scientists.

I am grateful to Professor Dr. Roland Benz of the Faculty of Biology, Chair of Biotechnology, Theodor-Boveri-Institut of the University of Wuerzburg, Germany, for his scientific support and his willingness to supervise this work of mine as an external student. Without his assistance I would not have terminated this work in time.

This study was granted by Fresenius Medical Care Deutschland GmbH, Bad Homburg, Germany. Without this support the study could not have been conducted. My special respect for supporting this work in an idealistic and practical way goes to

Mr. Spickermann, who gave me a lot of mental and practical support and simply was the best head of department I could imagine.

Dr. Malte Gross and Thomas Graf, who did a lot of the practical work and monitoring of the studies. They were simply wonderful colleges I could always rely on. In particular Malte who was always willing to sit in the rear of our car if the front glas was broken after Ionometer theft. Further to Achim Eberlein, Walter Ehrenberger, Wolfgang Stock, Helmuth Ender, Dr. Carsten Müller, Olaf Nicholas, Dieter Stöhlein, Gert Siebert and Johannes Fiedler, who all made their contribution to the final result as software- and quality-engineers and all were good ghost busters when the OCM machines died overnight without visible reason (Hope I forgot noone).

Dr. Giancarlo Orlandini and to Dr. Daniele Marcelli who initialised the study and made strong contribution to a good study protocol and gave enormous medical and scientific background support. Without their help and knowledge the CE approval for the dialysis machines that have been used in the studies would not have been achieved. Further they have simply been good friends in situations the problems were many and the solutions were few.

Last but not least I am grateful to my wife Monika Goldau. She gave me the peace and warmth that was the basement without which I could not have concentrated upon this work.

Rainer Goldau, January 2002

12. English abstract

During the last two decades an ongoing discussion about the necessary dose of dialysis brought the result that the urea based Kt/V value is significantly correlated to morbidity of the end stage renal disease (ESRD) patients. Even if it is not completely accepted, it seems to be more and more agreement of the nephrological community that for good dialysis practice Kt/V should be kept above 1.2 to 1.3 in the usual 3X4 hours per week dialysis schedule for patients without own residual clearance to assure long term quality of life, low morbidity and mortality. K is the clearance of urea the dialysis system can apply, t is the treatment time and V is the urea distribution volume of the patient, which is nearly equal to total body water. Kt/V has the unit of a drug dose (ml of drug per ml of patient volume) and therefore sometimes is called dialysis 'dose', even if this is subject of discussion because it implies that the dose can be described with only one urea related number.

This work does not participate in this discussion. The premise of this work is more technical: Whatever the final result of the above discussion will be, a patient-friendly, precise cost-neutral and handy technical solution should be given to the hand of the interested nephrologist to continuously supervise the urea based Kt/V that is applied to the patient. Of course this is combined with the hope that the long term mortality can be decreased if a covering online dialysis success control is facilitated.

The technical solution that has been chosen is based on the equivalence of the diffusion coefficients of sodium chloride and urea. It is central subject of the investigation if the diffusive behaviour of sodium is equal to that of urea crossing the dialysis filter membrane. The advantage that makes the principle so handy is that sodium can be measured very precise by standard conductivity cells as they are implemented in dialysis machines in large numbers. The only necessary hardware modification is a second conductivity cell downstream the dialyser to be able to measure the mass balance over the filter. This is more complicated with urea that can only be measured undergoing an enzymatic conversion to ammonium ions. The ammonium ions induce a membrane potential, which is measured with very sensitive amplifiers. A cooling chain for the enzyme must be maintained.

To find and approve the conductivity based technical solution two in-vivo studies have been conducted. In the first study a conductivity step profile, varying the conductivity in static levels in a baseline - 7 min high - 7min low- baseline shape, was applied that can be utilised to measure the urea clearance very accurate. This principle has been described in 1982 in a patent application [Pol2]. In a sequence of 206 computer recorded dialysis sessions with 22 patients it was found that urea clearance could be electrolytically measured with a mean error±standard deviation of -1.46±4.75% , n=494. The measurement of Kt/V according to a single pool model was of similar accuracy: 2.88±4.15% . Although in accordance with other studies these findings at an average confirmed the high correlation of ionic and urea based clearance measurements, an effect was found that was not consistent with the theory that was existent so far. It was found in the first study that the accuracy of the step profile measurements were dependent of the size of the patient, in particular of the urea distribution volume. Moreover it was of relevance which part of the step profile

was used: the high-low states, the baseline- low or the baseline-high states. This was a theoretical lack.

Careful analysis led to the result that sodium transfer from and into the patient was the reason for the dependence. This led to the enhancement of the theory that seems to correctly describe the nature of the effect. A new demand now was to minimise the sodium transfer. This was limited using static step profiles because in the time it needs to become stable sodium is shifted. In consequence non-stable, dynamic short conductivity boli were developed that allowed to minimise the amount of sodium to be shifted to the limits of the technical resolution of the measurement systems [RG3]. Also the associated mathematical tools to evaluate the boli had to be suited to the problem. After termination of this process a second study was conducted to approve the new method found.

In this study with 10 patients and 93 sessions, 264 step profile measurements and 173 bolus ionic dialysance measurements it was found that the bolus measurements matched their related blood side urea clearance references with the outstanding accuracy of (error±SD) 0.06±4.76%. The result was not significantly different (p=0.87) from the reference by student's t-test for paired data. The Kt/V reference according to the single pool variable volume urea kinetic model (sPVVUKM) was found to be matched by the bolus principle with 5.32±3.9% accuracy and a correlation of 0.98. The remaining difference of 5.32% can be attributed to the neglect of the urea generation rate. Also the step profile was found to be very precise here. The error versus sPVVUKM was 0.05%±5%, r=0.96. However it did not image the neglect of urea generation correctly.
Also a two pool modelling that comprises an internal compartimentation of the fluid pools of the patient was applied to the continuously recorded data. This two pool urea kinetic model (2PUKM) is regarded to be a more precise theoretical approach and now includes the urea generation. It found the bolus principle to deviate -3.04 ± 14.3%, n.s., p=0.13. The high standard deviation is due to the complexity of the model.
Further from the developed theory a simplified method to roughly measure the sodium distribution volume could be derived. This method was tested in-vitro versus a container with dialysate of known volume and in-vivo versus the urea distribution volume. The in-vitro results were -19.9±34%, r=0.92, n.s, p=0.916. In-vivo they were found to be -7.4±23.2%, r=0.71, n.s., p=0.39. Due to dilution theory the sodium and urea distribution volumes virtually appear to be very similar using this method, although they absolutely differ significantly. Facing the strong simplifications that were made before applying this theory these results seem to be very encouraging that it could be possible to develop a principle to measure not only K but also V electrolytically. This would allow a true Kt/V measurement.

The empirical urea distribution volume measurement using anthropometrical formulas has been compared to analytical methods. It has been found that the use Watson's formula with a –13% correction gives good results. The correction should be applied with great care because it increases Kt/V just on a arithmetical base to the disadvantage of the patient.

Also electrolytical plasma sodium measurement was evaluated and can be measured using a mixed analytic-empirical formula with an accuracy of 4.3±1.2%.

In summary, conductivity based methods seem to be a convenient method to measure several dialysis parameters of some clinical interest without effort. The results of this work meanwhile are implemented with substantial numbers into commonly available dialysis machines and the experience of the first time shows that the principle is well accepted by the clinicians.

13. Deutsche Zusammenfassung

Eine die letzten beiden Jahrzehnte anhaltende Diskussion über die erforderliche Dosis Dialyse, die ein Patient benötigt, ergab, daß der Harnstoff-basierte Kt/V-Wert signifikant mit der Morbidität von ‚end stage renal deasease' (ESRD)- Patienten korreliert. Wenn auch nicht vollständig akzeptiert, so scheint es doch zunehmende Übereinkunft zwischen Nephrologen, daß eine angemessene Dialysebehandlung von Patienten ohne Restdiurese im Rahmen eines 3x4 Stunden pro Woche- Schemas mindestens einen Kt/V-Wert von 1.2 bis 1.3 ergeben sollte, um auf lange Sicht die Lebensqualität zu sichern und die Morbidität und Mortalität niedrig zu halten. K ist die vom Dialysesystem erbrachte Clearance, t die Behandlungszeit und V das Harnstoff-Verteilungsvolumen, welches dem Gesamt-Körperwasser nahezu gleicht. Kt/V wird in der Dosiseinheit (ml Medikament pro ml Körperwasser) ausgedrückt und daher auch häufig als Dialyse-‚Dosis' bezeichnet, auch wenn darüber wegen der Zusammenfassung in nur einer Harnstoff-korrelierten Zahl konträr diskutiert wird.

Diese Arbeit besitzt eine eher technische Prämisse und möchte sich nicht an dieser Diskussion beteiligen. Sie möchte dem interessierten Nephrologen lediglich eine patientenfreundliche, präzise, kostenneutrale und leicht zu handhabende technische Lösung an die Hand geben, um kontinuierlich die in Kt/V ausgedrückte Dialysedosis zu überwachen. Natürlich ist damit auch die Hoffnung verbunden, daß die Langzeit-Sterblichkeit verringert werden kann, wenn eine flächendeckende, zeitnahe Erfolgskontrolle der Dialyse ermöglicht wird.

Die gewählte technische Lösung basiert auf der Äquivalenz der Diffusionskoeffizienten von gelöstem Harnstoff und Natriumchlorid. Es ist das zentrale Anliegen dieser Studie, festzustellen, ob das Diffusionsverhalten von NaCl und Harnstoff beim Durchtritt durch die Membran des Dialysefilters gleich ist. Der entscheidende Vorteil, der das Verfahren so leicht handhabbar macht, besteht darin, daß NaCl-Konzentrationen sehr genau durch die ohnehin in großer Zahl in Dialysegeräten verwendeten Leitfähigkeitsmeßzellen bestimmt werden können. Um die NaCl-Massenbilanz über den Dialysefilter zu bestimmen, benötigt man lediglich eine weitere Meßzelle, die stromab des Filters zu installieren ist. Die Messung von Harnstoff, die indirekt über die enzymatische Zerlegung in Ammonium-Ionen und das anschließende Erzeugen eines hoch zu verstärkenden elektrischen Potentials an einer Membran geschieht, ist komplizierter. Zudem ist eine geschlossene Kühlkette für das Enzym sicher zu stellen.

Um eine leitfähigkeitsbasierte technische Lösung abzusichern, wurden zwei klinische Studien durchgeführt. In der ersten Studie wurde die Leitfähigkeit in Form von konstanten Stufenprofilen variiert. Sie wurde ausgehend von der Grundlinie für 7 min erhöht und anschließend für 7 min erniedrigt. Das Prinzip einer solchen Messung wurde erstmals 1982 in einer Patentschrift beschrieben [Pol2]. In einer Sequenz von 494 solchen Messungen in 206 automatisch aufgezeichneten Dialysesitzungen an 22 Patienten wurde gefunden, daß sich die Harnstoff- Clearance elektrolytisch mit einer Genauigkeit von –1.46±4.75% (Fehler±Standardabweichung) messen ließ. Die Messung von Kt/V gemäß dem Ein-Kompartment-Modell ergab eine ähnliche Genauigkeit von 2.88±4.15%. Obgleich diese Ergebnisse in Übereinstimmung mit anderen Studien stehen, wurde ein Effekt bemerkt, der nicht in Einklang mit der zunächst bestehenden Theorie zu bringen war. Dieser Effekt besteht darin, daß die Genauigkeit der elektrolytischen Clearancemessung vom beim Patienten vorhandenen Harnstoff-Verteilungsvolumen abhängig war. Weiter war es nicht bedeutungslos, welchen Teil des dreiteiligen Stufenprofils man zur Auswertung heranzog: Den Grundlinie-Hoch- Übergang, den von der Grundlinie zum Niedrig-Nieveau oder den Hoch-Niedrig- Übergang. Dies deutete auf einen Mangel an theoretischem Verständnis hin.

Eine genaue weitere Untersuchung führte zu dem Ergebnis, daß unerwünschter NaCl-Transfer vom und zum Patienten die Ursache für die Abhängigkeit vom Verteilungsvolumen war. Es wurde daraufhin die Theorie dahingehend erweitert, daß dieser Effekt korrekt und plausibel beschrieben werden konnte. Aus dieser Erweiterung ergab sich die neue Forderung, den NaCl-Transfer bei der Messung weitestgehend zu minimieren. Die Benutzung von Stufenprofilen stellte hier jedoch an sich eine Limitierung dar, da in der zum Einnehmen eines stabilen Zustandes erforderlichen Zeit zuviel NaCl über die Membran transferiert wurde. Die Konsequenz war, von den Stufenprofilen auf kurze, dynamische Leitfähigkeitsboli überzugehen, die erlaubten, die NaCl-Gabe auf das Maß zu verringern, welches aufgrund der technischen Auflösung erforderlich war [RG3]. Hierzu mußten jedoch die notwendigen mathematischen Algorithmen neu zugeschnitten werden. Nach diesem Schritt wurde eine weitere klinische Studie gestartet, die den Zweck verfolgte, das neue Verfahren am Patienten zu verifizieren.

In dieser Studie mit 10 Patienten und 93 Dialysesitzungen, 264 Stufenprofil- und 173 Bolus-Dialysancemesssungen wurde gefunden, daß die Bolus-Messungen ihre zugehörigen blutseitigen Referenzmessungen mit außergewöhnlicher Genauigkeit von 0.06±4.76% trafen. Student’s t-Test für gepaarte Daten ergab, daß sich die Datensätze nicht signifikant unterschieden (p=0.87). Die blutseitige Kt/V-Referenz auf der Basis des equilibrierten Einkompartment-Modells mit variablem Volumen wurde mit 5.32±3.9% getroffen, wobei eine Korrelation von 0.98 erzielt wurde. Die verbleibende Differenz von 5.32% wird der Vernachlässigung der Harnstoff-Erzeugung während der Messung zugeschrieben. Auch das Stufenprofil zeigte trotz seiner Abhängigkeit vom Verteilungsvolumen gegenüber dem gleichen Modell einen mittleren Fehler von 0.05±5% bei einer Korrelation von 0.96. Jedoch konnte es die Vernachlässigung der Harnstoff-Erzeugung nicht korrekt abbilden.

Die kontinuierlich aufgenommenen Daten wurden auch nach dem 2-pool Modell untersucht, welches auch die Harnstoff-Erzeugung enthält sowie eine innere Kompartimentierung des Patienten annimmt und damit die tatsächlichen Verhältnisse

besser beschreibt. Danach weicht das Bolus-Meßprinzip -3.04±14.3% von der Referenz ab (n.s.,p=0.13). Die relativ hohe Standardabweichung wird mit der Komplexität des Modells erklärt.

Weiter ist aus der Theorie zum Na-Transfer eine vereinfachende Methode zur Messung des Na-Verteilungsvolumens abgeleitet worden. Diese Methode wurde in-vitro gegen ein Behältnis mit einer bekannten Menge an Dialysat geprüft. Es wurde ein mittlerer Fehler von -19.9±34% gefunden. Die Korrelation war 0.92 (n.s., p=0.916). Die gleiche Prüfung fand in-vivo gegen das Harnstoff-Verteilungsvolumen statt und ergab einen mittleren Fehler von -7.4±23.2% (r=0.71, n.s., p=0.39). Es hat den Anschein, als würden sich gemäß der Theorie der Dilution die Verteilungsvolumina für Natrium und für Harnstoff scheinbar nur wenig unterscheiden, obwohl sie sich absolut natürlich deutlich unterscheiden. In Anbetracht der erheblichen Vereinfachungen, die bei der Ableitung dieses Ansatzes gemacht wurden, scheint es ausgesprochen ermutigend, auf diesem Weg möglicherweise ein Verfahren entwickeln zu können, welches auf rein elektrolytischem Wege nun nicht mehr nur K, sondern auch V und damit alle zur Quantifizierung gesuchten Größen analytisch ermitteln kann.

Weiter wurde im Rahmen dieser Arbeit anhand der analytisch ermittelten Volumina verglichen, ob man mit Hilfe anthropometrischer Formeln zur Ermittlung des Harnstoff-Verteilungsvolumens zu einer guten Abschätzung kommen kann. Es wurde gefunden, daß man das Ergebnis der Watson-Formel um ca. 13% vermindern kann und dann zu einem recht guten Wert für das tatsächliche Harnstoff-Verteilungsvolumen gelangt. Dies ist jedoch mit Vorsicht und Erfahrung zu tun, da sich damit Kt/V rechnerisch zum Nachteil des Patienten verändert.

Auch ein elektrolytisches Verfahren mit empirischen Komponenten zur Ermittlung des Plasma-Natriums des Patienten wurde erprobt und konnte mit einer Genauigkeit von 4.3±1.2% den Laborwert vorhersagen.

Zusammenfassend kann gesagt werden, daß sich im Rahmen dieser Arbeit die leitfähigkeitsbasierten Methoden zur Messung von einigen wichtigen Dialyseparametern als sehr nützlich für die klinische Praxis erwiesen haben und zudem mit keinem Zusatzaufwand für die Beteiligten verbunden sind.

Das Ergebnis dieser Arbeit ist mittlerweile in größeren Stückzahlen in frei erhältliche Dialysegeräte implementiert worden. Die Erfahrung der ersten Zeit zeigt, daß das verwendete Prinzip von den Klinikern gut angenommen wird.

14. Ehrenwörtliche Erklärung

nach §4, Abs. 3, Ziffer 3, 5 und 8 der Promotionsordnung für die Fakultät für Biologie der Bayerischen Julius-Maximilians-Universität Würzburg i.d.Fssg. vom 15.3.1999.

Hiermit erkläre ich ehrenwörtlich, daß ich diese Dissertationsschrift eigenhändig und ohne Hilfe eines Promotionsberaters angefertigt habe. Ich habe keine anderen als die angegebenen Quellen benutzt.

Diese Dissertationsschrift hat bisher weder in gleicher noch in ähnlicher Form in einem anderen Prüfungsverfahren vorgelegen.

Am 12. Mai 1995 wurde mir von der Christian-Albrechts-Universität zu Kiel der akademische Grad ‚Diplom-Physiker' verliehen.

Am 11. Juni 1991 wurde mir von der Fachhochschule Lübeck der Hochschulgrad ‚Diplom-Ingenieur (Fachhochschule)' verliehen.

Darüber hinaus befand ich mich zwischen 1983 und 1985 an der Universität Hamburg in der Ausbildung zum Lehrer an der Oberstufe allgemeinbildender Schulen mit dem Ziel des ersten Staatsexamens. Ich habe jedoch das Studium vorzeitig aufgrund der damaligen Anstellungssituation für Lehrer abgebrochen, ohne den Versuch einer Abschlußprüfung zu machen.

Darüber hinaus habe keine weiteren akademischen Grade erworben oder versucht, zu erwerben.

Würzburg, den 8. Januar 2002

Rainer Goldau

15. CV

Schule / Dienst	
08/68 – 06/81	Kath. Grundschule HH-Farmsen – Kath. Jungengymnasium St. Ansgar –Gymn. Oldenfelde : Allgemeine Hochschulreife
09/81 – 12/82	Zivildienst
BERUFSAUSBILDUNG	
09/85 – 08/87	Fa. Müter, Hamburg: Gesellenbrief Zentralheizungs- und Lüftungsbau
STUDIUM	
03/83 – 07/85	Uni Hamburg: Lehramt Oberstufe – Fächer Physik, Biologie (Aufgabe aufgrund der damaligen schlechten Aussichten auf eine Anstellung)
09/87 – 06/91	FH Lübeck: Abschluß als Dipl-Ing. (FH) für physikalische Technik
10/91 – 05/95	Uni Kiel: Abschluß als Dipl.-Physiker Diplomarbeit: Darstellung von Oberflächenplasmawellen (Surface polaritons). Studienschwerpunkte: Laserspektroskopie, Mathematik, Optik, Elektronik.
PRAKTIKA	
03/90 – 04/91	Medizinisches Laserzentrum Lübeck: Fachpraktikum und externe FH-Diplomarbeit: Bau einer schnellen analogen Rechenschaltung zur Abschaltung von Festkörperlasern in der Laserchirurgie, um Perforationen zu vermeiden (Auge, Gefäße)
BERUFSTÄTIGKEIT	
07/91 – 12/93	Medizinisches Laserzentrum Lübeck: Projektingenieur Optik, Elektronik, klinische Versuche Mitarbeit in der Grundlagenforschung zur Laseranwendung in der Gefäßheilkunde sowie der Augenheilkunde, insbesondere bei der Anwendung spektroskopischer Methoden.
08/95 – 02/01	Fresenius Medical Care: Entwicklungsphysiker und Projektleiter in Abt. F&E, Gruppenleitung “Therapy Improvement” Entwicklung, klinische Erprobung und Serienimplementierung von neuen Verfahren der Dialysequantifizierung sowie weiterer Verfahren zur Messung von Dialyseparametern. Leitung einer Gruppe von

	Physikern, die sich mit der theoretischen Modellierung der Dialyse befaßt. Betreuung von Diplomarbeiten (FH, Uni) Berufsbegleitend: Verfassen der vorliegenden Dissertationsschrift
ab 03/01	Euroimmun GmbH: Entwicklungsabteilung Analysesysteme. Entwicklung immunologischer Analysesysteme. Berufsbegleitend: Verfassen der vorliegenden Dissertationsschrift
FAMILIENSTAND	
	Verheiratet, Änderung des Geburtsnamens Otto in den heutigen Namen Goldau. Vater von 3 Kindern.

16. Veröffentlichungen des Autors

Oral presentations

Goldau R, Twilfer H, Zwaan M, Morrin M, Marquardt U, Birngruber R.
Spectroscopical in vivo discrimination of artheriosclerotic plaque in human abdominal arteries. Held at Kongreß der Deutschen Gesellschaft für Lasermedizin, Nov. 1992

Kuhlmann U, Goldau R, Samadi N, Graf T, Orlandini G, Lange H.
Accuracy and safety of online clearance monitoring based on conductivity variation.
Held at the EDTA (European Dialysis and transplant assc.) convention, Madrid, 1999 (within best eight abstracts)
Published in Nephrol Dial Transplant (2001) 16: 1053-1058

Abstracts

Goldau R, Twilfer H, Zwaan M, Morrin M, Marquardt U, Birngruber R.
Spectroscopical in vivo discrimination of artheriosclerotic plaque in human abdominal arteries. In: Willital GH Maragakis RR Lehmann LASER 92 ABSTRACTS; Reihe Medizin, S109, Shaker Aachen

Di Filippo S, Goldau R, Manzoni C, Spickermann R, Marcelli D, Pontoriero G, Orlandini G, Locatelli F. Accurate and inexpensive online monitoring of hemodialysis performance. Abstract at the ASN (American Society of Nephrology) convention, Philadelphia, USA, 1998

Di Filippo S, Goldau R, Manzoni C, Spickermann R, Marcelli D, Pontoriero G, Orlandini G, Locatelli F. Effective Urea clearance and Ionic Dialysance by conductivity: What is the relationship. Abstract at the ASN (American Society of Nephrology) convention, Philadelphia, USA, 1998

Kuhlmann U, Goldau R, Samadi N, Graf T, Orlandini G, Lange H.
Online clearance monitoring of high-flux hemodialysis based on conductivity variation. Abstract at the ASN (American Society of Nephrology) convention, Philadelphia, USA, 1998

Kuhlmann U, Goldau R, Samadi N, Graf T, Orlandini G, Lange H.
Accuracy and safety of online clearance monitoring based on conductivity variation.
EDTA (European Dialysis and transplant assc.) convention, Madrid, 1999
Diese Kongreßveröffentlichung wurde mit einer Auszeichung versehen, da sie zu den 8 Besten von mehr als 1700 gehörte.

Publications

Goldau R, Twilfer H, Zwaan M, Morrin M, Marquardt U, Birngruber R . In-vivo fluorescence spectroscopy of abdominal and iliac arterial sclerosis in humans. Laser in medicine and surgery Vol.11, S212-218 (1995)

Wetzel W, Otto R*, Falkenstein W, Schmidt-Erfurt U, Birngruber R.
Development of a new Er:YAG laser conception for laser sclerostomy ab externo: experimental and first clinical results. German J Ophthalmol Vol. 4 S283-288 (1995)
** Hinweis: Der Geburtsname des Autors lautete Otto. Der Name Goldau wurde 1994 durch Heirat angenommen*

Kuhlmann U, Goldau R, Samadi N, Graf T, Orlandini G, Lange H
Accuracy and safety of online clearance monitoring based on conductivity variation. Nephrol Dial Transplant (2001) 16: 1053-1058

Goldau R, Kuhlmann U, Samadi N, Gross M, Graf T, Orlandini G, Marcelli D, Lange H. Ionic dialysance measurement is urea distribution volume dependent: A new approach to better results. Artif Organs 2002, in press, scheduled for April 2002

Patente

Hinweis: Diese Liste entstammt der Datenbank der Fa. Delphion, Lisle, IL, USA (ehemals IBM). Sie stellt den veröffentlichten Stand von 6/2001 dar. Aufgrund von Einsprüchen sowie eventuell nicht mehr entrichteter Patentgebühren können Patente erlöschen. Ebenso können neu aufgenommene Datensätze hinzukommen. Diese können hier jedoch nicht angegeben werden, da die Rechte an den Schriften nicht beim Autor liegen. Bei den meisten der Schriften ist der Autor Alleinerfinder, bei 3 Schriften wurde die Erfindung gemeinsam mit Anderen veröffentlicht.

Searchpattern: Goldau & A61

Reg Nr	Date	Title of patent
EP01062960A2	12/27/2000	METHOD FOR DETERMINING THE PERFORMANCE OF A DIALYSER AND DIALYSIS APPARATUS THEREFOR
DE19928407C1	10/26/2000	VERFAHREN ZUR BESTIMMUNG DER LEISTUNGSFAEHIGKEIT EINES DIALYSATORS EINER DIALYSEVORRICHTUNG UND DIALYSEVORRICHTUNG ZUR DURCHFUEHRUNG DES VERFAHRENS
US06126831	10/03/2000	METHOD AND DEVICE FOR DETERMINING HEMODIALYSIS PARAMETERS
US06077443	06/20/2000	METHOD AND DEVICE FOR MONITORING A VASCULAR ACCESS DURING A DIALYSIS TREATMENT
AU05156999A1	02/01/2000	METHOD FOR DETERMINING DIALYSANCE AND DEVICE FOR CARRYING OUT THE METHOD
DE19831385A1	01/27/2000	VERFAHREN ZUR BESTIMMUNG VON PARAMETERN DER HAEMODIALYSE UND VORRICHTUNG ZUR DURCHFUEHRUNG DES VERFAHRENS
EP00898974A3	10/27/1999	BLOOD TREATMENT APPARATUS INCLUDING A DEVICE TO DETERMINE HEMODIALYSIS PARAMETERS AND A METHOD FOR THEIR DETERMINATION
DE19746367C2	08/26/1999	VERFAHREN ZUR IN-VIVO-BESTIMMUNG VON PARAMETERN DER HAEMODIALYSE UND VORRICHTUNG ZUR DURCHFUEHRUNG DES VERFAHRENS
JP11137667A2	05/25/1999	METHOD FOR DECIDING PARAMETER OF LIQUID DIALYSIS AND BLOOD TREATING DEVICE WITH DECIDING FUNCTION OF PARAMETER OF LIQUID DIALYTIC
JP11104233A2	04/20/1999	MONITORING OF INLET AND OUTLET PORT OF BLOOD VESSEL DURING DIALYSIS TREATMENT AND APPARATUS FOR DIALYSIS TREATMENT HAVING EQUIPMENT FOR MONITORING INLET AND OUTLET PORT OF BLOOD VESSEL
EP00900094A1	03/10/1999	PROCESS AND DEVICE FOR MONITORING RECIRCULATION DURING AN EXTRACORPOREAL BLOOD TREATMENT
EP00898974A2	03/03/1999	BLOOD TREATMENT APPARATUS INCLUDING A DEVICE TO DETERMINE HEMODIALYSIS PARAMETERS AND A METHOD FOR THEIR DETERMINATION
EP00895787A1	02/10/1999	METHOD FOR MONITORING A BLOOD VESSEL ACCESS DURING A DIALYSIS TREATMENT AND APPARATUS FOR DIALYSIS TREATMENT WITH A DEVICE FOR MONITORING A BLOOD VESSEL ACCESS
DE19739100C1	02/04/1999	VERFAHREN ZUR BESTIMMUNG DER MAXIMALEN DIALYSANCE DES DIALYSATORS EINER DIALYSEVORRICHTUNG UND DIALYSEVORRICHTUNG ZUR DURCHFUEHRUNG DES VERFAHRENS
EP00894013A1	02/03/1999	PROCESS AND DEVICE FOR DETERMINING HEMODIALYSIS PARAMETERS
JP10314299A2	12/02/1998	METHOD FOR DETERMINING PARAMETER RELATING TO MATERIAL EXCHANGE OF BLOOD DIALYSIS AND DEVICE EXECUTING THE SAME
DE19734992C1	10/01/1998	VERFAHREN ZUR IN-VIVO-BESTIMMUNG VON PARAMETERN DER HAEMODIALYSE UND VORRICHTUNG ZUR DURCHFUEHRUNG DES VERFAHRENS
DE19734002C1	09/17/1998	VERFAHREN ZUR UEBERWACHUNG EINES GEFAESSZUGANGES WAEHREND EINER DIALYSEBEHANDLUNG UND VORRICHTUNG ZUR DIALYSEBEHANDLUNG MIT EINER EINRICHTUNG ZUR UEBERWACHUNG EINES GEFAESSZUGANGES
DE19746367A1	06/04/1998	VERFAHREN ZUR IN-VIVO-BESTIMMUNG VON PARAMETERN DER HAEMODIALYSE UND VORRICHTUNG ZUR DURCHFUEHRUNG DES VERFAHRENS
EP00845273A1	06/03/1998	METHOD AND MEANS FOR IN VIVO DETERMINING HAEMODIALYSIS PARAMETERS

17. Betreuende Hochschullehrer

Diese Arbeit wurde betreut von

Professor Dr. Roland Benz, Fakultät für Biologie, Lehrstuhl für Biotechnologie, Theodor-Boveri-Institut der Universität Würzburg (erster Gutachter).

sowie

Professor Dr. Harald Lange, Zentrum für Innere Medizin, Klinik für Nephrologie der Universität Marburg (zweiter Gutachter).