Biomedical Engineering
BSc Thesis

Investigating the difference in uremic toxin removal between single-pass and recirculation dialysis experiments, using in-house filters

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Abstract

For patients with chronic kidney disease (CKD) haemodialysis therapy is an often life-saving therapy which can replace the filtering capacities of the kidney. Although this therapy is mostly effective, it also greatly reduces the autonomy of patients. The patients have to be treated at the hospital multiple times a week for multiple hours. One important reason for this is the need of a large dialysis volume by current dialysis machines, making it very difficult to treat the patient at home. This study is concentrated on the improvement of current haemodialysis therapy, focusing on the usage of a smaller dialysate volume and investigating the influence on the effectiveness of the therapy. To do this, several experiments have been carried out using a smaller dialysate volume and a small-scale haemodialysis set-up. The current single-pass dialysis system, using a large amount of dialysate volume, is compared to a recirculation dialysis system, which reuses a smaller amount of dialysate.

Experiments with the toxins creatinine, hippuric acid (HA) and indoxyl sulfate (IS) have successfully been carried out. A small-scale Convergence set-up has been used in combination with in-house made modules with Fresenius FX1000 fibers and human plasma spiked with toxins. Measurements of four hours have been carried out and at different moments samples of the plasma and dialysate have been taken. The total removal after four hours, the clearance/dialysance and the toxin concentrations over time have been calculated using the obtained toxin concentrations. The creatinine measurements showed better removal by the single-pass system, compared to the recirculation system. The decrease in creatinine concentration in the plasma and the total removal of creatinine by the single-pass system were more efficient, compared to the recirculation system. This difference was significant. For the HA measurements the single-pass system showed a slightly better decrease of HA after four hours, however due to the large standard deviation this difference was not significant. This was also the case for the total removal of HA. For the IS measurements both systems showed similar removal. Due to the standard deviation being relatively large, no conclusion can be given about which system was more effective. However, the HA and IS results show that for these toxins the removal by the two different systems is very similar.

Contents

Acronyms

Aeff Effective surface area

ARF Acute renal failure

CAPD Continuous ambulatory peritoneal dialysis

CCPD Continuous cycling peritoneal dialysis

Cd Concentration of toxin in the dialysate solution

CKD Chronic kidney disease

CL-d Clearance for dialysis solution

CL-p Clearance for plasma

Cp Concentration of toxin in the plasma

CWF Clean water flux

DCT Distal convoluted tubule

DL-d Dialysance for dialysate solution

DL-p Dialysance for plasma

GFR Glomerular filtration rate

HA Hippuric acid

HPLC High-performance liquid chromatography

IOF Inside-out filtration

IS Indoxyl sulfate

MMM Mixed matrix membrane

OIF Outside-in filtration

PCT Proximal convoluted tubule

PD Peritoneal dialysis

PES Polyethersulfone

Psu Polysulfone

PVP Polyvinylpyrolidone

Qd Flow of dialysate solution

Qp Flow of plasma

TMP Transmembrane Pressure

Xd Amount of toxins removed from the dialysate solution

Xp Amount of toxins removed from the plasma

1 Introduction

1.1 Relevance

Chronic kidney disease (CKD) is a growing public health problem associated with high mortality, a low quality of life and high costs for adequate care [1][2]. It is estimated that currently around ten percent of the world population lives with a form of CKD. Next to this CKD is estimated to become the fifth largest cause of death in the world by 2040 [3]. The kidneys are critical for filtering toxins and excess fluid from the blood, next to other metabolic functions. For patients with CKD the kidney functions are lost, which can cause accumulation of toxins in the blood with all negative side-effects that come with this. For most patients with end-stage-renal disease, kidney transplantation would be the most effective solution. However, the number of available kidney transplants only amounts ten percent of the total amount of patients who need them [4]. As an alternative, haemodialysis using a semi-permeable membrane is used to replace the filtering functions of the kidney. Haemodialysis is an often life-saving therapy for patients, but negatively influences the patients autonomy because for many patients the treatment takes place at the hospital three times a week for four hours [5]. Also, the current haemodialysis treatment at the hospital uses a large volume of water to filter the blood [2]. When looking at the current importance of sustainability and waste-reduction, a smaller usage of water for the treatment would be favourable. A wearable-kidney-device with a smaller dialysate volume would be a viable solution, however adequate toxin removal using a smaller dialysate volume is still a challenge. Considering the worldwide magnitude of CKD, the high burden of disease for patients and the need for more sustainable treatments in the future, research is needed to improve the current haemodialysis treatment.

1.2 The healthy kidneys

The kidneys are located just below the rib-cage of the human body and carry out several important functions [6]. One of these functions is filtering the blood of toxins and excess fluid. The kidneys receive blood from the renal arteries, which arise from the abdominal aorta. The kidneys are made out of nephrons which filter the blood. The nephrons consist out of a glomerulus and a tubule. This is also shown in Figure 1. The glomerulus can be seen as a collection of small blood vessels, which receives blood from vessels that branch from the renal artery. The walls from the glomerulus are very thin, which makes is possible for small molecules, wastes and fluid to pass through the blood vessel wall to the tubule of the nephron. Larger molecules, such as red blood cells, are not able to pass the blood vessel wall. Hydrostatic pressure from the glomerular capillaries creates the filtration force, which pushes water and solute through the membrane [7]. The hydrostatic pressure is created by the difference in diameter of the afferent and efferent capillaries. The afferent capillaries, which carry blood to the glomerulus, have a bigger diameter than the efferent cappillaries, which drain the glomerulus of blood. This causes the pressure and filtration force which is needed. The volume of liquid that is filtered per minute is given bij the glomerular filtration rate (GFR).

The renal tubule now carries the small molecules, waste and fluid from the glomerulus [6][7]. The part that leaves the glomerulus is called the proximal convoluted tubule (PCT). Fluid in this tubule then flows to the descending limb and next to the ascending limb. Finally the fluid flows from the distal convoluted tubule (DCT) to the collecting duct. Next to the tubule a blood vessel is located. The fluid inside the tubule moves along the tubule and at the same time water and small molecules are reabsorbed into the blood vessel next to the tubule. The reabsorbed small molecules are molecules the human body needs, such as nutrients and minerals. The fluid and toxins that remain in the tubule become urine. The filtered blood leaves the kidney through the renal vein.

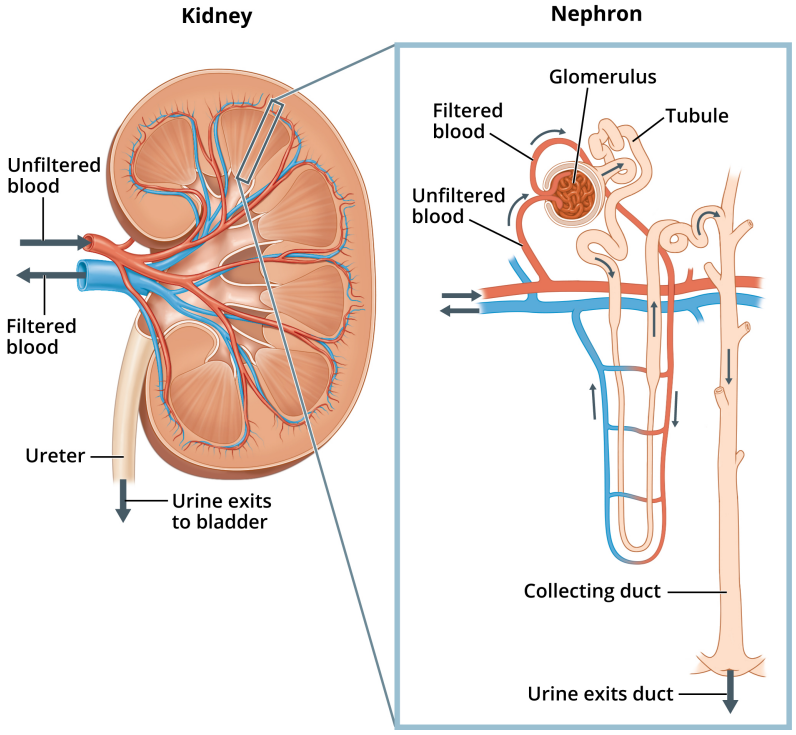


Figure 1: An image of the nephron and its place in the kidney [6].

Next to filtering the blood from toxins and excess fluids, the kidneys have more important functions [6]. They are also needed to remove acid that is produced by the body and influence the concentration of several salts and minerals in the blood. The formation of hormones is another important function of the kidneys [8]. Erythropoeitin is secreted by the kidney and needed for the formation of new red blood cells in the red bone marrow. Renin is another hormone secreted by the kidneys. This hormone is secreted if the blood pressure drops, because it causes blood vessels to constrict which increases the blood pressure. The kidneys are also used to activate vitamin D which is important for many functions, like maintaining a healthy bone structure and muscle function.

1.3 Kidney disease

Two main types of renal diseases can be described: acute renal failure (ARF) and chronic kidney disease (CKD) [9]. Acute renal failure is characterized by a sudden loss or decrease of renal functions, which causes a build up of fluid and waste products in the blood. Some causes of ARF can be inadequate blood flow to the kidney, nephron damage or obstruction of blood or fluid flow after the kidney. ARF is reversible, however in the case of inadequate recovery it can lead to CKD.

Chronic kidney disease is characterized by a gradual loss of kidney function, causing a decrease in the glomerular filtration rate (GFR) [9]. By international guidelines CKD is described as sustained damage to the kidney which can be seen by structural or functional changes to the kidney or/and a GFR of less than 60 ml/min per 1,73 m^2 body surface for three months or more. Risk factors which can cause the development of chronic kidney disease are diabetes, high blood pressure, heart disease and a family history of kidney disease [10]. Some other possible causes of CKD are glomerulonephritis or an infection of the urinary tract [9]. Diabetes and high blood pressure are the two most important causes of CKD [10]. For patients with diabetes, almost one in every three patients develops CKD. The high glucose level in the blood, which is associated with patients who have diabetes, damages the filtering capacities of the kidney. In the case of high blood pressure the higher pressure inside the blood vessels can damage the vessels inside the kidney, resulting in CKD.

Patients with CKD will only notice symptoms when more than 75 percent of the kidney function is lost [9]. When 90 to 95 percent of the kidney function is lost, renal replacement therapy is necessary to treat the patient. Chronic kidney disease is divided into five stages, to indicate the severity of the disease for each patient [11]. This can be seen in Figure 2.

By carrying out a blood and urine test a patient can be tested for kidney failure [10]. Using a blood test the GFR of a patient can be calculated, by looking at the amount of creatinine in the blood. Higher levels of creatinine indicate insufficient filtering of creatinine by the kidneys. Using a urine test the presence of albumin in the urine is examined. Damage to the kidney can cause it to let albumin pass into the urine, while healthy kidneys prevent this. Presence of albumin in the urine, also called albuminuria, therefore indicates kidney failure.

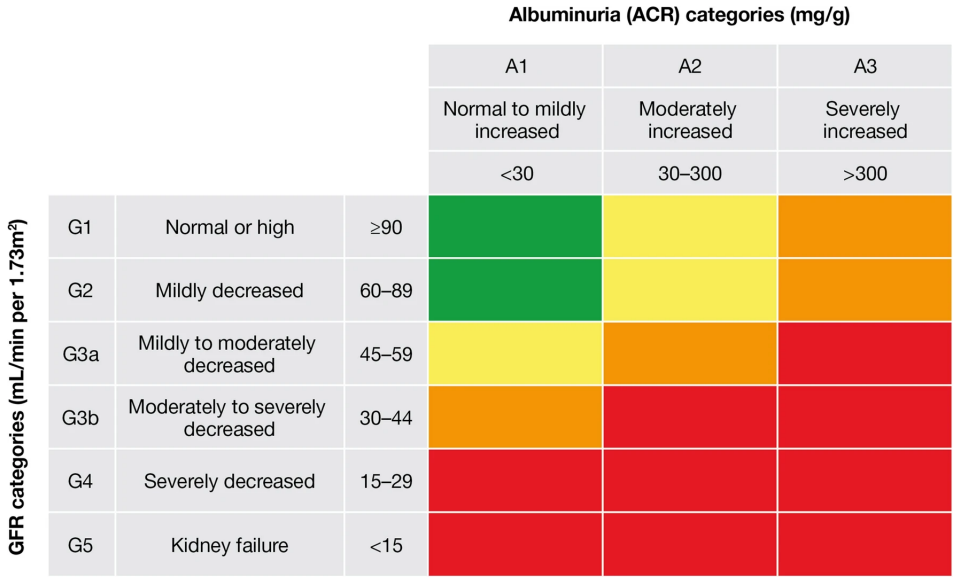


Figure 2: Different stages of chronic kidney disease according to the glomerular filtration rate (GFR) and albuminuria [11].

1.4 Types of treatments

For patients with relatively less severe CKD, the disease can often be helped to reduce symptoms and prevent further damage to the kidneys. This is often done with lifestyle changes, like diet, physical activity, and medication [12]. It is important for these patients to prevent a high blood pressure. When the kidneys have less than 15 percent left of the normal kidney function, the patient has end-stage-renal disease. To replace the function of the kidney the patient can receive haemodialysis, peritoneal dialysis or have a kidney transplant.

1.4.1 Haemodialysis

Haemodialysis is a therapy that replaces the filtering capacities of the kidneys, using a dialyzer which contains a filter [13]. The process can be seen in Figure 3 and 4. Two needles are placed in the arm of the patient, one carrying the blood towards the haemodialysis machine and one carrying the blood from the machine back to the patient. To gain access to the blood vessels an access needs to be made, which can be done using a fistula or graft [14]. For temporary access a catheter in the neck can also be used. With a small surgery a fistula can be made by joining an artery and vein in the arm. A graft can be made by surgically placing a soft tube inside the arm which connect a vein and an artery. When access to the blood

vessels in the arm is created, blood can flow from and to the dialysis machine. During haemodialysis the blood flows from the body to the dialyzer. It then flows through the hollow fibers of the filter, while a dialysis solution flows outside of the fibers in opposite direction. By diffusion waste products are removed from the blood and pass to the dialysate solution. The filtered blood then returns to the body. The haemodialysis treatment is for most patients carried out at the hospital, for three times a week for four hours [5].

Figure 3: Haemodialysis therapy at the hospital[15].

Dialysis filtration of toxins can be driven by the principle of diffusion or convection [16]. Current haemodialysis therapy uses diffusion as method to remove solutes and toxins from the blood. Solutes move from a high concentration to a lower concentration through a semipermeable membrane. This can also be seen in Figure 5. This method is effective for the removal of small water-soluble uremic toxins, but less effective for bigger and/or protein-bound uremic toxins. Hemodiafiltration uses a combination of diffusion and convection for the removal of uremic toxins, which can also be seen in Figure 5. Convection takes place when a transmembrane pressure is applied, causing fluid movement across the membrane from one side to the other [17]. In this situation the solutes which are dissolved in the fluid are also carried across the membrane. This type of removal is also called ultrafiltration. During hemodiafiltration plasma water volume is removed using ultrafiltration. The same volume of fluid is infused into the blood as replacement fluid. This infusion of fluid can be done at several places of the extracorporal circuit with the dialyzer [18]. Two possibilities of this are pre- and post-infusion. With pre-dilution hemodiafiltration the infusion occurs at the arterial side of the dialyzer, which runs towards the dialyzer. Post-dilution occurs at the venous side of the dialyzer, which runs away form the dialyzer to the body.

The hemodiafilter which is used in hemodiafiltration needs to have some specific characteristics to be able to be used for this technique [18]. The sieving coefficient, which indicates the potential for specific solutes to pass the membrane, needs to have a specific value which allows removal by convection, but reduces the removal of albumin from the blood. Next to this, the membrane should have a high permeability to allow ultrafiltration to occur. Finally, the fiber membrane should have an optimal geometry to reduce the effects of an increased concentration of cells and solids in the blood on the fiber, due to fluid loss. Next to the characteristics of the membrane, ultrapure water is also needed to perform hemodiafiltration treatment as substitution fluid [16]. Hemodiafiltration is said to be more effective for removal of middle- and large-molecular weight solutes, compared to haemodialysis, and is currently also used as a dialysis technique. However, more research needs to be done to gain more evidence for the full and accurate comparison of

haemodialysis and hemodiafiltration.

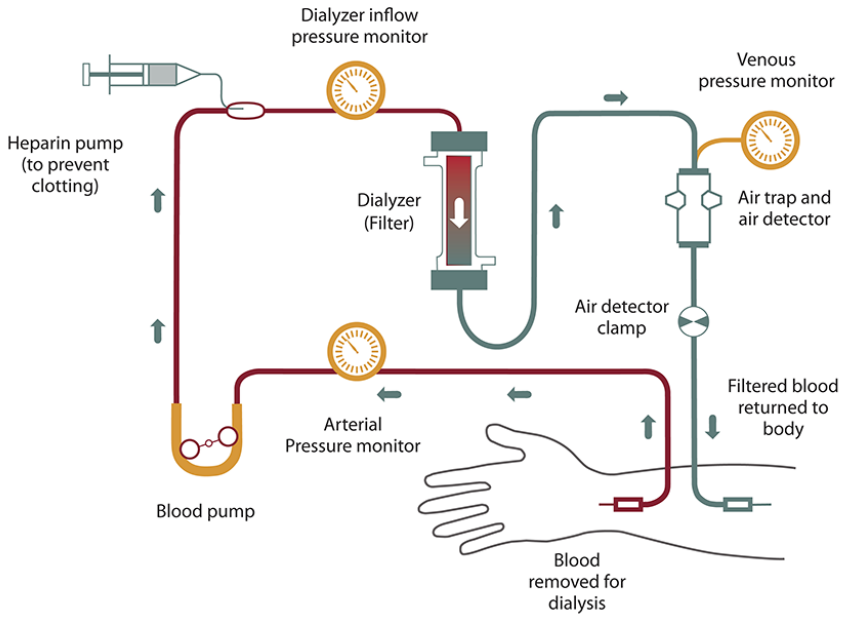


Figure 4: The process of haemodialysis therapy [13].

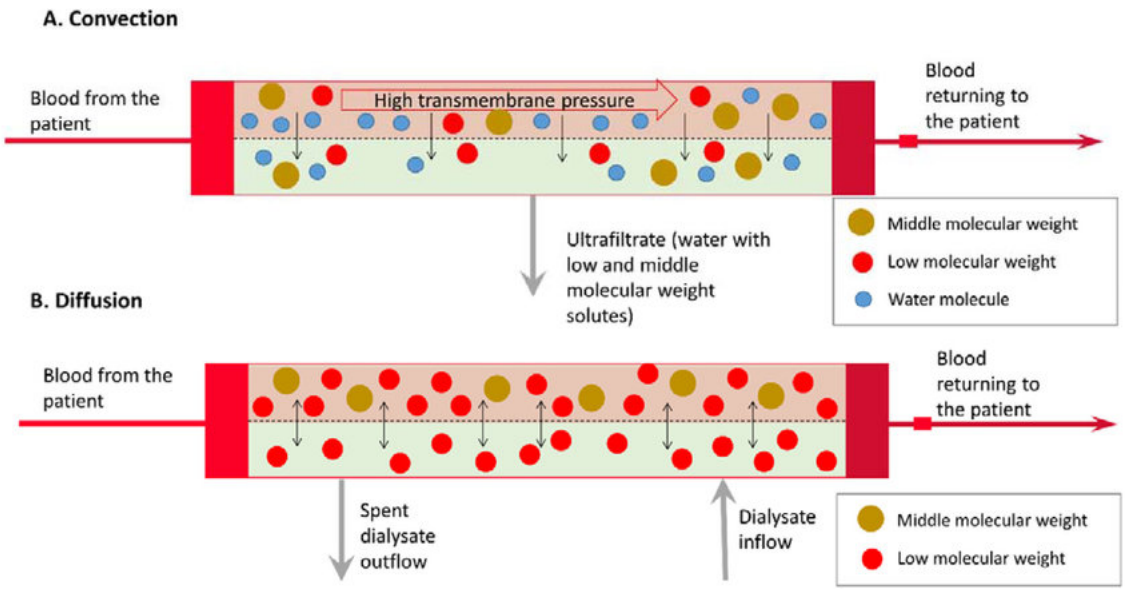


Figure 5: Toxin removal by diffusion or convection [19].

1.4.2 Peritoneal dialysis

Peritoneal dialysis (PD) is a type of kidney failure treatment which is partly carried out at home. Nine percent of all kidney replacement therapies globally is performed by peritoneal dialysis [20]. Peritoneal dialysis treatment uses the lining of the abdomen, also called the peritoneum, to filter the blood [21]. Some important advantages of PD compared to haemodialysis is the benefit of home-based treatment, technical simplicity, generally better cost-effectiveness, lack of need for vascular access in the arm and possibly improved survival in the first years of kidney replacement therapy. Disadvantages of this method include the risk of infection like peritonitis, the increased risk for a hernia and weight gain.

A couple of weeks before the patient can start peritoneal dialysis a catheter is placed in the abdomen using surgery [21]. For every therapy a bag of dialysate solution is connected to the catheter, which lets the solution pass into the abdomen. Fluid and waste products in the blood diffuse through the peritoneum, into the dialysate solution [22]. After a couple of hours the dialysate solution is drained out of the body. Two types of peritoneal dialysis can be used to treat a patient: continuous ambulatory peritoneal dialysis (CAPD) and continuous cycling peritoneal dialysis (CCPD). Using CAPD the patient fills and drains the abdomen using dialysate solutions for 3-4 times a day. During the night the solution is not drained, but stays in the abdomen until the morning. With CCPD the patient is connected to a machine which fills the abdomen with dialysate solution and drains it, multiple times during the night. The advantage of this method is that the patient does not need to drain the dialysate solution during the day.

1.4.3 Kidney transplantation

For patients with end-stage renal disease a kidney transplantation is a possible treatment for their kidney failure [23]. Kidney transplantation is often the preferred treatment for these patients, because it increases the chance for long-term survival compared to patients who only receive dialysis therapy. Next to this, CKD patients who receive a kidney transplantation as treatment often have a better quality of life. Patients who want to receive kidney transplantation are screened to determine whether the patient is able to tolerate surgery and immunosuppressive medication. If the patient is able to receive a kidney transplant he or she will be placed on the waiting list [24]. While a patient is on the waiting list dialysis might be needed to treat the patient during the waiting period. The kidney that is transplanted can be originated from a deceased or living donor. Diseased donors can either donate after reaching the criteria for being brain dead, or after cardiac death [23]. Receiving a kidney from a living donor creates the highest chance for survival of the patient and the organ. When a patient has received a kidney he or she will have to start taking immunosuppressive medication, to prevent the body from rejecting the donor kidney [24].

1.5 Haemodialysis filter membranes

As explained before, haemodialysis is a therapy which replaces the filtering capacities of the kidney. To perform haemodialysis therapy, a dialyzer containing hollow fibers is used [5]. The hollow fibers are made of a specific kind of membrane, which should have good blood compatibility, selectivity and resistance to fouling. Dialysis membranes can be classified according to their material composition [25]. They are categorized as cellulosic or synthethic, with synthetic membranes as the kind of membranes that are currently most often used. Next to this, dialysis filters can be classified as high- or low-flux. Dialysis filters are categorized as high flux if they have an Kuf-value of more than 14 ml/h/mmHg and can clear β-2-microglobulin with more than 20 ml/min. Dialysis filters are categorized as low-flux if they have a β-2-microglobulin clearance of less than 10 ml/min. The Kuf gives the value of the hydraulic permeability of the membrane, which is defined as the level of water permeability across the surface of the membrane.

When comparing cellulosic and synthethic dialysis membranes, several differences can be seen [26]. Cellulosic membranes are often relatively thin, around 10 μm, while synthetic membranes are often around 20 μm. Next to this cellulosic membranes have a symmetric structure of the fiber wall, with an equal pore size in all the membrane layers. Synthethic membranes can have either a symmetric or asymmetric structure of the fiber wall. The asymmetric membrane structure of synthetic membranes is characterized by a thick outer support layer, with often a sponge-like structure, and a thin high-density inner layer. This can also be seen in Table 4 (Methods). The specific rippled surface of synthetic membranes creates a better dialysate flow, which increases dialysis efficiency.

Most synthethic polymers which are currently used for membrane fabrication are hydrophobic, which makes it necessary to use co-polymers or additives to make them more hydrophilic [26]. The most com-

monly used polymers to create this type of membrane are polysulfone (Psu) and polyethersulfone (PES) [5]. These two hydrophobic polymers are often blended with hydrophilic polymers like polyvinylpyrolidone (PVP). The hydrophobic and hydrophilic areas on the membrane are effectively distributed, to prevent adsorption of blood proteins. In the experiments of this study, modules based on the hollow fibers of the FX1000 dialyzer from Fresenius Medical Care are used (Table 1). This dialyzer contains a specific Helixone ® membrane, which is based on a polysulfone membrane [5]. This Helixone ® membrane is an improved version of the previous polysulfone membrane. The improved membrane has a smaller wall thickness (35 nm) and diameter (185 μm). By using new nanotechnology process membranes with a more uniform pore distribution and homogeneous pore size can be created. The average pore size of the membrane is also increased from 3.1 nm to 3.3 nm.

Figure 6: Cross-section image of the FX1000 dialyzer.

Figure 7: Cross-section image of the FX1000 dialyzer, with a better view of the dialyzer fibers.

Figure 8: The Fresenius Medical Care FX1000 Dialyzer.

Table 1: Characteristics of the Fresenius Medical Care FX1000 hemodiafilter.

Hemodiafilter FX1000	
Ultrafiltration coefficient (Kuf) (ml/h*mmHg)	75
Effective surface area (m^2)	2.2
Membrane material	Helixone ®
Housing material	Polypropylene
Sterilization method	INLINE steam
Application	HD/HDF
Clearance (QB 300ml/min, QF 75ml/min)	
Creatinine	280 ml/min

With the use of hollow-fiber spinning, the polymers are used to fabricate dialyzer fibers. Several spinning techniques are available [27]. In the process of spinning a spinneret is used, which contains a needle through which a polymer solution is ejected. The polymer is ejected in a hollow cylindrical shape and solidifies into a membrane. This process is also called phase inversion. One type of fiber-spinning technique which can be used to fabricate hollow fiber membranes, is solution spinning [27]. This type of spinning can be either wet or dry-wet spinning. In wet-spinning the polymer solution, also called the polymer dope, is pushed out of the spinneret needle in a hollow cylindrical shape. Inside the cylindrical shape a fluid is pushed out, also called the bore fluid. The hollow fiber moves from the spinneret into a coagulation bath which contains a non-solvent liquid. In this process solvent is exchanged from the polymer solution to the coagulant, causing phase inversion and solidification of the fiber.

During the solidification in the wet-spinning process irregularities in the fiber can be formed [27]. This formation of irregularities is reduced by using another technique, the dry-wet spinning. In dry-wet spinning a polymer solution is also pushed through the needle of the spinneret into the coagulation bath, just like with wet-spinning. However the set-up contains an air-gap between the coagulation bath and spinneret. This air-gap in dry-wet spinning causes stress-relaxation of the polymer chains, reducing the formation of irregularities in the fiber. The fiber moves from the spinneret into the coagulation bath to the godet rollers and is finally collected. An overview of the dry-wet spinning technique can be seen in Figure 9. Several spinning conditions can influence the quality of the hollow fibers, such as the spinneret design, type of bore fluid and the travelling distance. Currently the dry-wet spinning technique is often used for the fabrication of dialyzer hollow-fiber membranes, also due to its improved characteristics compared to wet spinning.

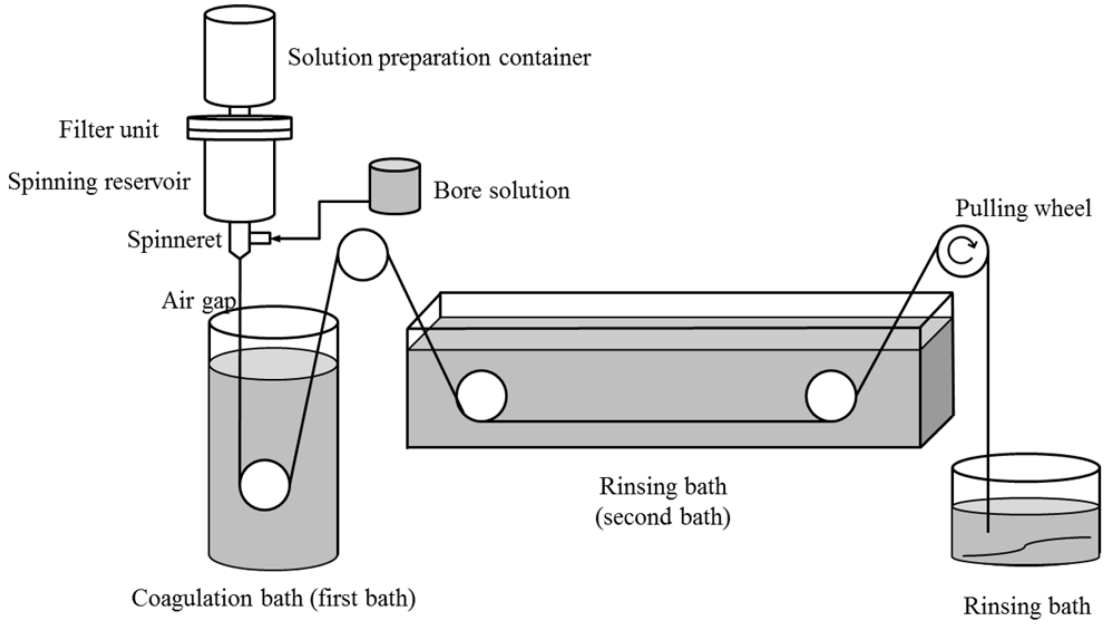


Figure 9: Overview of the dry-wet spinning method used to fabricate hollow fiber membranes [28].

A lot of improvements can still be made to the current haemodialysis membranes to further better the quality of life of CKD patients. Current haemodialysis membranes for example have a high removal of small water-soluble solutes and toxins, but are not able to sufficiently remove middle and large solutes and toxins [2]. Next tot this protein bound toxins are also still difficult to remove using current membranes. Ideally patients with CKD would also receive continuous haemodialysis, just like a healthy kidney. This would reduce the negative side effects that come with discontinuous haemodialysis treatment, like the accumulation of fluid and toxins in the body and the burden of travelling for treatment CKD patients currently have. To be able to provide continuous treatment, haemodialysis membranes should have long-term blood compatibility and should prevent blood clotting.

1.6 Uremic toxins in the blood

A loss of kidney function due to chronic kidney disease can cause the accumulation of uremic toxins in the blood. In a healthy patient these toxins would be removed by the kidneys, however in a patient with CKD this removal is not sufficient. According to the European Uremic Toxins Work group (EUTox) database more than 153 uremic compounds are characterised. These uremic toxins can be divided into three groups: small water-soluble compounds with a molecular weight of less than 500 Da, middle molecules with a molecular weight of more than 500 Da and protein-bound compounds [29].

1.6.1 Small water-soluble and middle molecules

The small water-soluble toxins do not bind to proteins and can be easily removed using haemodialysis. Examples are creatinine and urea. Creatinine is formed by creatine and the creatine phosphate metabolism [30]. Most of the creatine in the human body can be found in skeletal muscle, which causes most of the creatinine to be formed here. In the healthy kidney creatinine can be easily removed from the blood using glomular filtration, due to its low molecular weight. Next to this, creatinine can also be removed using tubular secretion. Of all uremic toxins creatinine is the most often used to assess the kidney function. This is also why the toxin creatinine will be used in the experiments described in this study. Compared to small water-soluble molecules middle molecule toxins, such as β-2-microglobulin, are more difficult to remove using haemodialysis. They require membranes with large pores to be able to remove them. Middle

molecule toxins are an important cause of the high mortality rate of CKD patients and can negatively influence many organs and bodily functions. Some middle-molecule toxins can for example contribute to structural damage of the kidney or can contribute to cardiovascular complications [29].

1.6.2 Protein-bound uremic toxins

Protein-bound uremic toxins are formed due to the metabolism of dietary proteins [29]. These toxins can have many harmful effects in the body. They are also difficult to remove using haemodialysis, because they can stay bounded to the proteins in the blood. The membrane pores of the haemodialysis filter are too small for complete proteins to pass, therefore the toxins bound to proteins are not removed using diffusion in haemodialysis. Only the unbound fraction of the toxins is removed. Examples of protein-bound uremic toxins are indoxyl sulfate and hippuric acid, which are both also used in the experiments of this study.

Indoxyl sulfate (IS) is originated from the degradation of the amino acid tryptophan in the intestine [29]. 90 percent of IS in the blood is bound to the protein albumin, which makes it difficult to remove during haemodialysis. In the healthy kidneys IS is removed using tubular secretion [31]. An equilibrium for bound and unbound IS exists in the blood. In a healthy kidney the unbound IS is transported from the blood to the proximal tubule of the kidney, using organic anion transporters. When an unbound IS molecule is transported out of the blood, the equilibrium causes another IS molecule to separate from the protein, also becoming an unbound toxin. The tubular secretion that is performed by the kidney is not yet possible to perform using haemodialysis. High concentrations of IS in the blood of CKD patients have been associated with several negative effects on the body. It can for example be a contributing factor to kidney disease progression and is associated with vascular disease.

Hippuric acid (HA) is originated from the conversion of dietary polyphenols by bacteria in the intestine [32]. The toxin is for approximately 34 percent bound to albumin in the bloodstream. Considering the relatively big amount of toxin that is bound to proteins not all of the HA can be removed using haemodialysis. In the healthy kidneys HA is also removed using tubular secretion next to the glomular filtration [33], which was also the case for indoxyl sulfate. Organic anion transporters are here also involved in tubular secretion [32].

1.7 Single-pass vs recirculation dialysis

Currently the most conventional type of dialysis therapy is haemodialysis, carried out at the hospital. Patients receive this treatment three times a week for four hours. The haemodialysis therapy at the hospital uses a single-pass dialysate solution stream to filter the blood. For a single 4h dialysis session 120-150 l of dialysate is used [2].

In a single-pass system the dialysate solution is passed through the filter, gaining toxins from the blood by diffusion (Figure 11), and is afterwards removed from the system. In a recirculation system the dialysate that is passed through the system is kept inside the system, to be used again. The two different situations can be seen in Figure 10. In the situation of the recirculation system (a) the same volume of dialysate is continuously used to filter the blood, which means the concentration of toxins in the dialysate will increase while filtering. In the situation of the single-pass system (b), the concentration of toxins in the dialysate will not increase and will have a more or less constant value. This value will be relatively low compared to the recirculation system, because the dialysate solution is constantly refreshed.

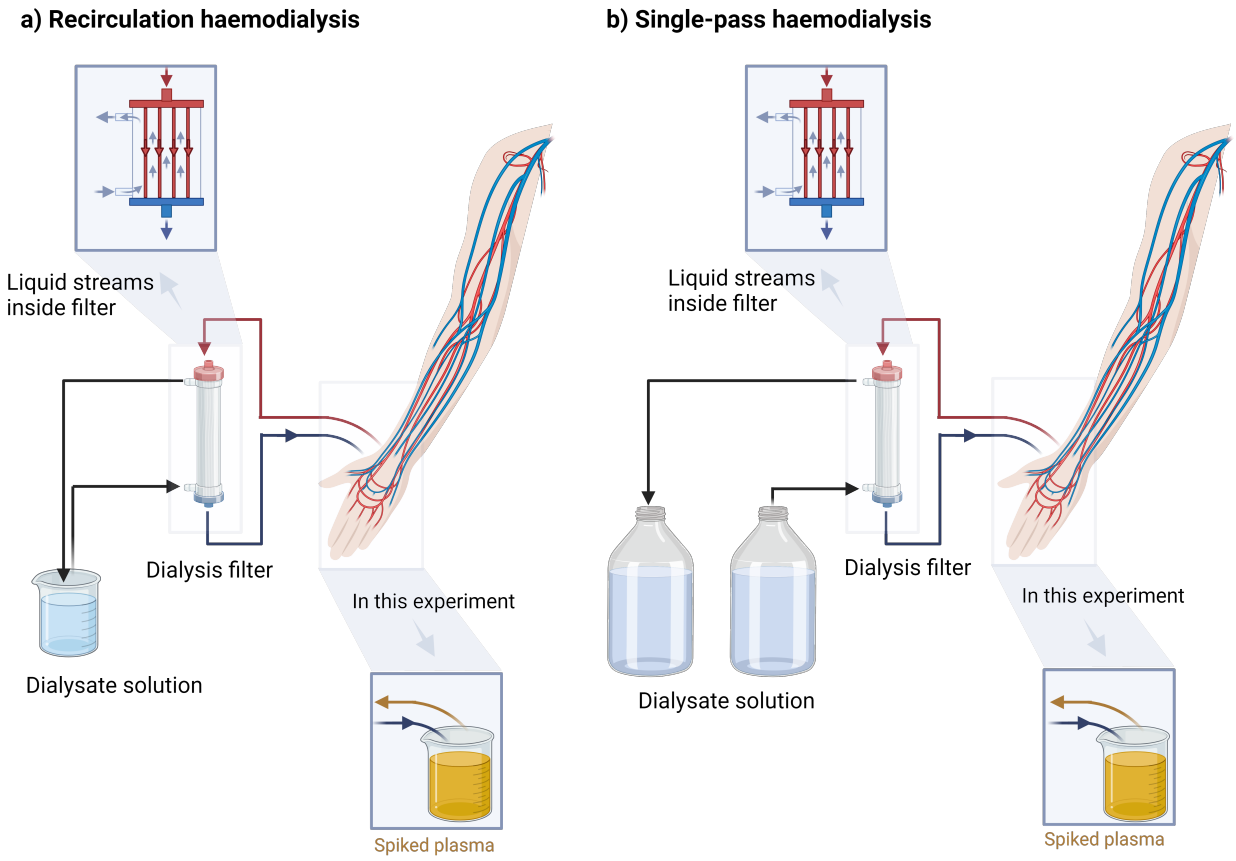


Figure 10: (a) Recirculation dialysis, compared to (b) Single-pass dialysis [34].

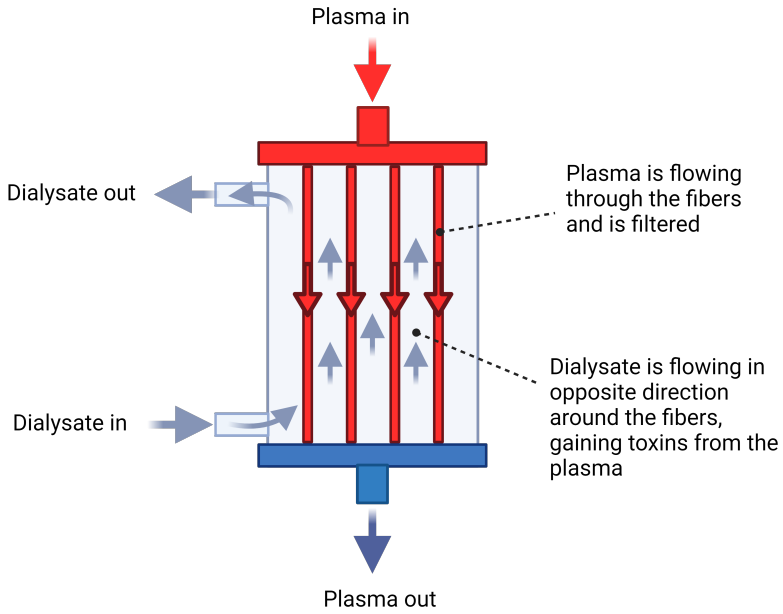


Figure 11: Overview of fluid flows inside the dialysis filter [34].

1.8 Previous studies

1.8.1 Previous studies: Kuf values

Several studies have already been carried out using a recirculation dialysis set-up. For many of these studies, in-house made modules are also used for the measurements. The Kuf values which are obtained using CWF measurements with these modules can be used to compare to the CWF results of this current study. Three previous studies have used the same kind of commercial fibers, from the Fresenius FX1000, for the modules as used in the experiments of this study. One study [35] used 10 fibers inside the module, which is less than the 31 fibers used in the modules of this current study, and reported a Kuf value of 136 ml/m^2*h*mmHg. Another study [36] from a student at the University of Twente used 25 fibers in the modules and reported a Kuf value of 115 ± 29 ml/m^2*h*mmHg. The last study [37] used 31 fibers inside the modules, which is identical to the modules this study, and reported a Kuf value of 156 ± 43 ml/m^2*h*mmHg.

1.8.2 Previous studies: Total creatinine removal

The total creatinine removal using a recirculation dialysis set-up can also be found in previous studies (Table 2). One study [38], which is using a similar recirculation set up as explained in this current study, has reported a total creatinine removal of 3732 ± 915 mg/m^2 after four hours. This study used 50 ml plasma, which is similar to what is used in this study, but used an outside-in filtration method and a different kind of fiber. Two similar studies have also been carried out, using a recirculation set-up with 50 ml plasma, but again with different types of fibers. The first study [39] has reported a total creatinine removal of 2549 mg/m^2. This study used an in-house made mixed-matrix-membrane fiber (MMM-fiber). The other study [39] had a creatinine removal of 3420 mg/m^2 after four hours. This study used a commercial fiber for the experiments.

To determine the reliability of the single-pass results of this study, these measurements are also compared to previous studies. One in vivo study [40] has reported a total creatinine removal of 779 ± 381 mg/m^2 after four hours. This study was carried out using a single-pass dialysis machine and dialyzer which are also used in hospitals. However, the set-up in hospitals is very different from the small-scale set-up used in this current study. This makes it also important to compare the results of this study to results from previous studies which have used a similar small-scale set-up. One study [36] by a student at the University of Twente has also used 50 ml plasma and used the same type of fibers as the one used in this current study. This measurement was also carried out with a small single-pass set-up, but a lower amount of fibers was used. This study reported a total creatinine removal of 2020 ± 179 mg/m^2 by diffusion.

The clearance and dialysance values of previous studies can also be compared to the results of this current study. One study [38] which is also using 50 ml plasma and a recirculation set-up has reported a creatinine dialysance of 200 ml/min*m^2. This paper used a different kind of fiber and an outside-in filtration method for this measurement, which is not used in this current study. One other study [36] from a student at the University of Twente used a similar single-pass haemodialysis set-up, compared to the one used in this study. It also used 50 of plasma and reported a creatinine clearance of 563 ml/min*m^2. Several in vivo studies have also been carried out, using a single-pass set-up similar to the ones used in hospitals. One of these studies [40] carried out single-pass haemodialysis therapy and and reported a creatinine clearance of 61 ± 16 ml/min*m^2. Another in vivo single-pass study [41] reported a creatinine clearance of 106 ± 28 ml/min*m^2 after only one hour. These studies were carried out in vivo with several patients and used a different kind of fiber, compared to the one used in this current study. This should be taken into account, when comparing the results.

1.8.3 Previous studies: Total Indoxyl Sulfate (IS) and Hippuric Acid (HA) removal

The total amount of indoxyl sulfate and hippuric acid that is removed can also be compared to previous studies (Table 3). One study [35] has been carried out using a recirculation set-up, 50 ml plasma and with the same kind of fiber that is used in the measurements of this current study. This study reported a IS removal of 377 ± 91 mg/m^2 and a HA removal of 2674 ± 564 mg/m^2 after four hours. One other study [37] has been carried out under the same conditions and reported a IS removal of 92 ± 10 mg/m^2 and a HA removal of 1454 ± 626 mg/m^2. Two other studies which used a different kind of fiber, but the same set-up, have also been found. One of these studies [38] reported a IS removal of 374 ± 12 mg/m^2 and a HA removal of 1562 ± 263 mg/m^2. The other study [35], again using another fiber, reported a IS removal of 272 ± 43 mg/m^2 and a HA removal of 2611 ± 582 mg/m^2.

One in vivo single-pass measurement has been found which has used the toxins HA and IS. This study used a normal dialysis machine with FX800 fibers, which are similar to the FX1000 fibers. This study reported a total HA removal of 377 mg/m^2 and a total IS removal of 46 mg/m^2 after four hours.

The clearance and dialysance values of previous studies can also be compared to the results of this study. One previous study [38] which is also using 50 ml plasma and a recirculation set-up, has reported an IS dialysance of 145 ml/min*m^2 and a HA dialysance of 370 ml/min*m^2. This paper used a different kind of fiber and an outside-in filtration method for this measurement. The same paper also carried out experiments which also used a recirculation set-up with 50 ml plasma, but this time with the same dialysis method as used in this current study. The study reported for these measurements a dialysance of 63 ml/min*m^2 for IS and a dialysance of 112 ml/min*m^2 for HA. One in vivo single-pass study [42] has been found that can be used to compare to the IS and HA single-pass results. This study reported a IS clearance of 15 ± 3 ml/min*m^2 and a HA clearance of 73 ± 7 ml/min*m^2. This study was carried out using a normal dialysis machine and FX800 fibers.

1.8.4 The Genius at home system

One very different study [43] looks at the Genius single-pass batch system, which is a recirculation-dialysis system that is produced for patients to use at home. In this study the blood is spiked with indoxyl sulfate and hippuric acid, and a 4.5 hour dialysis treatment is carried out with a blood flow of 300 ml/min. This resulted in a dialysis efficiency of around 1.18 Kt/V. According to European guidelines the standard for dialysis adequacy is an average of 1.2 Kt/V[44], for patients who receive haemodialysis therapy three times a week. According to this, the Genius is efficient enough for dialysis treatment. This shows that recirculation systems could be efficient enough to be used in haemodialysis therapy.

Table 2: Comparison of the results of several similar creatinine haemodialysis studies.

Dialysis method	**Type of fiber**	**Creatinine conc.**	**Flow rate (ml/min)**	**Surface area (m^2)**	**Removal (mg/m^2)**
OIF Recirculation [38]	MM-membrane	0.1 g/L	Qp: 10 Qd: 1	1.6×10^{-9}	3732 ± 915
IOF Recirculation [39]	MM-membrane	0.1 g/L	Qp: 1 Qd: 10	4.2×10^{-4}	2549
IOF Recirculation [39]	Fresenius F8HPS	0.1 g/L	Qp: 1 Qd: 10	6.3×10^{-4}	3420
IOF Single-pass [36]	Fresenius FX1000	0.1 g/L	Qp: 1 Qd: 10	8.3×10^{-4}	2020 ± 179, by diffusion
Single-pass in vivo study [40]	Fresenius FX80	Patient Cr. level (often between 6 mg/L to more than 14 mg/L [45])	Qp: 350 Qd: 350	1.8	779 ± 381

Table 3: Comparison of the results of several similar IS and HA haemodialysis studies.

Dialysis method	**Type of fiber**	**IS and/or HA conc.**	**Flow rate (ml/min)**	**Surface area (m^2)**	**Removal (mg/m^2)**
IOF Recirculation [38]	Polyflux 2H	IS: 40 mg/L HA: 110 mg/L	Qp: 10 Qd: 1	1.6×10^{-9}	IS: 374 ± 12 HA: 1562 ± 263
IOF Recirculation [37]	FX1000	IS: 40 mg/L HA: 110 mg/L	Qp: 1 Qd: 10	1.0×10^{-3}	IS: 92 ± 10 HA: 1454 ± 626
IOF Recirculation [35]	FX1000	IS: 40 mg/L HA: 110 mg/L	Qp: 1 Qd: 10	6.0×10^{-4}	IS: 377 ± 91 HA: 2674 ± 564
IOF Recirculation [35]	F8HPS	IS: 40 mg/L HA: 110 mg/	Qp: 1 Qd: 10	5.9×10^{-4}	IS: 272 ± 43 HA: 2611 ± 582
Single-pass in vivo study [42]	FX800	Patient IS and HA level (IS: ± 15.1 mg/L / HA: ± 24.1 mg/L)	Qp: 300 Qd: 700	1.8	IS: 46 HA: 377

1.9 The aim of this study

As mentioned before, Chronic kidney disease (CKD) is an increasingly big public health problem. Associated with a lower quality of life, high mortality and high costs for care [1][2], the disease impacts patients as well as society. Current therapy still has its limitations, one of them being that patients are still forced to go to the hospital to receive treatment multiple times a week, which negatively influences the patients quality of life. Next to this, limiting water consumption is an increasingly important topic considering the necessary sustainability goals set by governments and companies.

Portable haemodialysis systems with a limited amount of dialysate solution would be the ideal solution to improve CKD therapy. However, decreasing the amount of dialysate that is used is difficult, considering the current 120-150 L of dialysate that is used for dialysis. Recirculating and re-usage of the dialysate solution will be necessary to reduce the amount of dialysate solution that is needed and to make the dialysis system portable. Recirculating the dialysate solution will cause an increase of waste products in the solution, lowering the concentration gradient between patient's blood and the dialysate, which is probably

slowing down the toxin removal process. In this study the current single-pass system is compared to the recirculation system, using the Convergence set-up, to investigate the difference in uremic toxin removal of the system.

2 Methods

In-house filters are prepared by making "modules" which represent small-scale filters. These modules can be used in the Convergence set-up, which is a small-scale dialysis set up. The modules are prepared using commercial hollow fiber membranes of the type Fresenius FX1000. Using a scanning electron microscope (SEM) images are made of the fibers, to have a better understanding of the memebrane structure. The in-house made modules are tested using the clean water flux (CWF). The outcome of these measurements gives the permeability of the membranes, which is used to check whether the modules have a sufficient permeability to be used in the dialysis experiments. The values of the CWF measurements are also used as a reference, to compare with the results when using the modules with plasma in the Convergence set-up. The samples which are taken during the dialysis measurements are later analyzed using different analyzation techniques.

2.1 Materials

The following materials are used in the experiments:

- Fresenius FX1000 hemodiafiltration commercial fibers
- Two-component griffon fast glue (Klium)
- 6 mm diameter plastic tubes
- Festo T-connectors
- Scalpel
- MiliQ water
- CWF set-up
- Converence set-up (with connection tubes)
- Plasma
- Dialysate components (explained in section 2.4)
- 1 L glass bottles
- Greiner bio-one Easy StrainerTM 70 microliter sterile
- Eppendorfs
- Eppendorfs stand
- Pipette
- Pipette tips
- Microcentrifuge tubes with filter
- Water bath
- SEM
- PH-meter
- HPLC vials
- Nanodrop
- HPLC
- Scales
- Stirring bars for magnetic stirrer
- Magnetic stirrer
- 50 ml plastic bottles
- 100 ml glass bottles

2.2 Preparation of the hollow fiber modules

The small modules containing the hollow fibers are prepared using the hollow fibers of the type FX1000, 6 mm polyethylene tubing, Festo T-connectors and Griffon combi fast two-component glue. Each module contains 31 fibers. The distance between the outer parts of the Festo T-connectors is 6.5 cm, which makes the length of each fiber which participates in the diffusion process inside the module also 6.5 cm. The total effective surface area of the membrane of the fibers is 0.001 m^2. An example of a module can be seen

in Figure 12.

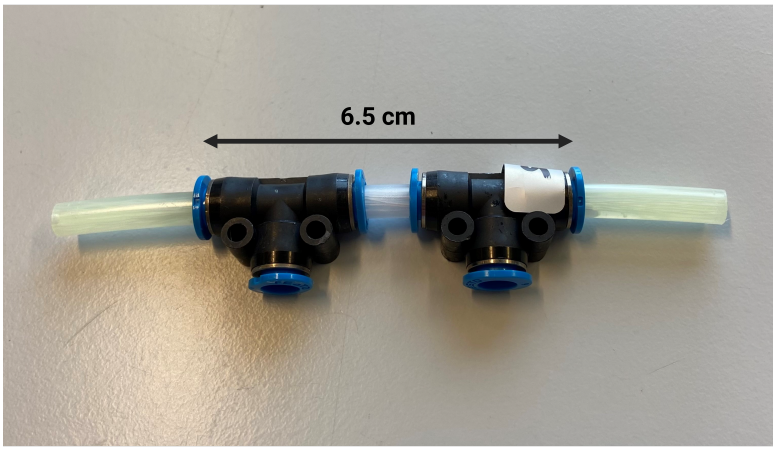


Figure 12: An in-house made module with commercial fibers.

2.3 Permeability measurements using CWF

The permeability of the modules is determined by using the clean water flux (CWF) in dead-end mode. The CWF set-up can be seen in Figure 13 and 14. For this set-up ultra-pure water is used. The permeability is determined by measuring the water outflow of the module. The outflow is measured in a situation where the water can only flow through the fiber membrane. This situation is created by blocking the fiber-outflow side of the module, which forces the water to leave the module by passing through the membrane. The measurement is done under water pressures of 1, 0.25, and 0.5 bar. Water passes the module and falls onto the scale, which is used to determine the amount of water that passed the module. During the measurement a specific pressure is applied and the amount of water that has passed the module during ten minutes is noted. Every two minutes, for ten minutes, the weight of the scale is noted. The permeability is calculated by looking at the applied water pressure, the mass that is written down and the time that has passed.

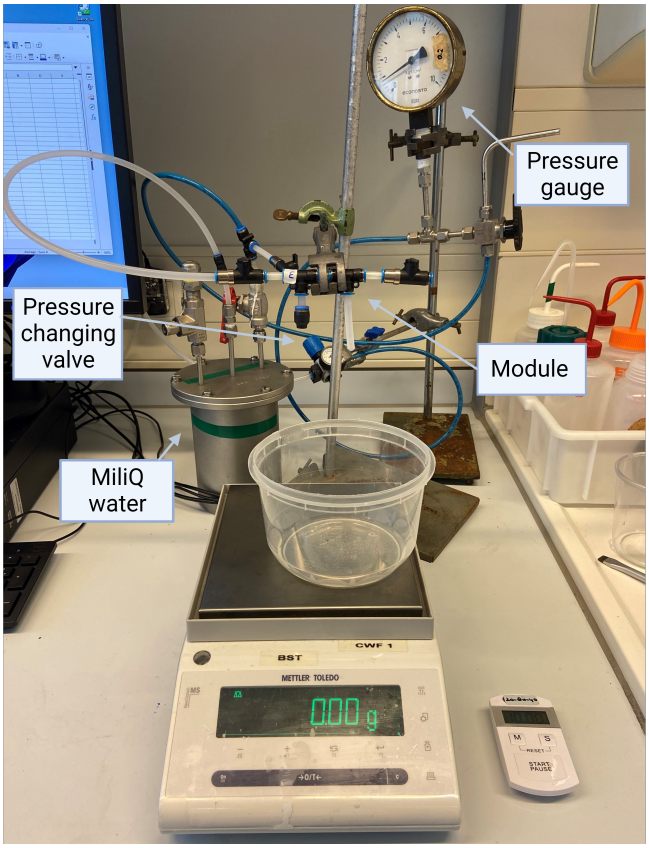


Figure 13: The CWF set-up.

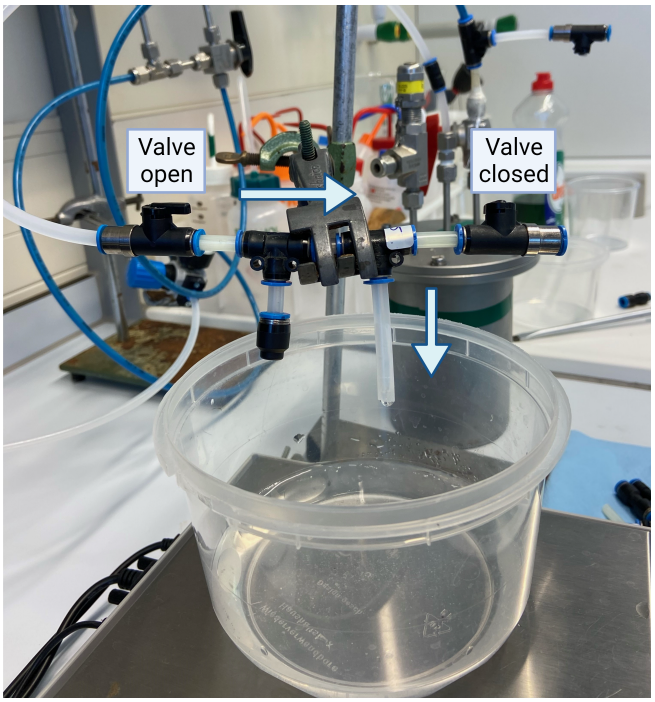


Figure 14: Module in the CWF set-up. The blue arrows show the flow of the MiliQ water inside the module.

2.4 Convergence dialysis measurements

For this experiment a Convergence Investigator set up is used, which is a small-scale dialysis machine set up. An overview of the used set-ups can be seen in Figure 15 and 16. Two types of dialysis experiments are carried out, recirculation-dialysis experiments and single-pass-dialysis experiments. Every experiment with a certain type of dialysis-method and dialysis volume is carried out three times.

2.4.1 Preparation of dialysate

First dialysate is prepared by dissolving 2mM KCl, 140 mM NaCl, 1.5 mM CaCl2, 0.25 mM MgCl, 2.35 mM NaHCO3 and 5.5 mM glucose in ultrapure MiliQ water. This is then mixed using a magnetic stirrer at 300 rpm. If the added substances do not dissolve, the dialysate solution is filtered using a Sartorius Minisart single use filter unit non-pyrogenic and a syringe. After mixing the pH is checked, as it needs to be around 7.4. Using HCl or NaCl the pH could be changed if necessary. Finally the dialysate is stored in the refrigerator until usage.

2.4.2 Preparation of human plasma spiked with toxins

The human plasma that is used in the experiment is provided by the blood bank Sanquin and stored in a -80 °C freezer. To spike the plasma, the frozen plasma is first put in a water bath, until reaching 37 °C. It is then filtered using a Greiner bio-one Easy Strainer 70 nm. Two 1 ml of unspiked plasma are put in eppendorfs for control. These are stored in the -20 °C freezer. Next the plasma is spiked with the toxin that will be used. For the creatinine measurements the plasma is spiked to reach a creatinine concentration of 0.1 g/L. This is done with solid creatinine. After spiking, the plasma is placed on the stirrer at 200 rpm for one hour. After this, it is placed in the water bath at 37 °C for four hours. For the

hippuric acid (HA) and indoxyl sulfate (IS) measurements, the plasma is spiked to reach a concentration of 0.61 mmol/l HA and 0.19 mmol/l IS. To do this, pre-made ultra-pure water with dissolved toxins is used. After spiking the plasma with the required amount of ultra-pure water with toxins, the plasma is finished. The spiked plasma, with creatinine or IS and HA, is finally stored in the -20 °C freezer until usage.

2.4.3 The dialysis experiments

Before starting the Convergence experiments, the plasma and dialysate solution are taken out of the fridge to reach room temperature. They are put into the water-bath to defrost. For the experiments a volume of 45 ml plasma spiked with creatinine or IS and HA is used. The dialysate volume that is used, depends on the type of measurement. For the recirculation measurements a volume of 100 ml is used and for the single-pass measurements a volume of 3 L. During the experiment a sample is taken from the plasma and dialysate containers at time t=0, t=30 min, t=1h, t=2h, t=3h and t=4h to be able to determine the change in toxin concentrations. In the set-up of the single-pass measurements the dialysate sample is taken from the tube that carries the dialysate from the module to the container with used dialysate. The plasma sample is again taken from the plasma container, in the same way which is was done during the recirculation measurements.

During the experiment the volume of the plasma and dialysate are monitored, to check whether filtration of fluid from the plasma to the dialysate does not take place. The plasma and dialysate volumes are checked before and after every time a sample is taken. The transmembrane pressure (TMP) is monitored and should stay around zero for the haemodialysis experiment. The pressure value of the plasma before the module is also monitored. This pressure should remain more or less the same. For the recirculation experiments the same volume of dialysate solution is reused and recirculated in the set-up. During the single-pass dialysis experiment a volume of 3 L dialysate is used and is not recirculated. The set-up of the recirculation and single-pass measurements can be seen in Figure 15 and 16.

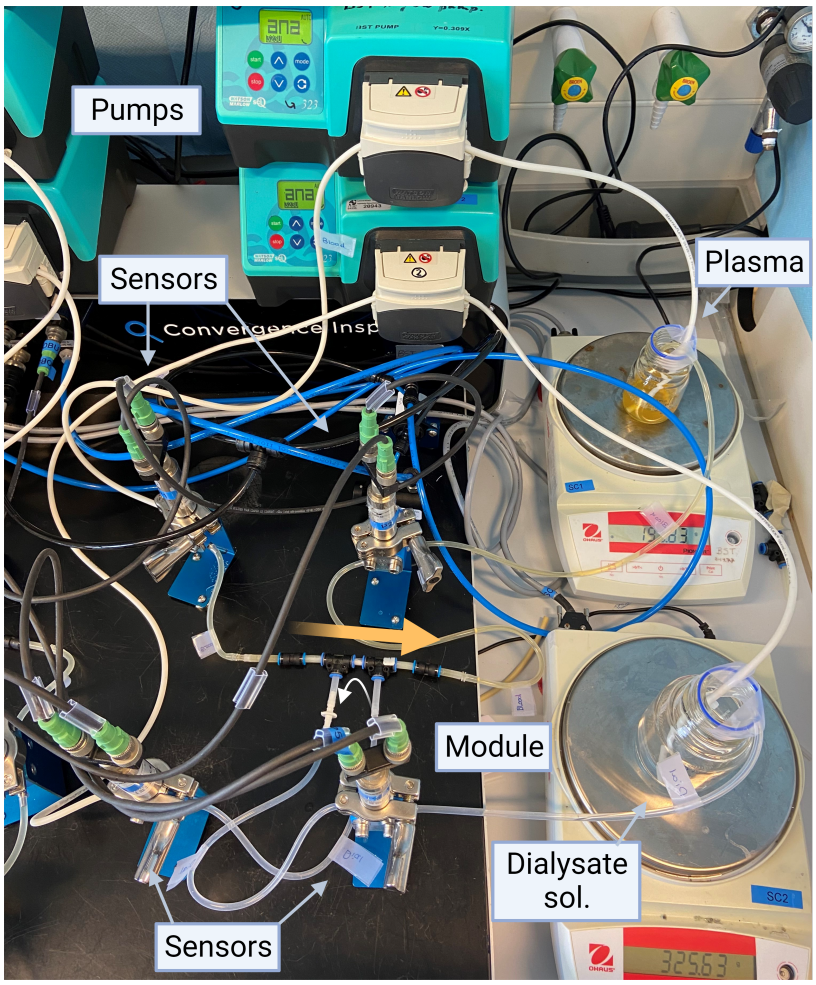


Figure 15: Overview of the Convergence recirculation set-up. The yellow arrow indicates the plasma flow inside the module. The white arrow indicates the dialysate flow inside the module.

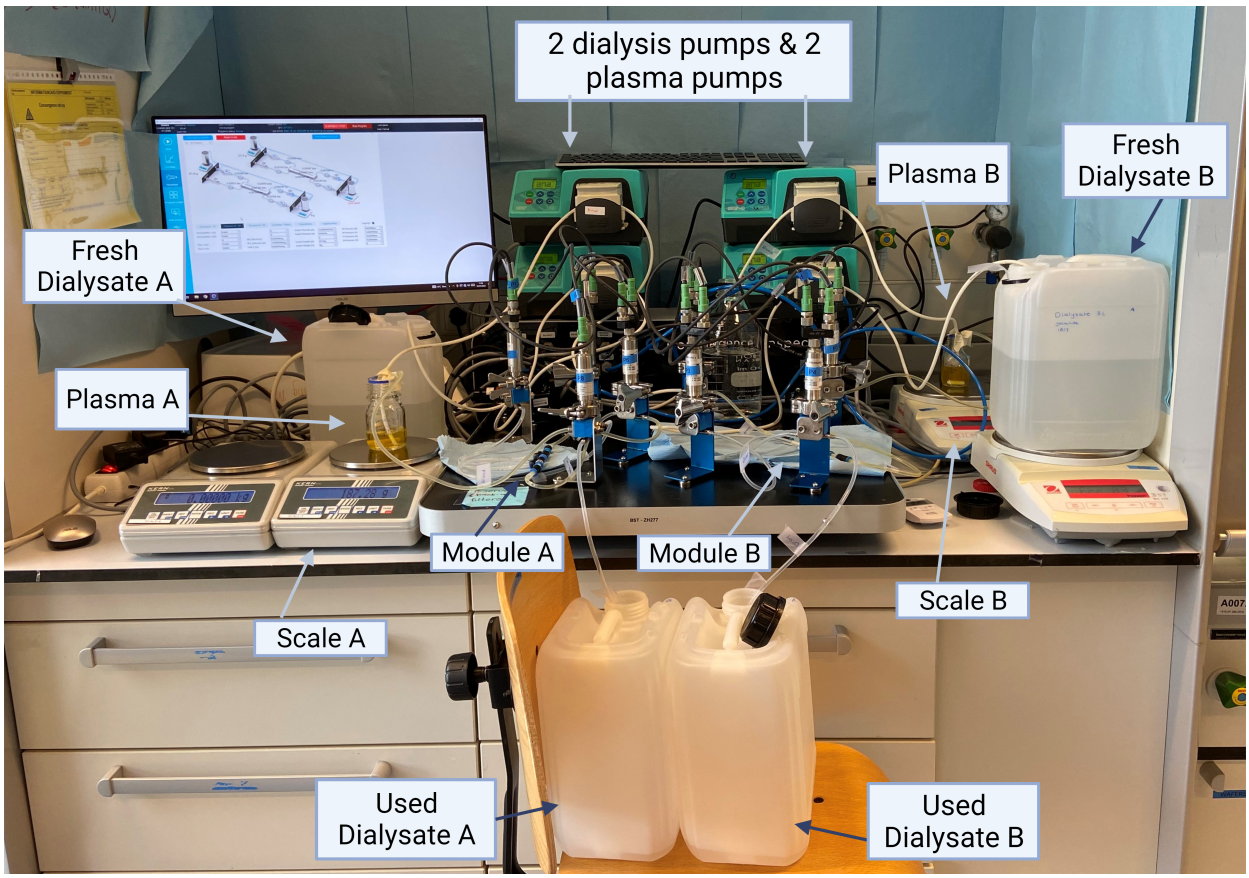


Figure 16: Overview of the Convergence single-pass set up. On the picture two set-ups are shown. Set-up A an B, which are identical.

2.5 Analyzation of samples

After the measurements, the samples that are taken are analyzed. The analyzation technique that is used is dependent on the type of toxin that was added to the plasma. The two techniques that are used are the nanodrop and the HPLC.

2.5.1 The nanodrop

The creatinine samples are examined using the Nanodrop. The Nanodrop uses the principle of the Beer-Lambert law (Equation 1) to calculate the concentration of a specific substance in the sample. The Beer-Lambert law states that the amount of light that is absorbed (A) by the sample is proportional to the concentration (c) of the specific substance that is measured in the sample. In this case the pathlength (b) and extinction coefficient (ε) stay constant.

$$c = \frac{A}{\varepsilon * b} \tag{1}$$

A callibration curve is first made by using a known concentration of creatinine in solution on the Nanodrop. After the dialysis experiment the samples are moved to filter eppendorfs and centrifuged at 14000 rpm for 15 min. Using the Nanodrop the absorption of the samples is determined. A wavelength of 230 nm [38] is used for the nanodrop. The measured adsorption is later used to calculate the concentration toxins, using the callibration curve.

2.5.2 The HPLC

The indoxyl sulfate and hippuric acid samples are examined using high-performance liquid chromatography (HPLC). The HPLC is used to separate and identify compounds in liquid samples [46]. The HPLC contains a mobile and a stationary phase. The stationary case is located inside the column of the HPLC. The mobile phase carries the sample through the column towards the detector. The compounds inside the sample separate from each other due to the fact that each type of compounds has a different interaction with the stationary phase. A detector measures the compounds that are separated by the stationary phase. Several different detectors can be used, depending on the compounds that are analyzed. Two types of detectors are for example UV/VIS detectors and fluorescence detectors. A UV/VIS detector [47] uses light to detect the compounds inside the sample. The absorption of light by the sample at different wavelengths is measured and this can be used to identify the compound. A fluorescence detector [48] excites the sample that leaves the column with light and measures the fluorescence light emission of the sample. The light that is emitted from the sample gives information about which compounds the sample contains.

Before analyzing, the samples are first diluted four times with MiliQ water. One extra plasma sample is made with the plasma t=0 sample, named t=0n. This sample is identical to the t=0 plasma sample, with the only difference being that this sample is not heated in a later step. Using this sample the free fraction of the toxins can be measured. After diluting the samples, all samples, except the t=0n sample, are heated at 95 °C for 30 minutes. All samples are then put in ice for 10 minutes. As the next step 400 μL of each sample is put into a filter Eppendorf and centrifuged at 14000 rpm for 15 minutes. 250 μL of each sample is then put into a HPLC vial for HPLC analysis. Before analysis all HPLC vials are checked to prevent air bubbles from remaining at the bottom of the vials.

Before HPLC analysis two types of eluent are made, which are used during the analysis. Eluent A is made using 3.153 g ammonium formate and 1000 ml MiliQ water. This solution is set to pH 3 using a 4M HCl solution. 100 ml methanol is added to 900 ml of this solution and finally put into the sonicator to remove air bubbles. Eluent B is made similarly. 3.153 ammonium formate is added to 1000 ml MiliQ water. This solution is again set to pH 3 using 4M HCl. 900 ml methanol is added to 100 ml of this solution. This mixture is also put into the sonicator to remove air bubbles.

After analysis of all samples, a chromatogram for each sample and each type of detection method is obtained as the output [46]. This chromatogram shows the time of the measurement and the intensity of the signal that is generated. The chromatogram shows a peak at a specific time, which is specific for each type of compound. The area underneath the peak can be used to calculate the concentration of the compound in the sample. For indoxyl sulfate the fluorescence detector is used and for hippuric acid the UV/VIS detector. For both indoxyl sulfate and hippuric acid a callibration curve is made, using known concentrations of the toxins in MiliQ water. Using the callibration curves the concentrations of the toxins in the samples can be determined.

2.6 Clearance and Dialysance

To be able to compare the Convergence results with literature, the dialysance and clearance are calculated. The dialyance is calculated for the recirculation-dialysis and the clearance for the single-pass dialysis. The dialysance for the toxin in the plasma (DL_p) and in the dialysate solution (DL_d) is calculated using equation 2 and 3 [38]. The clearance for the toxin in the plasma (CL_p) and dialysate solution (CL_d) is calculated using equations 4 and 5 [38]. In the formulas Xp is the amount of toxins removed from the plasma and Xd is the amount of toxins transported to the dialysis solution after a certain time t in minutes. Cp is the concentration of toxins inside the plasma and Cd is the concentration of toxins inside the dialysate solution. Aeff represents the effective surface area of the module.

$$DL_p = \frac{\frac{X_p}{t \cdot A_eff}}{(C_p - C_d)} \tag{2}$$

$$DL_d = \frac{\frac{X_d}{t \cdot A_eff}}{(C_p - C_d)} \tag{3}$$

$$CL_p = \frac{\frac{X_p}{t \cdot A_eff}}{C_p} \tag{4}$$

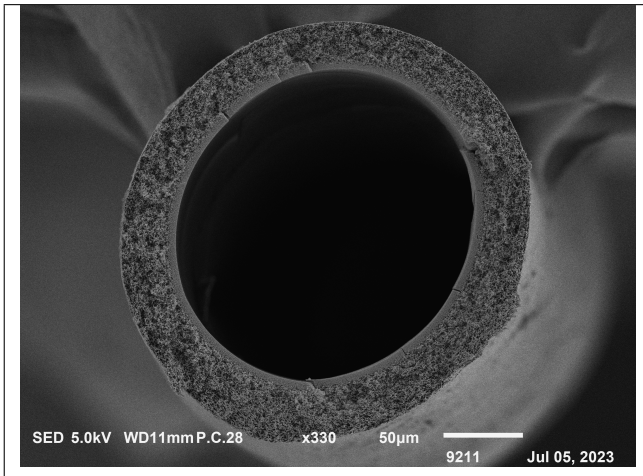
$$CL_d = \frac{\frac{X_d}{t \cdot A_eff}}{C_p} \tag{5}$$

3 Results and discussion

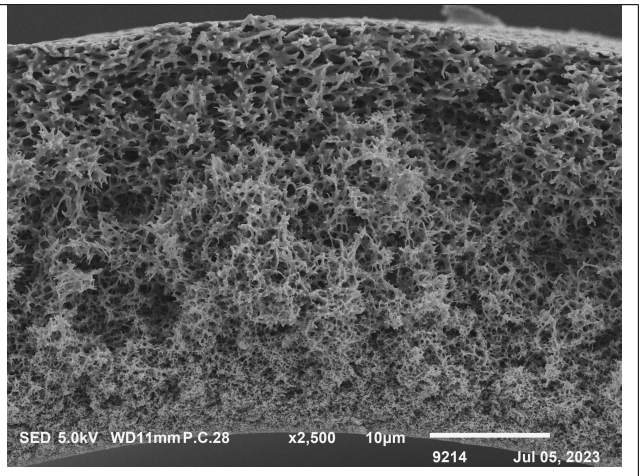
3.1 SEM images

Images of the hollow fibers are made using a scanning electron microscope (SEM). The images can be seen in Table 4. The commercial FX1000 fibers have a sponge-like structure with a thin selective layer on the lumen side.

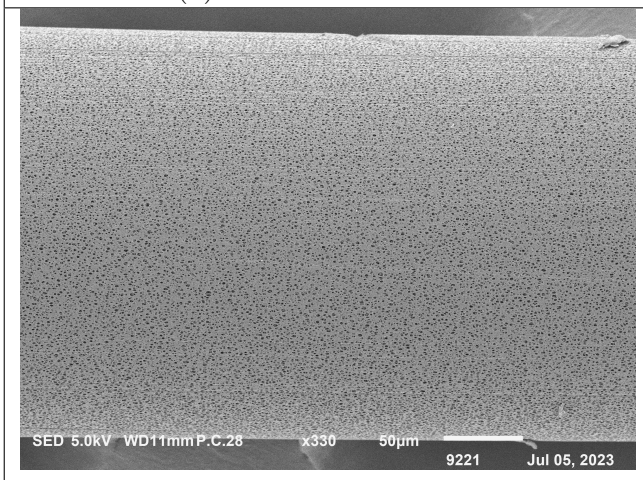
Table 4: SEM-pictures of hollow fiber Fresenius FX1000. (a) Fiber cross-section image with magnification 330x. (b) Cross-section of the fiber wall with magnification 2500x. (c) Image of the membrane surface with magnification 330x. (d) Image of the membrane surface with a higher magnification of 3300x.



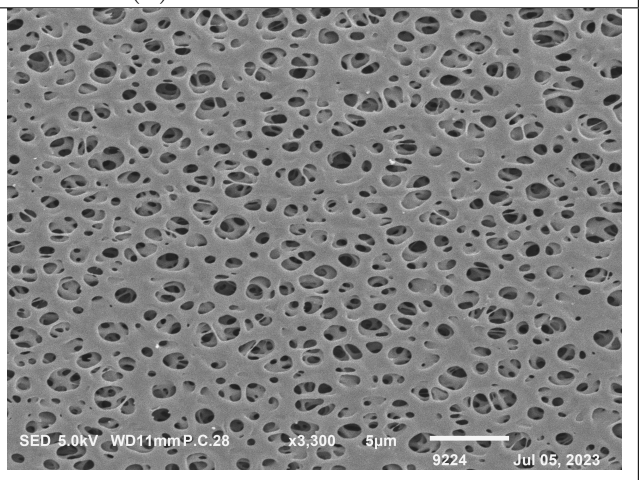
(a) Fiber cross-section



(b) Fiber wall cross-section



(c) Membrane surface



(d) Membrane surface close-up

3.2 Clean water flux measurements

The permeability of the modules is measured using the CWF. An example of this is shown in Figure 17. The slope of the graph gives the permeance of the module in [L/m^2*h*bar]. This calculation is carried out for every module.

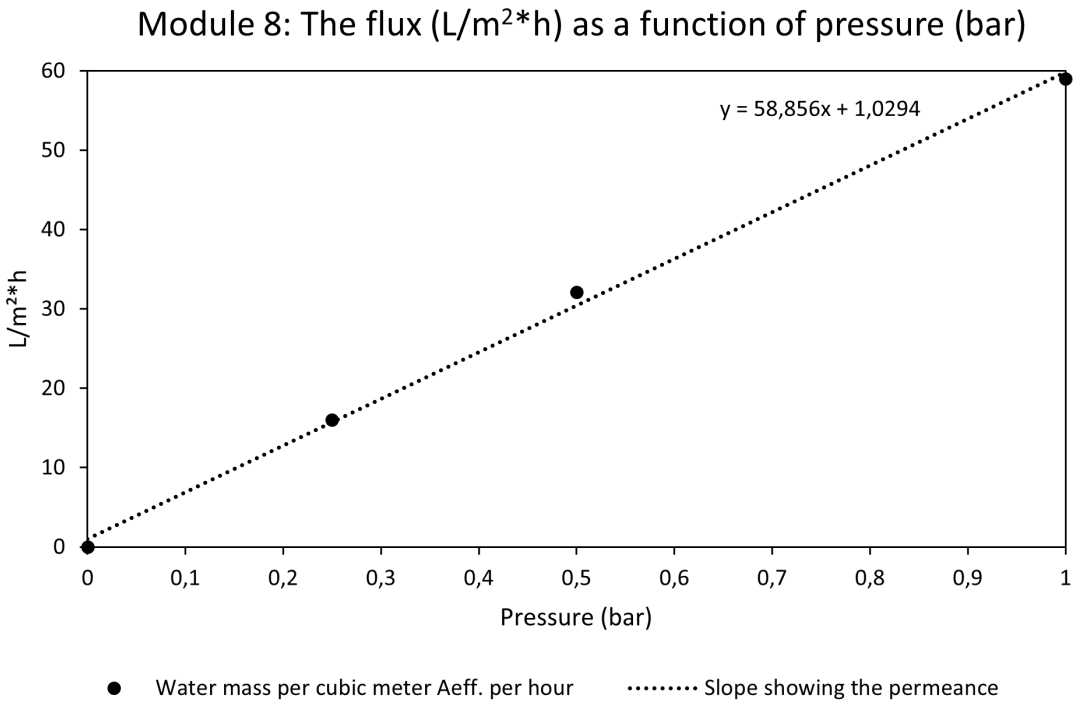


Figure 17: The water flux of module 8 in [L/m^2*h] as function of the pressure (bar), in this case 59 [L/m^2*h*bar].

The permeability and Kuf value of all modules have been measured and calculated. This is shown in Table 5 and 6. An average permeability of 55 ± 4 L/m^2*h*bar and an average Kuf value of 72 ± 5 ml/m^2*h*mmHg are calculated for the modules used for the creatinine measurements. For the HA and IS experiments an average permeability of 70 ± 7 L/m^2*h*bar and an average Kuf value of 93 ± 10 ml/m^2*h*mmHg are calculated. The modules which are used have specifically been selected by their permeability and Kuf value out of a larger number of created modules. This has been done to perform the experiments with modules which have a maximum variation of 30 percent in their permeability, to prevent this from influencing the results. However, most modules which have been made had a permeability within the range of 50 to 80 L/m^2*h*bar. In previous studies, Kuf values of 136 ml/m^2*h*mmHg for 10 fibers [35], 115 ± 29 ml/m^2*h*mmHg for 25 fibers [36] and 156 ± 43 ml/m^2*h*mmHg for 31 fibers [37] have been reported. When these results are compared with the Kuf values of the modules used in the experiments of this current study, it is clear that the modules of this study have a lower Kuf value. It is not certain why the values are different, considering that the same methods for assembling the modules are used and the same type of fibers are used. Some factors could have influenced the assembly of the modules, like the amount of glue that has been used in the tubes, which could lead to more or less area of the fibers being exposed to the dialysis fluid inside the module. Another explanation could be that the dialyzer manufacturer has made some changes to the fibers in the time between this experiment and previous experiments, which could therefore influence the permeability of the fibers.

Table 5: Permeability and Kuf for every module that is used for the creatinine measurements.

Module Nr.	**Permeability [L/m²*h*bar]**	**Kuf [ml/m²*h*mmHg]**
Module 8	59	78
Module 1	57	76
Module 6	51	68
Module 9	56	74
Module 7	49	65
Module 15	56	75
Average	**55 ± 4**	**73 ± 5**

Table 6: Permeability and Kuf for every module that is used for the HA and IS measurements.

Module Nr.	**Permeability [L/m²*h*bar]**	**Kuf [ml/m²*h*mmHg]**
Module 16	68	91
Module 23	79	105
Module 25	79	105
Module 27	65	87
Module 3	68	91
Module 17	62	82
Average	**70 ± 7**	**93 ± 10**

3.3 Creatinine experiments

3.3.1 Recirculation measurement results

As the first step of the measurements, recirculation haemodialysis measurements are carried out using the Convergence set-up. Three recirculation experiments are carried out and the average of these measurements is taken. In Figure 18 the average concentration of creatinine in the plasma and dialysate over the time of four hours can be seen. The figure shows a decrease of the creatinine concentration in the plasma of approximately 110 mg/L and an increase of creatinine in the dialysate of approximately 41 mg/L after four hours.

In Figure 19 the normalized concentrations over time can be seen, which shows the percentage of the total amount of creatinine that is gained or lost by the dialysate and plasma. The figure of the normalized concentration shows that the creatinine concentration in the plasma decreases and the creatinine concentration in the dialysate increases over time. In the recirculation measurements the creatinine concentration in the plasma decreases on average with 57 percent. The creatinine concentration in the dialysate increases on average with 22 percent. The plasma increase and dialysate decrease is the highest at the beginning of the measurement and becomes less as the measurement continues. At the end of the measurement, around 3/4 hours, the concentration line becomes more horizontal, which indicates that the concentrations stay more constant at this time.

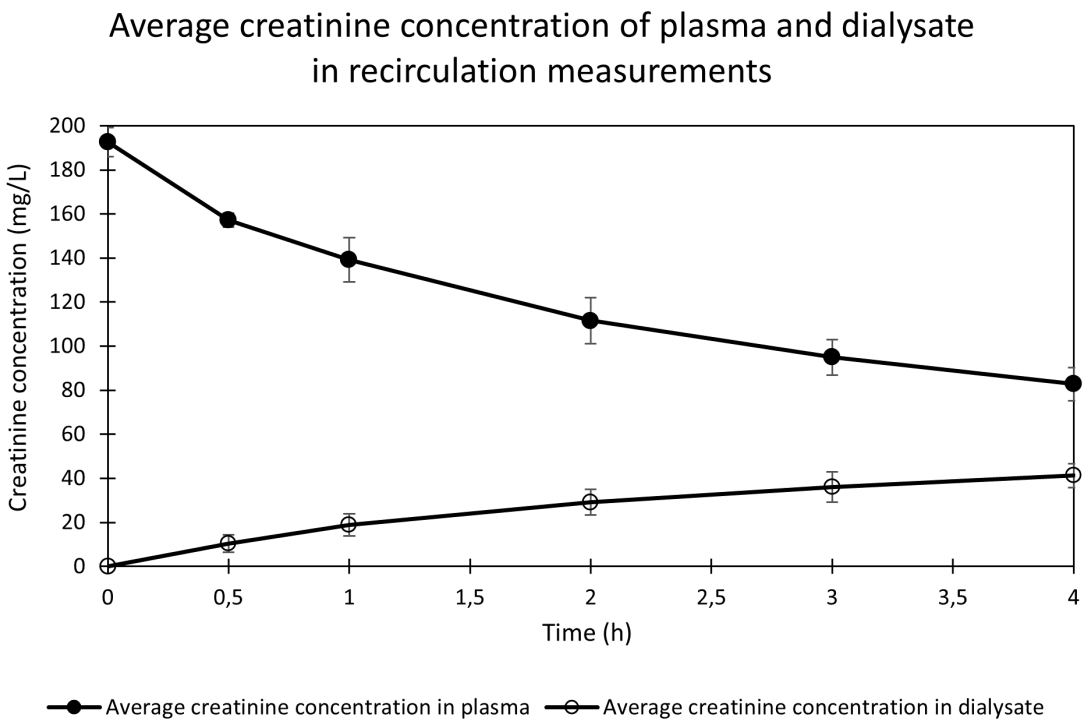


Figure 18: Average creatinine concentration in the plasma and dialysate during the recirculation measurements (n=3).

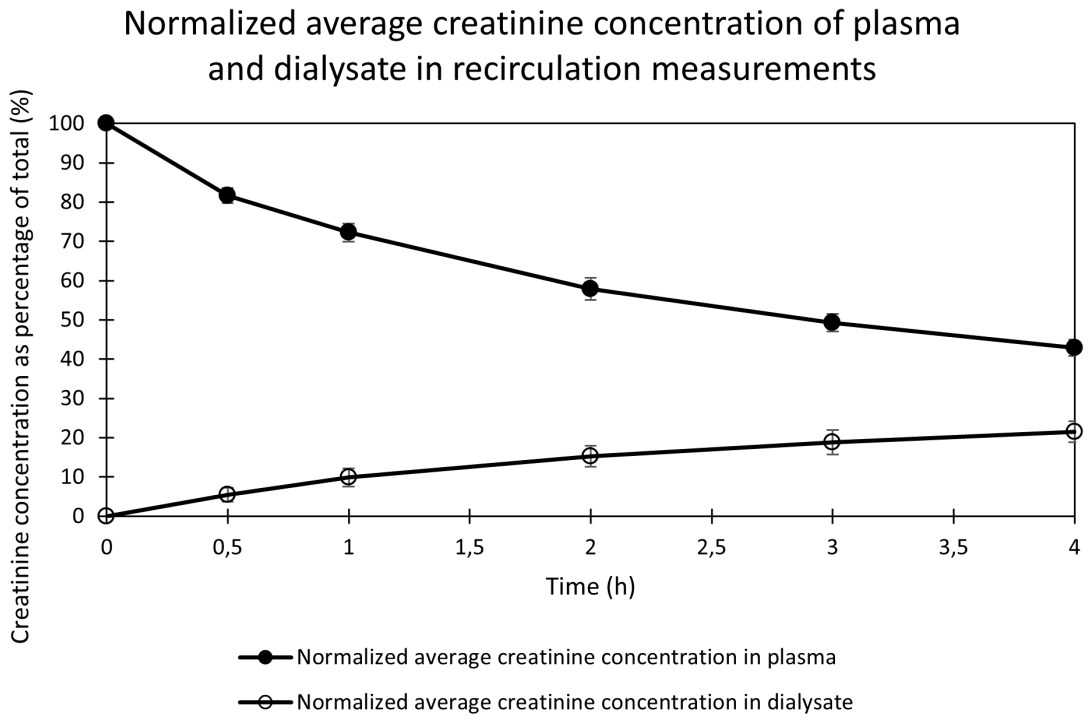


Figure 19: Normalized average creatinine concentration in the plasma and dialysate during the recirculation measurements (n=3).

When looking at Figure 18 and 19, a couple of things are noticeable. From the results it is clear that the decrease in creatinine concentration in the plasma is the highest at the beginning of the four hour measurement and slowly becomes less. This can be explained by the lowering concentration gradient between the

dialysate solution and the plasma, caused by the accumulation of toxins in the dialysate solution. After three hours the decrease of creatinine concentration in the plasma is very low, which means that if the measurement had been extended for more than four hours this had probably not had a large effect on the final results.

To examine the efficiency of the recirculation measurements, the total amount of creatinine removed after four hours is calculated. Using this value and the effective surface area (Aeff) of the module, the average percentage of creatinine removed from plasma after four hours using the recirculation set-up is 56 percent per square meter. The total amount of mg creatinine removed from the plasma after four hours is 4644 $\pm$ 54 mg/m^2.

The dialysance for the recirculation creatinine measurements is calculated using Equations 2 and 3, as mentioned in the introduction of this thesis. The average amount of creatinine removed from the plasma per square meter Aeff is plotted against the time, as can be seen in Figure 20. The slope gives the calculated dialysance for the removal of creatinine from the plasma (DL_p). This value is 27 L/m^2/h, or also 450 L/m^2/h. The average amount of creatinine gained in the dialysate per square meter Aeff is also plotted against the time, as can be seen in Figure 20. The slope gives the calculated dialysance for the gain of creatinine in the dialysate (DL_d). This value is 23 L/m^2/h, or also 383 ml/m^2*min.

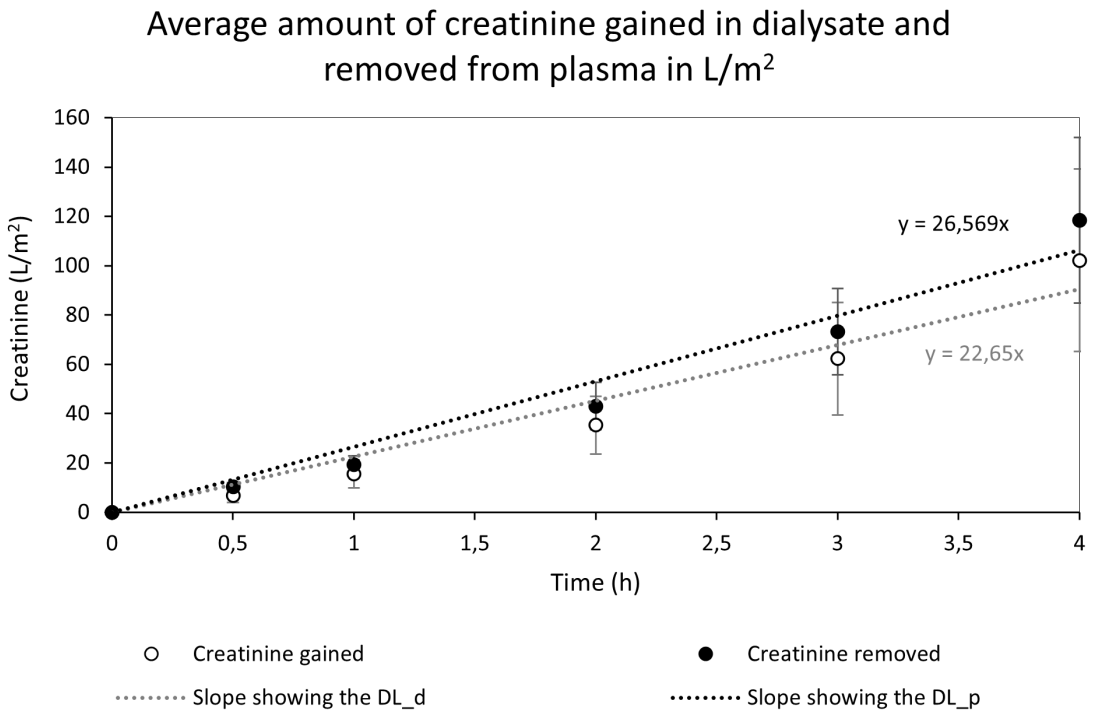


Figure 20: Dialysance of the removal of creatinine from plasma and the gain of creatinine by the dialysate in the recirculation measurements (n=3).

In Figure 19 with the normalized creatinine concentrations, it is visible that the percentage of creatinine in the plasma has decreased by a greater amount than the percentage of creatinine that is gained by the dialysate solution. This is also shown in the results of the dialysance, in Figure 20. The total removal from plasma in this situation is higher than the creatinine gained by the dialysate. It would be expected that the DL_d and DL_p are similar, considering that no creatinine can leave the circuit. However, considering the large error margins of the dialysance calculations, it could be possible that the difference in DL_d and DL_p is caused by the error margins being too large. This does however not explain the difference in

creatinine removed and gained in Figure 19. It is not certain why this difference is measured. A possible explanation is the absorption of creatinine to the fiber membranes or to other surfaces inside the dialysis circuit. When the calculated dialysance of this study is compared to a previous study, the obtained results are relatively high. One previous study reported a dialysance of 200 ml/m^2*min. The dialysance of 450 ml/m^2*min reported here is higher, however it is still in the same range as the previously reported dialysance.

3.3.2 Single-pass measurement results

After the recirculation measurements, the single-pass measurements are carried out. Three single-pass measurements are carried out and the average of these measurements is taken. In Figure 21 the average concentration of creatinine in the plasma and dialysate over time can be seen. This Figure shows a decrease of 123 mg/L creatinine in the plasma after four hours, while the creatinine concentration in the dialysate remains relatively low and has a value of approximately 2.2 mg/L. Figure 22 shows the normalized concentrations over time. In Figure 22 it can be seen that the creatinine concentration in the plasma decreases with 69 percent after four hours. The creatinine concentration in the dialysate has a relatively constant value of approximately 1.2 percent. The decrease in creatinine is the highest at the beginning of the measurement and becomes less over time. However, the line is still not horizontal after four hours, so the creatinine concentration was still decreasing at the end of the measurement.

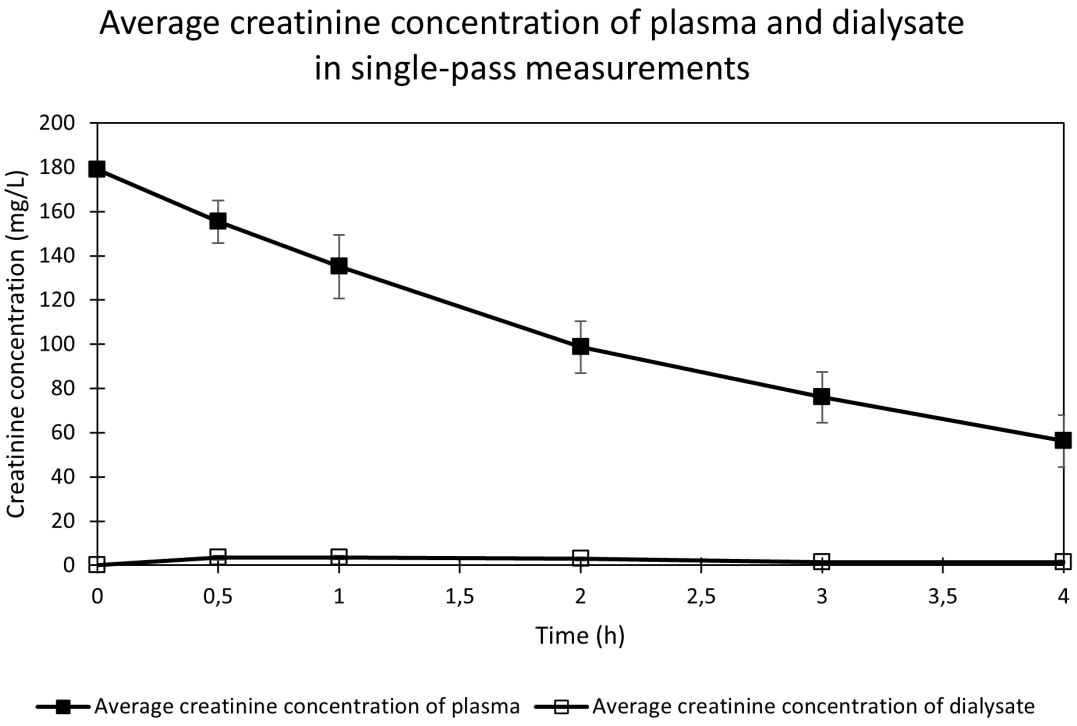


Figure 21: Average creatinine concentration in the plasma and dialysate during the single-pass measurements (n=3).

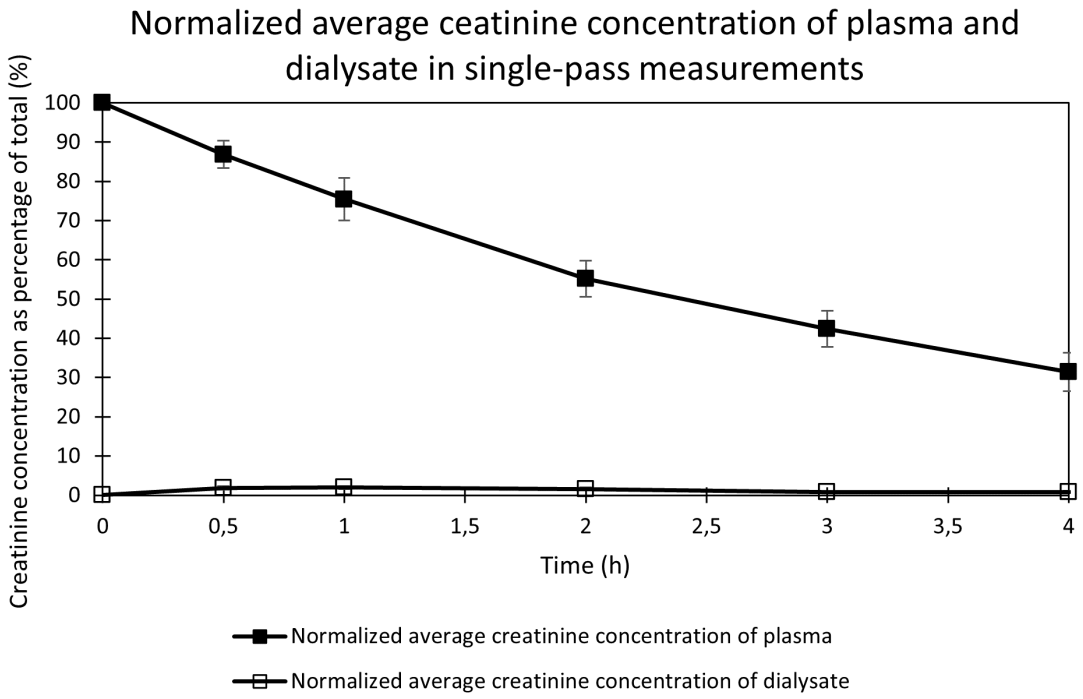


Figure 22: Normalized average creatinine concentration in the plasma and dialysate during the single-pass measurements (n=3).

The single-pass results from the creatinine measurements (Figure 21 and 22) show a decrease of creatinine in the plasma which is more linear during the four hour measurement, compared to the recirculation results. This also means that increasing the time of the single-pass measurement for more than four hours could possibly lead to an even better removal of creatinine. The more efficient and more linear removal of creatinine by the single-pass measurement is probably caused by the fact that there is no toxin accumulation in the dialysate solution. Which leads to a more or less constant and relatively large concentration gradient between the plasma and the dialysate solution.

To examine the efficiency of the single-pass measurements, the total amount of creatinine removed after four hours is calculated. Using this value and the effective surface area (Aeff) of the module, the average percentage of creatinine removed from plasma after four hours using the single-pass set-up is 67 percent per square meter. The total amount of mg creatinine removed from the plasma after four hours is 5159 $\pm$ 395 mg/m^2.

The clearance for the single-pass creatinine measurements is calculated using Equations 4 and 5. The average amount of creatinine removed from the plasma per square meter Aeff is plotted against the time, as can be seen in Figure 23. The slope gives the calculated dialysance for the removal of creatinine from the plasma (DL_p). This value is approximately 21 L/m^2/h, or also 350 L/m^2/h. The average amount of creatinine gained in the dialysate per square meter Aeff is also plotted against the time, as can also be seen in Figure 23. The slope gives the calculated dialysance for the gain of creatinine in the dialysate (DL_d). This value is approximately 22 L/m^2/h, or also 367 ml/m^2*min.

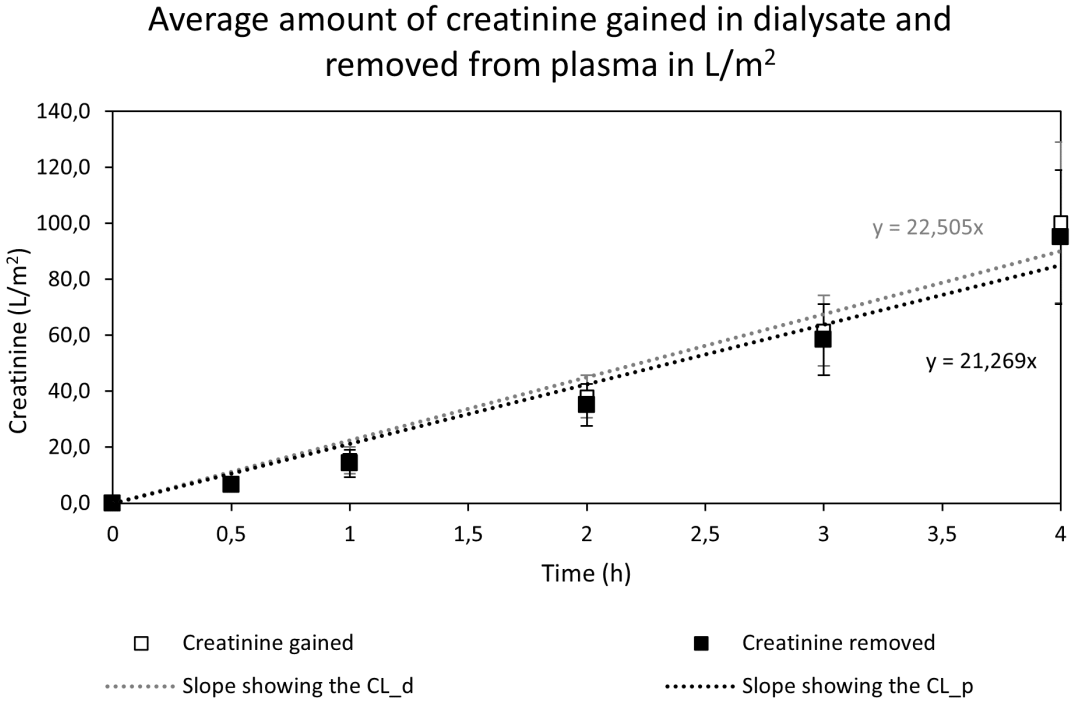


Figure 23: Clearance of the removal of creatinine from plasma and the gain of creatinine by the dialysate in the single-pass measurements (n=3).

In Figure 23 it is shown that the clearance for the removal of creatinine from the plasma and the amount of creatinine gained by the dialysate is more or less the same. This is also expected, considering that all creatinine is expected to be removed by diffusion. When the clearance is compared to a previous study which is carried out with the same Convergence set-up, the obtained values from the measurements of this current study are lower than expected. One paper reported a creatinine clearance of 563 ml/m^2*min and one other in vivo study reported a creatinine clearance of 106 $\pm$ 28 ml/m^2*min after only one hour. The results of this last study are comparable with the results of this current study, however this is an in vivo study. The results of this current study are in the same order of magnitude as the previous studies and therefore seem reliable.

3.3.3 Single-pass compared to recirculation

There is a significant difference (p=0.0381) in creatinine removal from the plasma by the FX1000 fibers, when the recirculation mode is compared to the single-pass mode. In Figure 24 the normalized recirculation and single-pass results are shown together. The first clear difference that can be seen is the significantly lower creatinine concentration in the single-pass dialysate, compared to the recirculation dialysate. Next to this a difference can also be seen in the decreasing creatinine concentration of the plasma. The single-pass measurement has an average decrease of the creatinine concentration in the plasma of 69 percent, after four hours. For the recirculation measurements this value is 57 percent. This makes the single-pass set-up more effective in decreasing the creatinine concentration in the plasma, compared to the recirculation set-up. The figure also shows that the single-pass and recirculation measurements are almost even effective during the first hours. The recirculation set-up is slightly more effective during this time. However, after 1.5 hours the single-pass set-up becomes more effective leading to a larger decrease in the creatinine concentration of the plasma.

A difference can also be seen in the increase and decrease of the creatinine in dialysate and plasma after

approximately 2.5 hours. In the recirculation situation the line which represents the plasma creatinine concentration becomes less steep towards the end, compared to the beginning of the measurements. In the single-pass situation this line at the end of the measurement is steeper, compared to the recirculation situation. This difference shows that the single-pass set-up will probably lead to an even bigger decrease in the creatinine concentration of the plasma if the measurement would be extended to more than four hours. For the recirculation set-up the creatinine concentration in the plasma would probably not decrease much further if the length of the measurement would be extended.

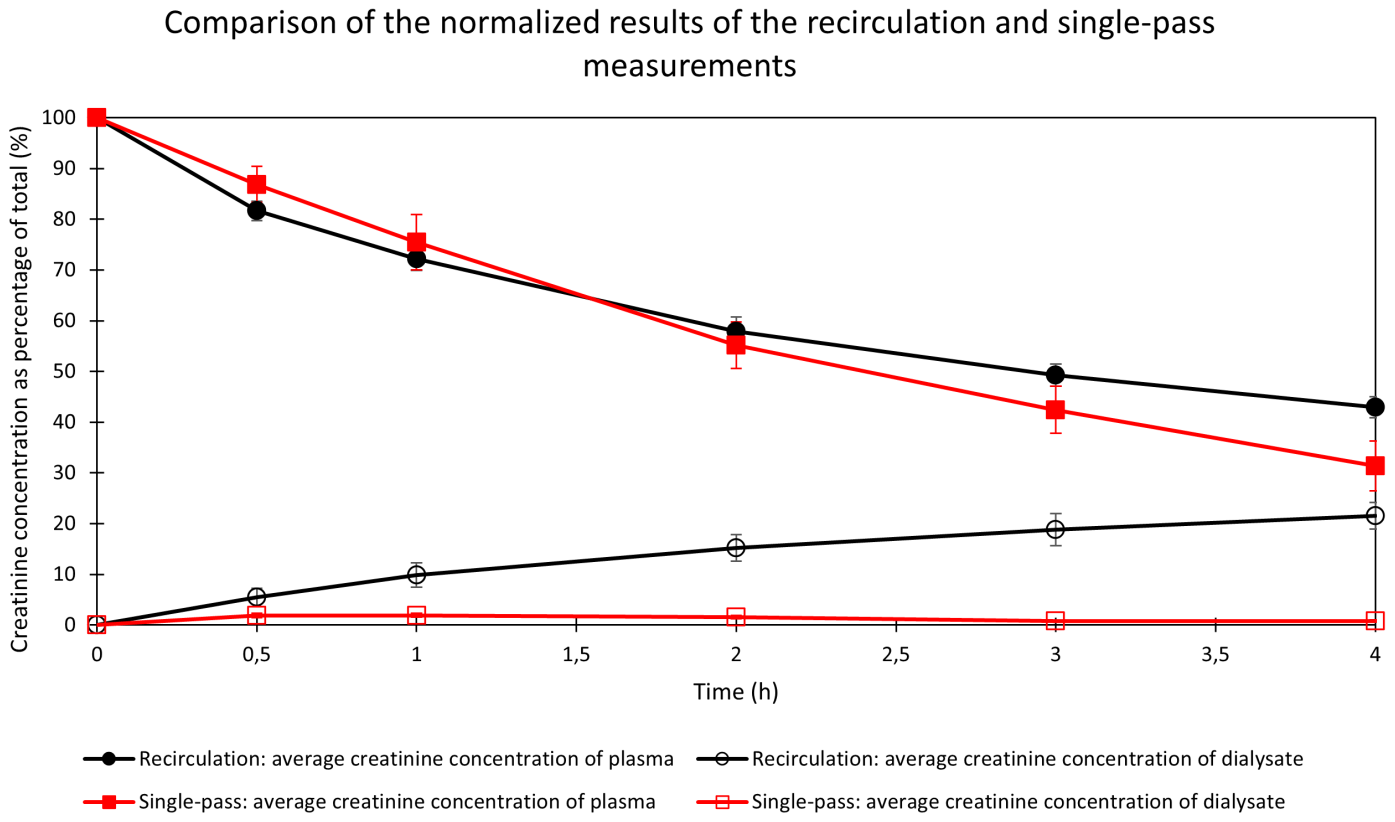


Figure 24: Comparison of creatinine concentration of the plasma and dialysate in the recirculation (n=3) and single-pass (n=3) measurements.

The total creatinine removal from plasma by the recirculation and single-pass set-ups are compared in Figure 25. For the recirculation measurements an average of 56 percent creatinine per square meter is removed after four hours. Using the single-pass set-up an average of 67 percent creatinine per square meter is removed after four hours. This means the single-pass set-up removes approximately 11 percent more creatinine after four hours, compared to the recirculation set-up. The difference between the removal of the two set-ups is significant (p=0,0423). This figure shows a similar curve as Figure 24. During the first hours the recirculation set-up is more efficient, however after 1.5 hours the single-pass set-up becomes more efficient. The single-pass set-up has the highest removal after four hours, because it can maintain its efficiency while the recirculation set-up becomes relatively less efficient after some time.

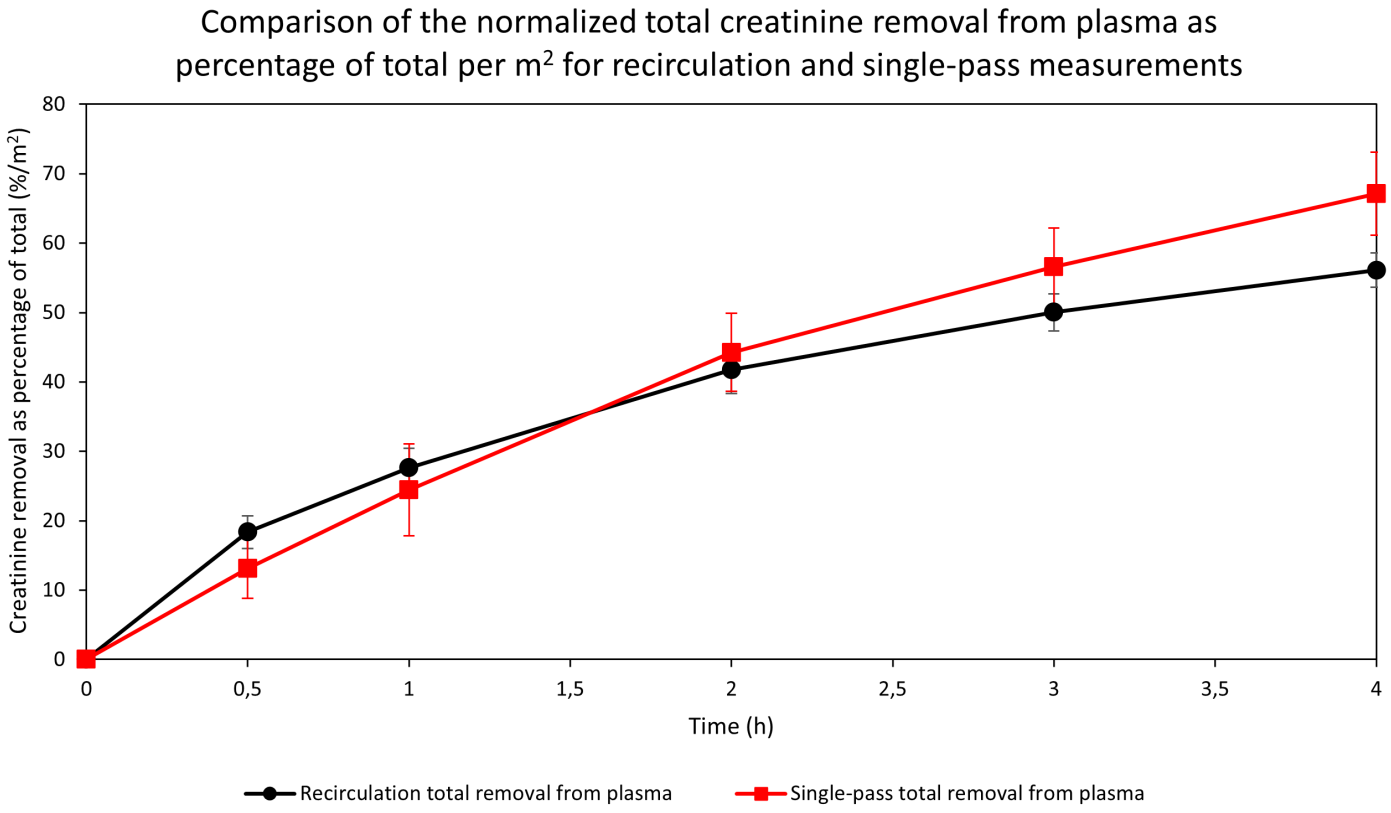


Figure 25: Comparison of the total removal of creatinine from plasma in the recirculation (n=3) and single-pass (n=3) measurements.

Given the results of Figure 24 and 25, it can also be concluded that for shorter measurements, of less than approximately two hours, the single-pass and recirculation set-ups have more or less the same efficiency. This is probably caused by the concentration gradient which is still relatively large for the recirculation set-up at this time. Hence, for shorter creatinine measurements both types of set-ups could be used, while for longer measurements (of more than 2 hours) the single-pass set-up is shown to be more effective.

The total amount of creatinine removed in mg/m² from this study and similar previous studies can be seen in Table 7. The recirculation results of this study can be compared to the three recirculation studies in the table. From the table can be seen that the recirculation total removal of this study is relatively high, compared to previous studies. A possible explanation for this difference is the use of a different fiber membrane. Two studies used a MMM-fiber and one of these studies also used an outside-in filtration method. The use of this new type of fiber and new type of dialysis method, can be an explanation for the total removal being lower compared to this current study. The third similar study used Fresenius F8HPS fibers, which are low-flux fibers. These low-flux fibers have a lower clearance, compared to hemodiafilter fibers like the FX1000. This can cause the removal of the previous study to be less compared to the removal of this study. One other possible explanation for the higher removal of this current study compared to previous studies is the starting creatinine concentration. In all studies a creatinine concentration of 0.1 mg/L is used. This means that for this study 0.1 mg/L creatinine has been added to the plasma. However, the plasma that has been used in this current study already contained a relatively high amount of creatinine. This caused the creatinine concentration in the plasma at the beginning to be on average 193 mg/L, which can explain the higher removal after four hours.

Two previous studies can be used to compare to the total removal single-pass results of this study (Table 7). One study reported a total removal of 2020 ± 179 mg/m². This study also used the small-scale Con-

vergence set-up. One other in vivo study, using a normal dialysis machine, reported a total removal of 779 ± 381 mg/m^2. Compared to these previous studies, the results of this current study are relatively high. It is not certain why this difference is observed between the two small-scale set-ups. However, the results of this study and the previous study using the small-scale set-up are still in the same order of magnitude. The difference with the in vivo study could be explained by the different set-up that has been used. The in vivo study used a normal dialysis machine, a different fiber and patients with full human blood instead of plasma.

Table 7: Comparison of the results of several similar creatinine haemodialysis studies.

Dialysis method	**Type of fiber**	**Creatinine conc.**	**Flow rate (ml/min)**	**Surface area (m^2)**	**Removal (mg/m^2)**
OIF Recirculation [38]	MM-membrane	0.1 g/L	Qp: 10 Qd: 1	1.6×10^{-9}	3732 ± 915
IOF Recirculation [39]	MM-membrane	0.1 g/L	Qp: 1 Qd: 10	4.2×10^{-4}	2549
IOF Recirculation [39]	Fresenius F8HPS	0.1 g/L	Qp: 1 Qd: 10	6.3×10^{-4}	3420
IOF Single-pass [36]	Fresenius FX1000	0.1 g/L	Qp: 1 Qd: 10	8.3×10^{-4}	2020 ± 179, by diffusion
Single-pass in vivo study [40]	Fresenius FX80	Patient Cr. level (often between 6 mg/L to more than 14 mg/L [45])	Qp: 350 Qd: 350	1.8	779 ± 381
IOF Recirculation (this study)	Fresenius FX1000	0.1 g/L	Qp: 1 Qd: 10	1.03×10^{-3}	4644 ± 54
IOF Single-pass (this study)	Fresenius FX1000	0.1 g/L	Qp: 1 Qd: 10	1.03×10^{-3}	5159 ± 395

3.4 Hippuric Acid (HA) and Indoxyl Sulfate (IS) experiments

3.4.1 HA recirculation measurement results

After the creatinine measurements, recirculation and single-pass measurements have been carried out with HA as toxin in the plasma. Three recirculation measurements have been carried out and the average of these measurements is taken. In Figure 26 the average concentration of HA in the plasma and dialysate over the time of four hours can be seen. Figure 26 shows an average decrease of the HA concentration in the plasma of approximately 67 mg/L and an average increase of HA in the dialysate of approximately 27 mg/L.

Figure 27 shows the normalized concentrations over time, which shows the percentage of the total amount of HA that is gained or lost by the dialysate and plasma. From the figure can be seen that the concentration of HA in the plasma decreases on average with 43 percent. The concentration of HA in the dialysate increases on average with 17 percent. The decrease of HA in the plasma and the increase of HA in the dialysate is the largest at the beginning of the measurements. This becomes less as the measurements continue.

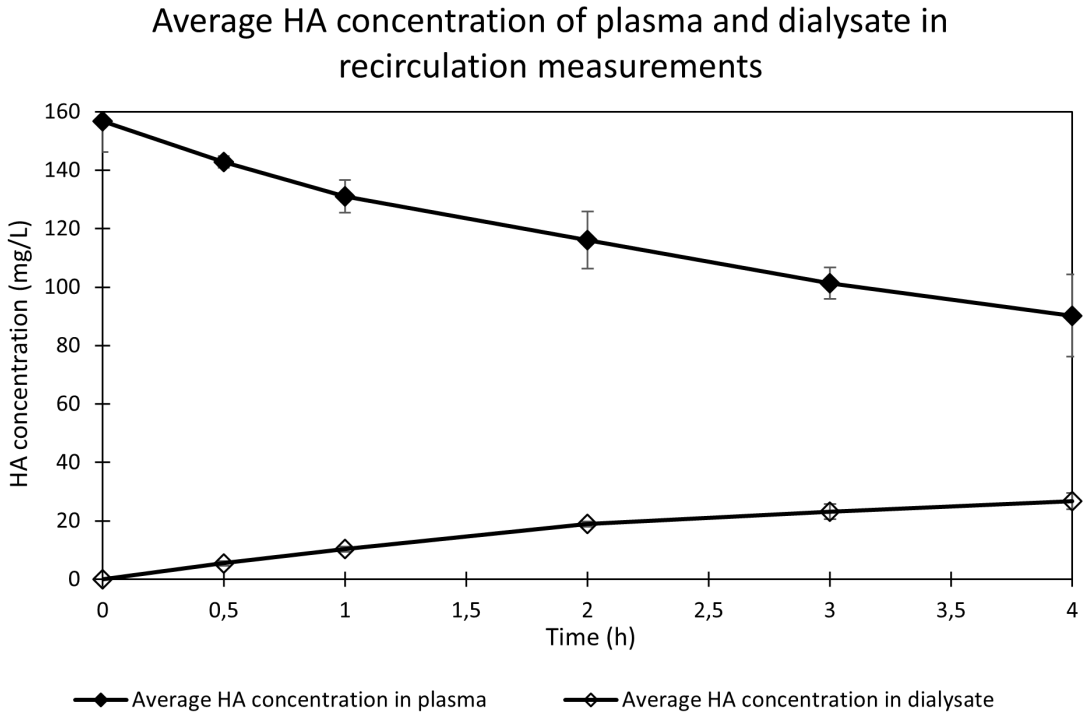


Figure 26: Average HA concentration in the plasma and dialysate during the recirculation measurements (n=3).

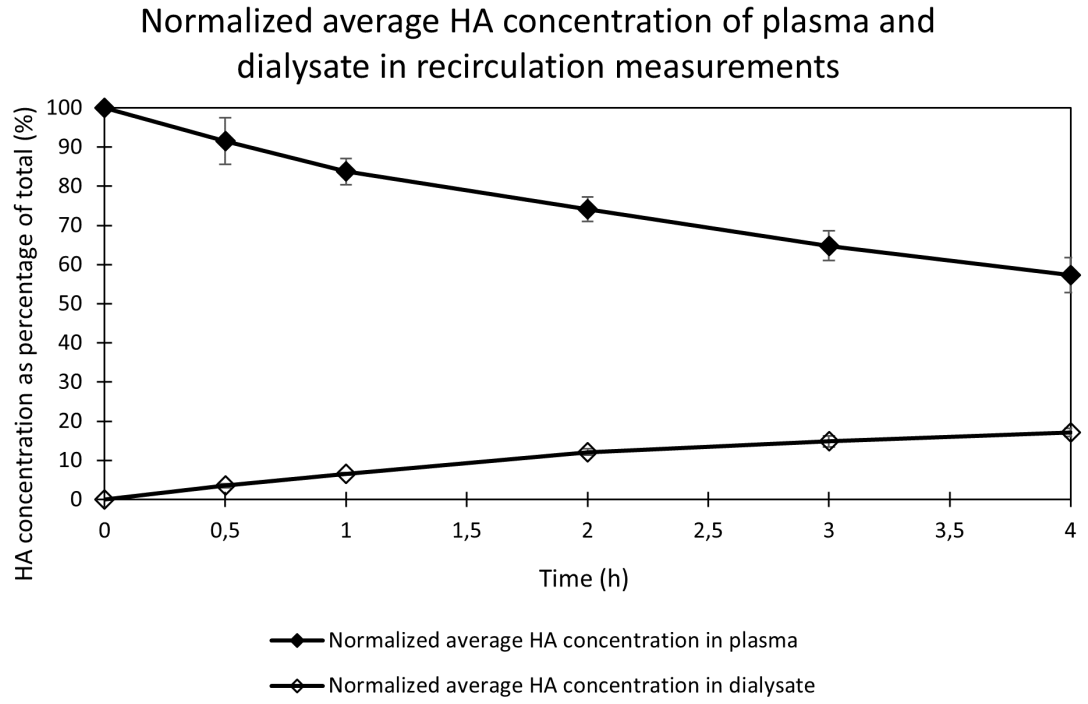


Figure 27: Normalized average HA concentration in the plasma and dialysate during the recirculation measurements (n=3).

Figure 27 with the normalized results of the recirculation measurements shows a decrease of HA in the plasma which was the biggest at the beginning of the measurement and becomes less when the measure-

ments progresses. This can be explained by the accumulation of toxins in the dialysate container, reducing the concentration gradient between the plasma and dialysate solution. From Figure 27 it is also noticeable that the HA concentration decreases with 43 percent, while the HA concentration in the dialysate increases with only 17 percent. Possible explanations for the difference between the removal of HA from the plasma and the gain of HA by the dialysate are a loss of HA toxins in the measurement circuit or adsorption of the toxin to the fibers. By protein-fouling a part of the protein-bound fraction of HA could also be removed by adsorption of the proteins with bound toxins to the fibers. This could lead to a higher removal of HA from the plasma compared to the gain of HA by the dialysate.

To examine the efficiency of the recirculation measurements, the total amount of HA removed from the plasma after four hours is calculated. Using the effective surface area of the module (Aeff), the average amount of HA removed from plasma after four hours is 2799 ± 179 mg per square meter. When looking at the total amount of HA at the beginning of the measurement, it is calculated that 42 percent HA per square meter is removed from the plasma after four hours.

The dialysance for the HA recirculation measurements is calculated using Equations 2 and 3. The average amount of HA removed from plasma per square meter Aeff is plotted against the time. This is shown in Figure 28. The slope gives the calculated dialysance for the removal of HA from the plasma (DL_p). This value is 10.6 L/m²/h, or also 177 ml/m²*min. The average amount of HA gained in the dialysate per square meter Aeff (DL_d) is also plotted against the time. The calculated dialysance is 9.9 L/m²/h, or also 165 ml/m²*min.

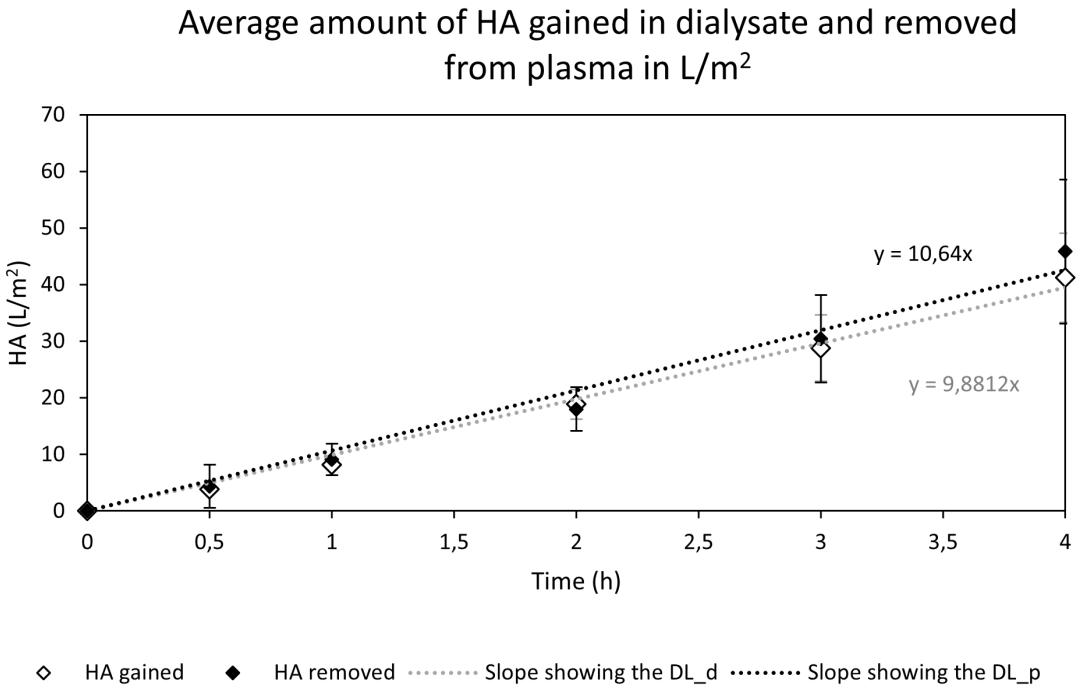


Figure 28: Dialysance of the removal of HA from plasma and the gain of IS by the dialysate in the recirculation measurements (n=3).

The calculation of the dialysance (Figure 28) shows a similar value for the DL_d and DL_p, as would be expected. When the dialysance for the removal of HA from the plasma of study is compared with previous studies, the calculated dialysance is in the same order of magnitude. The calculated value is similar to the dialysance of one previous study, which reported a dialysance of 122 ml/m²*min. This study was

using the same dialysis method, but used a different fiber. One other study using an outside-in filtration method and a MMM-fiber has reported a significantly higher HA dialysance. However, this difference can be explained by the use of a different dialysis method and an in-house made MMM-fiber with possibly different characteristics.

3.4.2 HA single-pass measurement results

After the recirculation measurements with HA as toxin, single-pass measurements have been carried out. Again three measurements are done and the average of these measurements is taken. In Figure 29 the average concentration of HA in the plasma and dialysate over the time of four hours can be seen. The figure shows an average decrease of HA concentration in the plasma of approximately 77 mg/L and an average HA concentration in the dialysate that has a relatively constant value of approximately 1.4 mg/L.

In Figure 30 the normalized concentrations over time can be seen, which shows the percentage of the total amount of HA that is gained or lost by the dialysate and plasma. The figure shows an average decrease of the HA concentration in the plasma of 45 percent after four hours. The average dialysate HA concentration has a relatively constant value of approximately 0.8 percent.

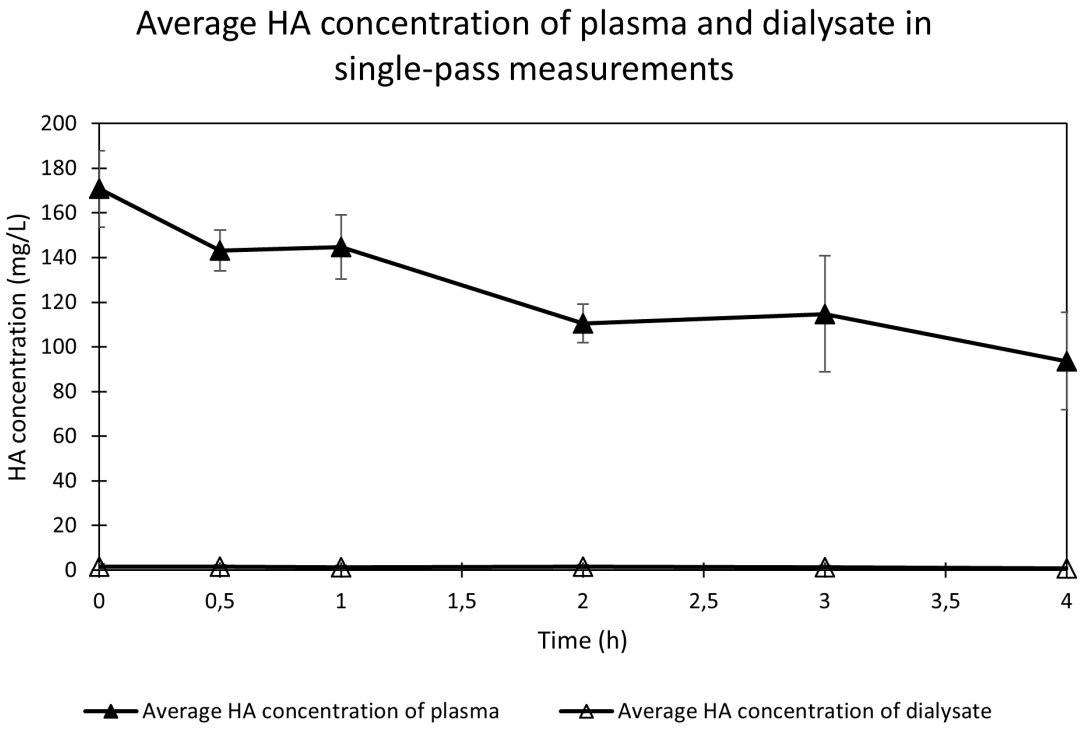


Figure 29: Average HA concentration in the plasma and dialysate during the single-pass measurements (n=3).

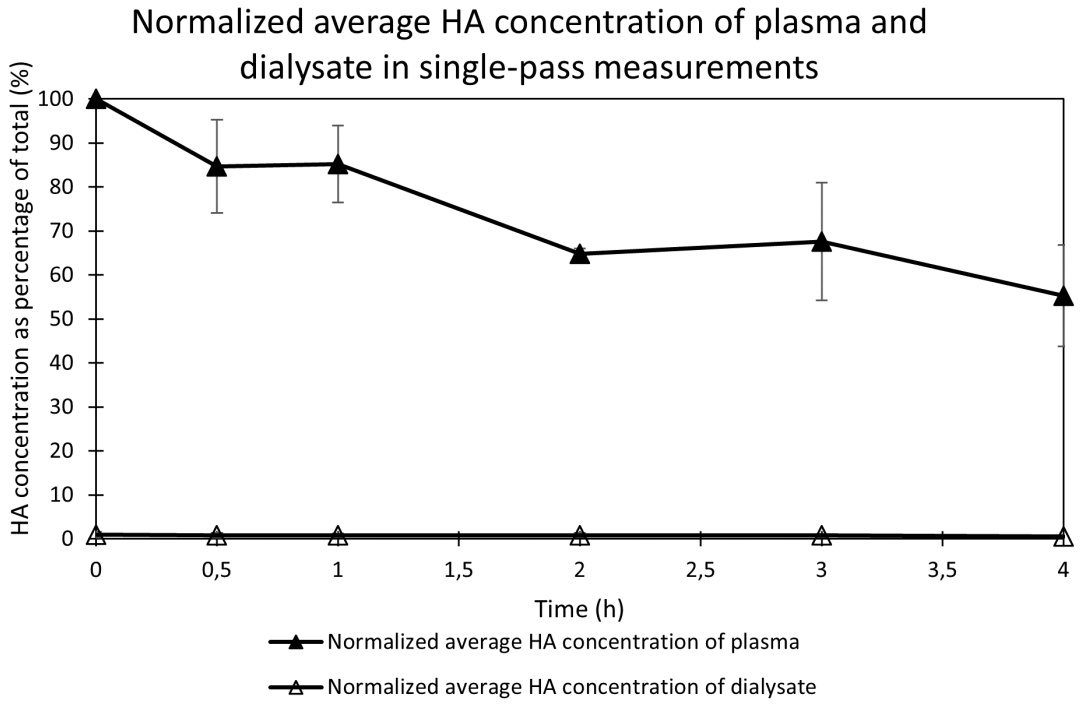


Figure 30: Normalized average HA concentration in the plasma and dialysate during the single-pass measurements (n=3).

Figure 30 shows a decreasing HA concentration in the plasma which is not smooth and which has relatively large error bars. This can be explained by the big variance in efficiency of the single-pass measurements that have been carried out. One of the three measurements that have been carried out had a relatively smaller removal of HA from the plasma, therefore influencing the results of the average values. However, from the figure can still be seen that all HA measurements showed a graduate decrease of HA over the time of four hours.

The total removal of HA from the plasma has also been calculated, using the effective surface area of the module (Aeff). After four hours an average of 3250 $\pm$ 1229 mg/m^2 HA has been removed from the plasma. When looking at the total amount of HA at the beginning of the measurement, it is calculated that 44 percent HA per square meter is removed from the plasma after four hours.

The clearance for the HA single-pass measurements is calculated using Equations 4 and 5. The average amount of HA removed from plasma and gained by the dialysate per square meter Aeff are plotted against the time. This can be seen in Figure 31. The calculated clearance for the removal of HA from the plasma is 9.3 L/m^2/h, or also 155 ml/m^2*min. The calculated clearance for the gain of HA by the dialysate is 8.4 L/m^2/h, or also 140 ml/m^2*min.

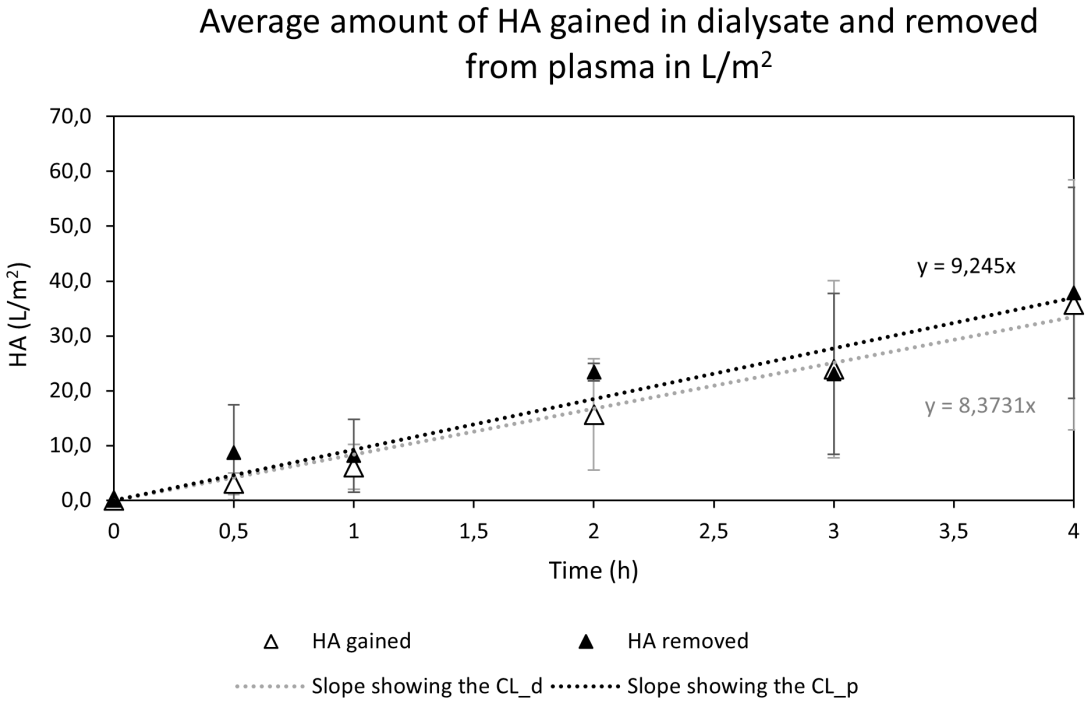


Figure 31: Clearance of the removal of HA from plasma and the gain of HA by the dialysate in the single-pass measurements (n=3).

The calculation of the clearance (Figure 31) shows a similar value for the CL_d and CL_p, as would be expected. The calculated clearance can be compared to an in vivo single-pass study, which reports a HA clearance of 73 ± 7 ml/m²*min. This reported clearance is in the same order of magnitude, but lower compared to the clearance of this current study. This could possibly be explained by the use of a different set-up in this in vivo study. The study used a normal dialysis machine, a different type of fiber and patients with full human blood. Another possible explanation for this variation is the manner in which the CL_d is calculated. Considering the fact that the HA concentration of the dialysate container in the single-pass measurements was unknown, the concentration of the container could not be used to calculate the mass of HA that had passed the module. To be able to calculate the clearance, the HA mass is approximated by using the dialysate HA concentration at every time period and the total time that had been passed. The use of this different way of calculating the HA mass, could have been less accurate.

3.4.3 HA single-pass compared to recirculation

In Figure 32 the normalized recirculation and single-pass results of the HA measurements are shown together. The figure shows that the removal of HA from the plasma is approximately the same for both the recirculation and single-pass measurements. The single-pass measurement has an average decrease of the HA concentration in the plasma of 45 percent, after four hours. For the recirculation measurements this value is 43 percent. The difference between the removal of the two set-ups is not significant (p=0.8232).

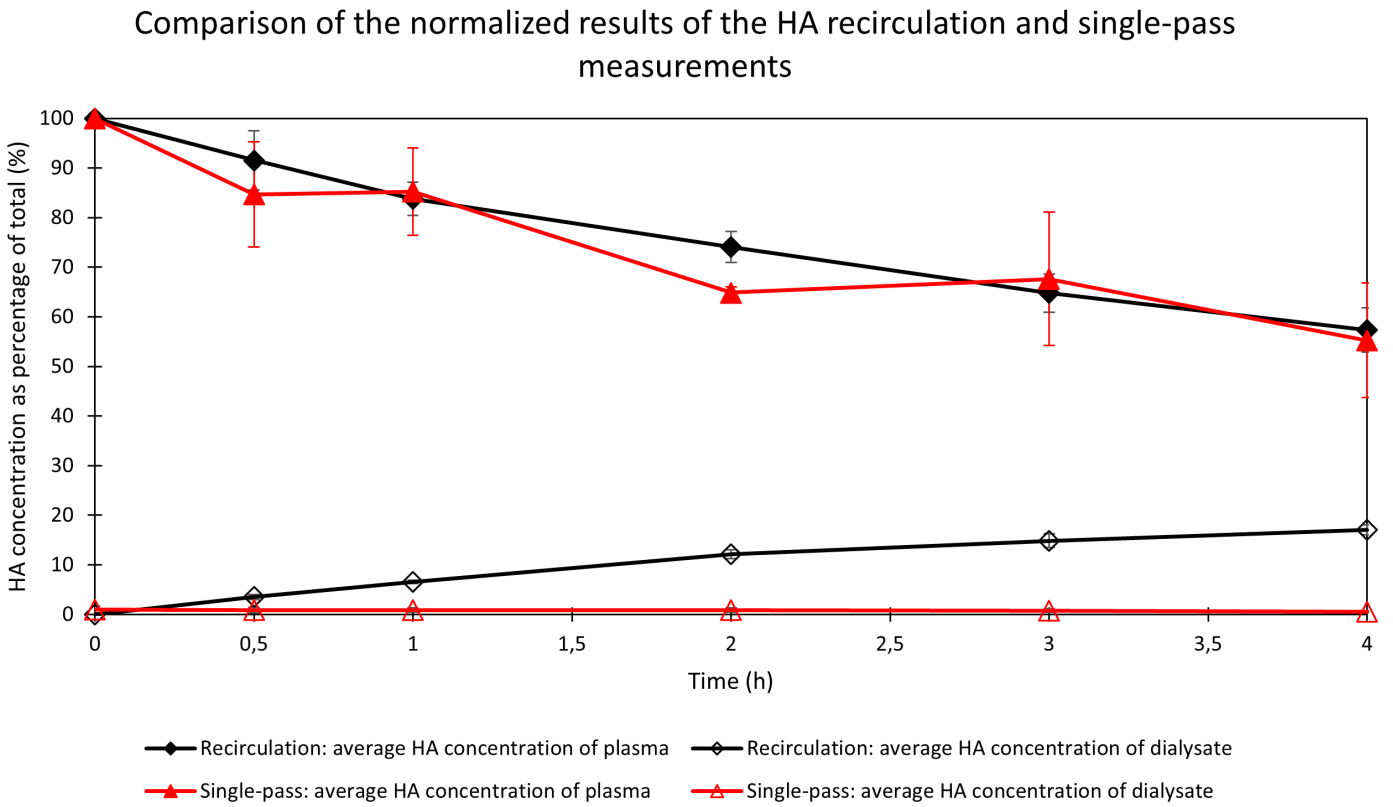


Figure 32: Comparison of the recirculation (n=3) and single-pass (n=3) results of the HA measurements.

When the single-pass and recirculation results are compared (Figure 32), it is shown that the recirculation and single-pass measurements have a similar removal of HA from the plasma. This is different from the creatinine measurements, which showed a more efficient removal by the single-pass set-up. A possible reason for this can be the smaller free fraction of HA, compared to creatinine. The smaller free fraction makes it more difficult to remove HA from the plasma, which causes the accumulation of HA in the dialysate to be less. The reduced accumulation of HA in the dialysate causes the concentration gradient between the plasma and dialysate to remain efficient for the recirculation measurements. This can explain why the accumulation of toxins in the dialysate does not greatly influence the removal efficiency, which therefore makes the single-pass set-up not more efficient.

The total removal of HA from plasma by the recirculation and single-pass set-ups are also compared. This is shown in Figure 33. An average of 42 percent HA per square meter is removed after four hours by the recirculation measurements. By the single-pass measurements an average of 44 percent HA per square meter is removed after four hours. This makes the single-pass set-up slightly more effective, compared to the recirculation set-up. However, the standard deviation for the calculation is relatively high. Considering the relatively high standard deviation and the fact that the total removal of the two set-ups is more of less the same after four hours, the removal of the two set-ups can be considered similar according to these results.

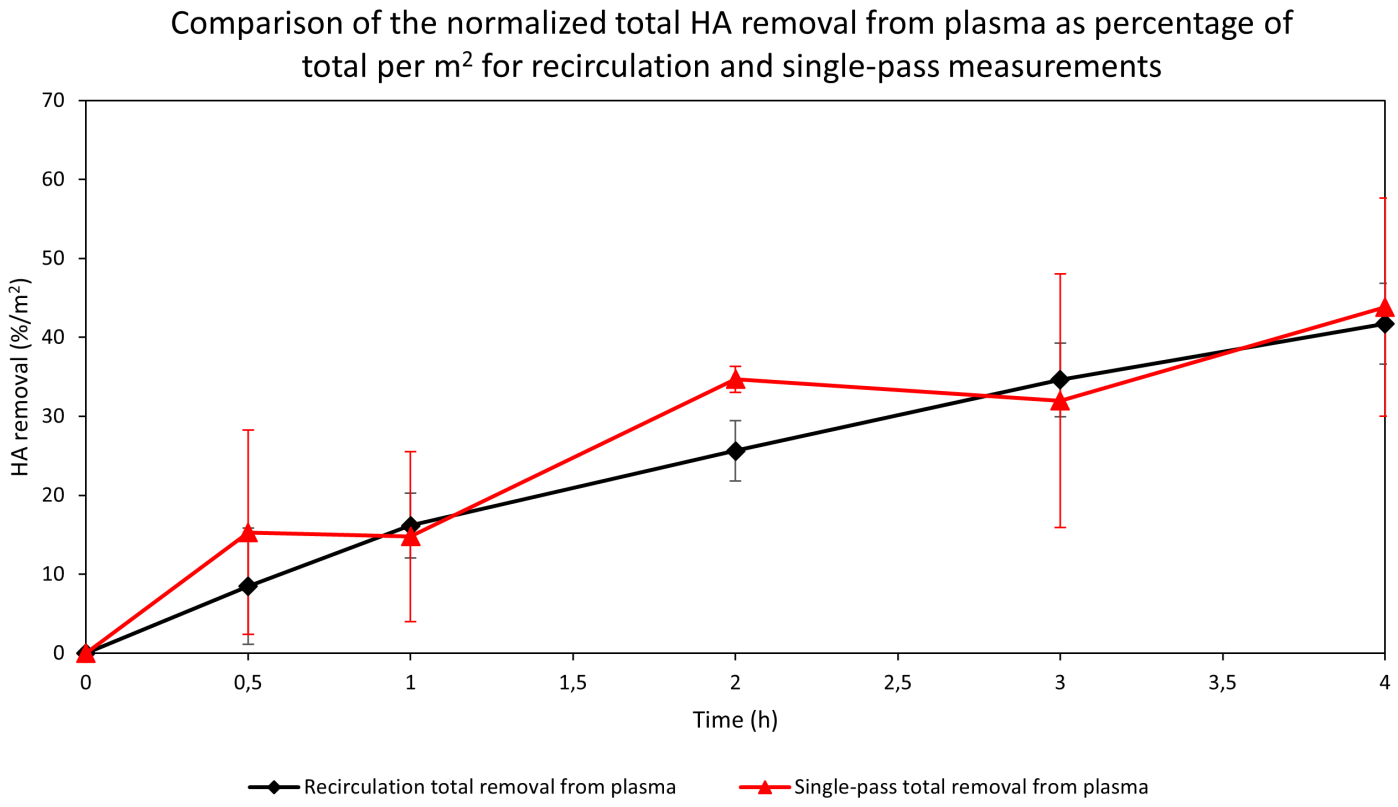


Figure 33: Comparison of the total removal of HA by the recirculation (n=3) and single-pass (n=3) measurements.

The measured total removal of HA can be compared to previous studies, to see if these results have been similar. In Table 8 the results of this study are compared to previous studies. From the table can be seen that the removal of the measurements of this current study are slightly higher, compared to previous studies. Which was also the case with the creatinine measurements. However, when the recirculation measurement results are compared to the previous studies the results are in the same order of magnitude. Especially one study [35], which also used the same type of fiber, reported a HA total removal of 2674 ± 564 mg/m². This is very similar to the removal of 2799 mg/m² of this current study. The other study [37] using the same type of fiber reported a relatively low HA removal. It is not certain why this study reported a lower removal, considering it used the same toxin concentrations and fiber as this current study. One other study [38] also reported a lower removal, which can be explained by the usage of a different fiber with a lower clearance for many toxins compared to the FX1000, according to the manufacturer. The last recirculation study [35] which is mentioned in the table reported a slightly lower removal of 2611 ± 582 mg/m². In this study also another fiber had been used with a slightly lower clearance, which can explain the lower removal. One in vivo single-pass study using HA has been found that can be used to compare to the single-pass measurements of this current study. The previous study reported a HA total removal that is significantly lower than the total removal calculated in this study. This can possibly be explained by the different set-up of the in vivo study. The in vivo study used a normal dialysis machine with different fibers and patients with full human blood instead of plasma.

Table 8: Comparison of the results of several similar IS and HA haemodialysis studies.

Dialysis method	**Type of fiber**	**IS and/or HA conc.**	**Flow rate (ml/min)**	**Surface area (m^2)**	**Removal (mg/m^2)**
IOF Recirculation [38]	Polyflux 2H	IS: 40 mg/L HA: 110 mg/L	Qp: 10 Qd: 1	1.6×10^{-9}	IS: 374 ± 12 HA: 1562 ± 263
IOF Recirculation [37]	FX1000	IS: 40 mg/L HA: 110 mg/L	Qp: 1 Qd: 10	1.0×10^{-3}	IS: 92 ± 10 HA: 1454 ± 626
IOF Recirculation [35]	FX1000	IS: 40 mg/L HA: 110 mg/L	Qp: 1 Qd: 10	6.0×10^{-4}	IS: 377 ± 91 HA: 2674 ± 564
IOF Recirculation [35]	F8HPS	IS: 40 mg/L HA: 110 mg/L	Qp: 1 Qd: 10	5.9×10^{-4}	IS: 272 ± 43 HA: 2611 ± 582
Single-pass in vivo study [42]	FX800	Patient IS and HA level (IS: ± 15.1 mg/L / HA: ± 24.1 mg/L)	Qp: 300 Qd: 700	1.8	IS: 46 HA: 377
IOF Recirculation (this study)	Fresenius FX1000	IS: 40 mg/L HA: 110 mg/L	Qp: 1 Qd: 10	1.03×10^{-3}	IS: 247 ± 26 HA: 2799 ± 179
IOF Single-pass (this study)	Fresenius FX1000	IS: 40 mg/L HA: 110 mg/L	Qp: 1 Qd: 10	1.03×10^{-3}	IS: 169 ± 177 HA: 3250 ± 1229

3.4.4 Free fraction of HA

The free fraction of HA is calculated from the results. For HA a free fraction of approximately 66 percent is found in literature. From the results of the HA recirculation measurements an average free fraction of 66 ± 9 percent is calculated. For the single-pass measurements this value is 69 ± 10 percent.

3.4.5 IS recirculation measurement results

After using creatinine and HA as toxin, recirculation and single-pass dialysis experiments have been carried out with IS as toxin. Again three experiments have been carried out and the average has been taken. In Figure 34 the average IS concentration of the plasma and dialysate over the time period of four hours can be seen. Figure 35 shows the normalized results of the recirculation measurements. The figures both show a small but constant decrease of IS concentration in the plasma and a smaller increase of IS concentration in the dialysate. From Figure 34 an average decrease in IS concentration in the plasma of 5,93 mg/L after four hours is calculated. The IS concentration in the dialysate has an average increase of 1.32 mg/L. From Figure 35 an average decrease of IS concentration in the plasma of 17 percent can be seen. The IS concentration of the dialysate increases on average with 3.5 percent.

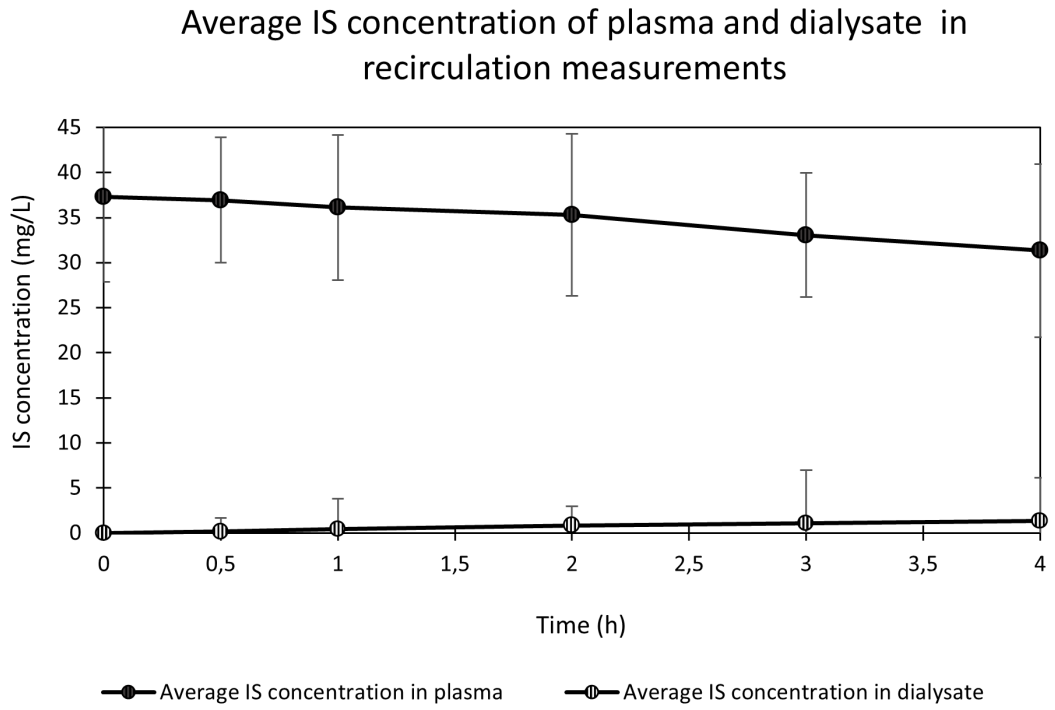


Figure 34: Average IS concentration in the plasma and dialysate during the recirculation measurements (n=3).

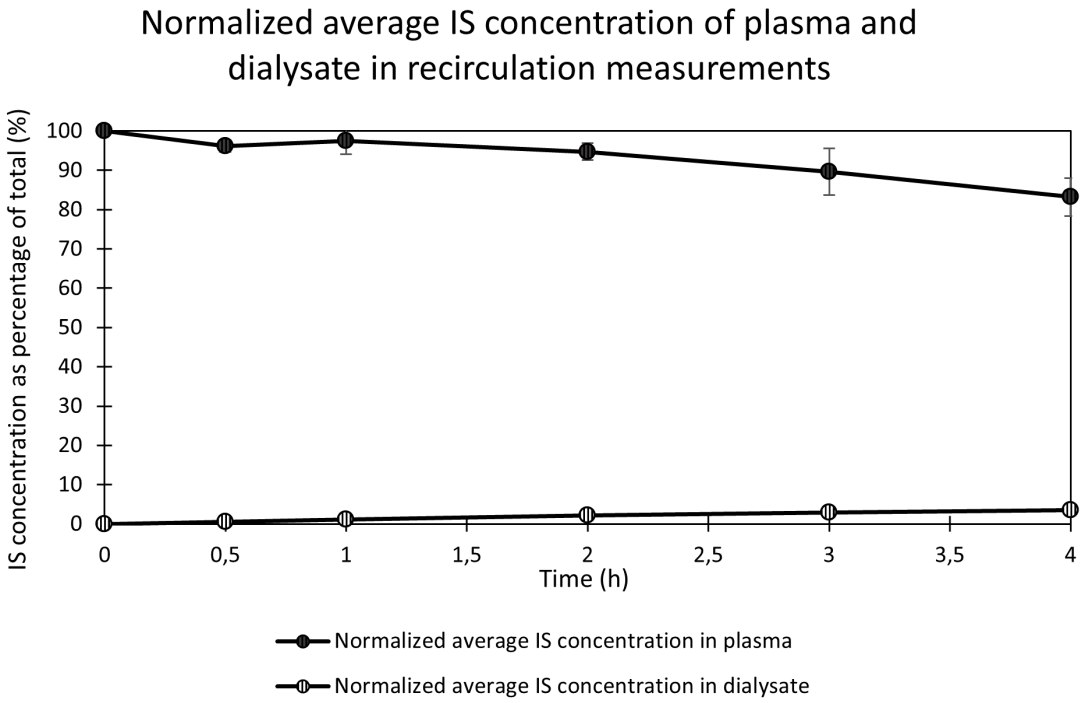


Figure 35: Normalized average IS concentration in the plasma and dialysate during the recirculation measurements (n=3).

From Figure 34 can be seen that there is a relatively large variation in the concentration between the different measurements. This can be seen by the large error margin. The variation in the concentration

is partly caused by a relatively large variation in the IS starting concentration in the plasma. However, the average of the measurements shows a graduate decrease in IS of the plasma, which was also expected. Figure 35 with the normalized results shows a small error margin, which makes the results very reliable.

The total removal of IS from the plasma has also been calculated, using the effective surface area of the module (Aeff). After four hours a total of 247 ± 26 mg/m^2 IS has been removed from the plasma. When looking at the total amount of IS at the beginning of the measurement, it is calculated that 16 percent IS has been removed from the plasma after four hours.

These results of the calculations for the dialysance be seen in Figure 36. The calculated dialysance for the removal of IS from the plasma is 1.9 L/m^2/h, or also 32 ml/m^2*min. The calculated dialysance for the gain of IS by the dialysate is 1.1 L/m^2/h, or also 18 ml/m^2*min.

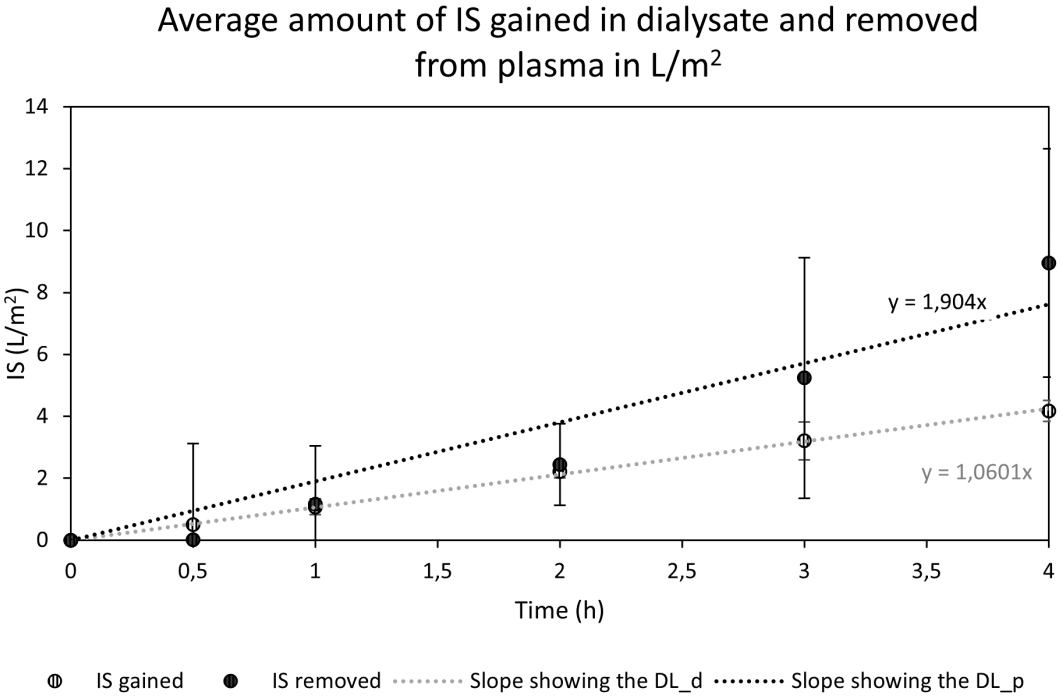


Figure 36: Dialysance of the removal of IS from plasma and the gain of IS by the dialysate in the recirculation measurements (n=3).

In Figure 34 and 35 it is visible that the gain of IS by the dialysate is lower than the removal of IS from the plasma. This can also be seen when looking at the results of the dialysance, shown in Figure 36. The dialysance of IS removed from the plasma is higher than the dialysance of IS gained by the dialysate. Considering no toxins can leave the circuit, these two were expected to be the same. However, the calculated dialysance values are still close to each other and the error margins of the calculation are relatively big. This makes the results of the dialysance calculation less reliable. A possible explanation for the smaller gain of IS by the dialysate, compared to the removal of IS from the plasma, is the adsorption of proteins with bounded IS toxins to the fibers. The absorption of the proteins to the fiber membrane can cause additional removal of IS toxins in this situation. Another explanation can be the that toxins might be left behind on a surface inside the circuit.

The calculated dialysance of the recirculation measurements can be compared to previous studies. The calculated IS dialysance of 32 ml/m^2*min is significantly smaller than the IS dialysance reported by a

previous study using the same set-up. This study reported a dialysance of 63 ml/m^2*min. A possible explanation for this can be the usage of MMM-fibers by this previous study. Another experiment mentioned in the same paper reported an even higher dialysance of 145 ml/m^2*min. However, this study used an outside-in filtration method which could have caused a different outcome and different results.

3.4.6 IS single-pass measurements

After the recirculation measurements, the single-pass measurements have been carried out. In Figure 37 the average IS concentration of the plasma and dialysate during the time period of four hours can be seen. The IS concentration of the plasma is decreased by 3.99 mg/L after four hours. The IS concentration of the dialysate stays relatively low and has a more or less constant value of 0.04 mg/L. The relatively large standard deviation also shows that there is much variation in the IS concentration over time between the different measurements. This is probably partly caused by the relatively large variation in IS concentration of the plasma after spiking. In Figure 38 the normalized results of the single-pass measurements can be seen. After four hours the IS concentration in the plasma has decreased with approximately 12 percent. The IS concentration of the dialysate in this figure has a relatively constant value of approximately 0.12 percent.

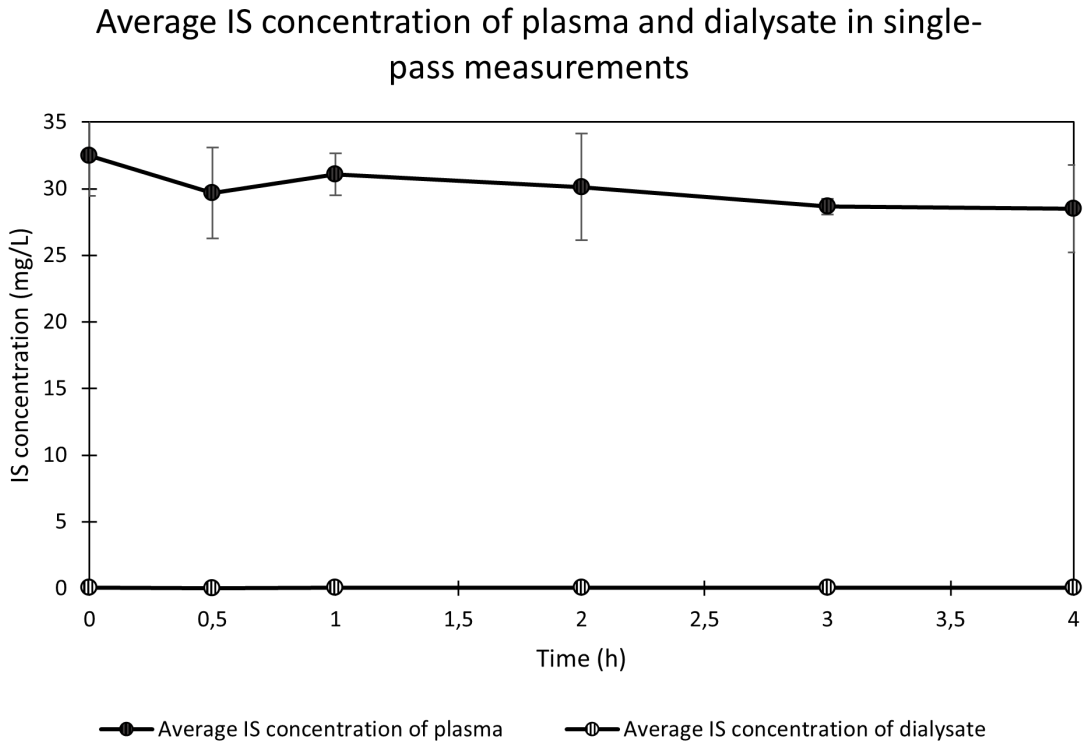


Figure 37: Average IS concentration in the plasma and dialysate during the single-pass measurements (n=3).

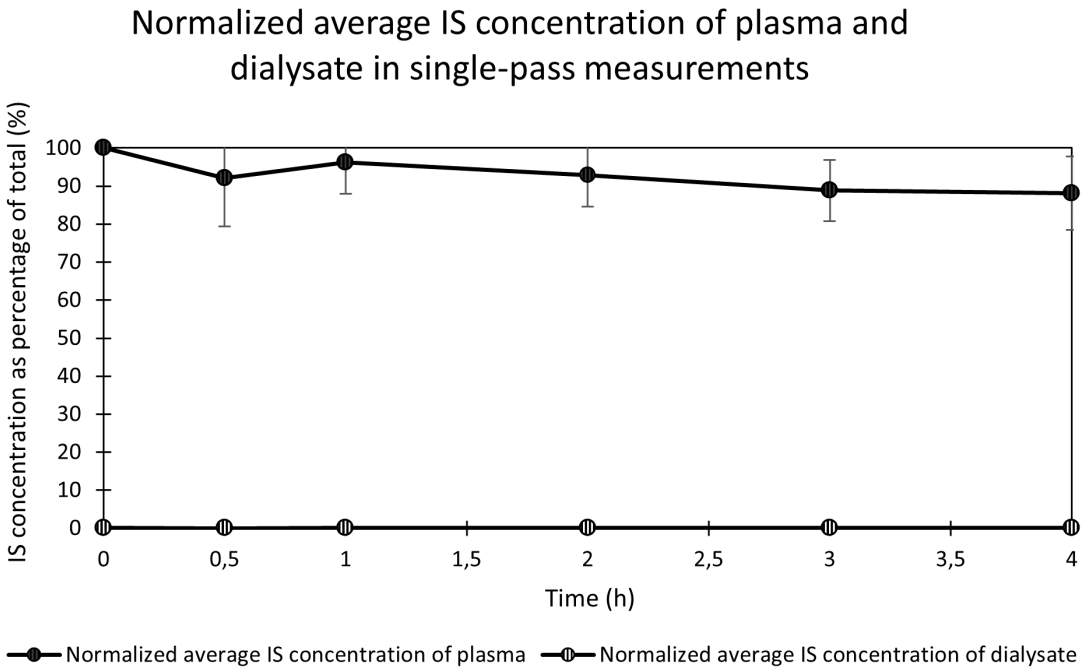


Figure 38: Normalized average IS concentration in the plasma and dialysate during the single-pass measurements (n=3).

Figure 38 shows that after four hours the IS concentration in the plasma has gradually decreased, however the relatively large standard deviation makes the results less reliable. In the figures can also be seen that the IS concentration in the dialysate remains low and relatively constant, which is expected for the single-pass measurement.

The total removal of IS from the plasma has been calculated to examine the efficiency of the measurement. This has been done using the effective surface area of the module (Aeff). After four hours a total of 169 $\pm$ 177 mg/m^2 IS has been removed from the plasma. When looking at the total amount of IS at the beginning of the measurement, it is calculated that 12 percent of IS has been removed from the plasma after four hours.

The clearance of the removal of IS from the plasma and the gain of IS by the dialysate has been calculated. This can be seen in Figure 39. The figure shows that the clearance of the removal of IS from the plasma is 1.8 L/m^2/h, or also 30 ml/m^2*min. The clearance of the gain of IS by the dialysate is calculated to be 0.8 L/m^2/h, or also 13 ml/m^2*min. The standard deviation of the calculations is relatively high.

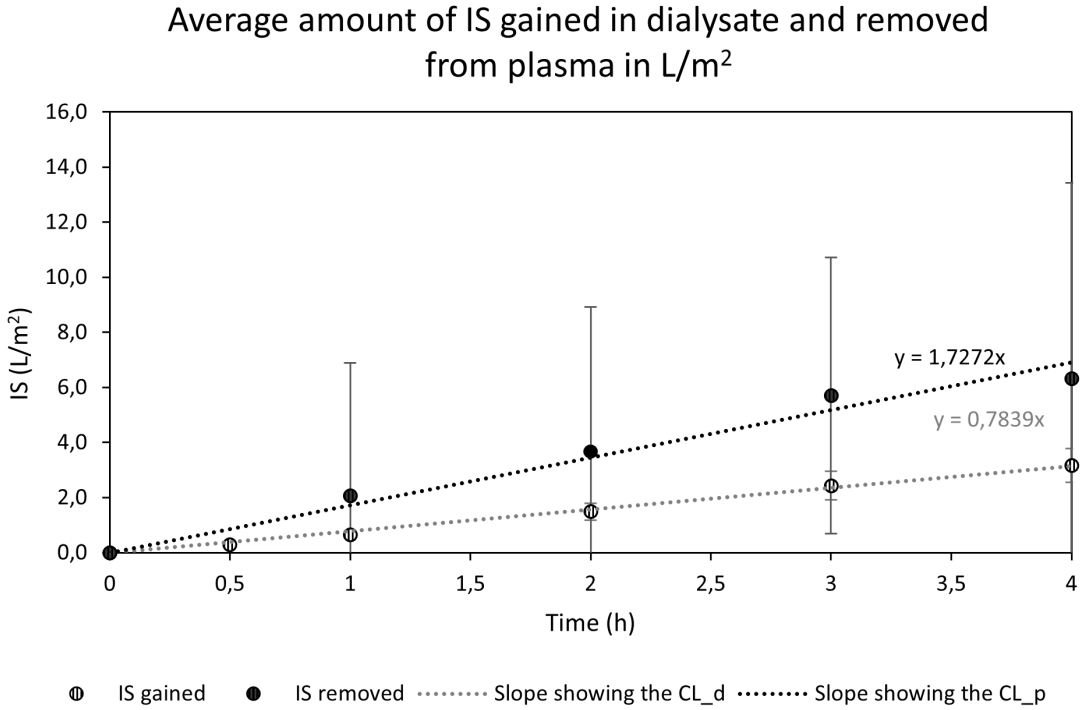


Figure 39: Clearance of the removal of IS from plasma and the gain of IS by the dialysate in the single=pass measurements (n=3).

The calculated IS clearance (Figure 39) shows a relatively low clearance for the gain of IS by the dialysate, considering the fact that the CL_d and CL_p are expected to be similar. A possible explanation for this is the adsorption of proteins with bounded IS toxins to the fibers, or toxins being left behind inside the set-up circuit. Another possible cause for this variation is the manner in which the CL_d is calculated. This has been done in the same manner the CL_d of HA has been calculated. The IS concentration of the dialysate container in the single-pass measurements was unknown, so the concentration of the container could not be used to calculate the mass of IS that had passed the module. To calculate the clearance the IS mass is approximated by using the dialysate IS concentration at every time period and the total time that had been passed. This different way of calculating the IS mass, could have been less accurate. When the calculated IS clearance is compared to a previous study, the clearance of this value is in the same order of magnitude. The previous study [42] reported an IS clearance of 15 $\pm$ 3 ml/m^2*min, which is lower than the clearance of 30 ml/m^2*min of this study. However, the standard deviation of the calculation of the clearance in this current study is relatively high, making the results of this study less reliable.

3.4.7 IS single-pass compared to recirculation

To compare the single-pass and recirculation results of the IS measurements, the normalized results of both different set-ups are shown in Figure 40. The recirculation and single-pass set-ups both show a similar graduate decrease of IS in the plasma. However, the large error margins of the single-pass measurements should be taken into account. The standard deviations of the calculations are too large to be able to accurately conclude which set-up is more efficient. However, the results do show that the removal of IS using the single-pass and recirculation set-ups is very similar.

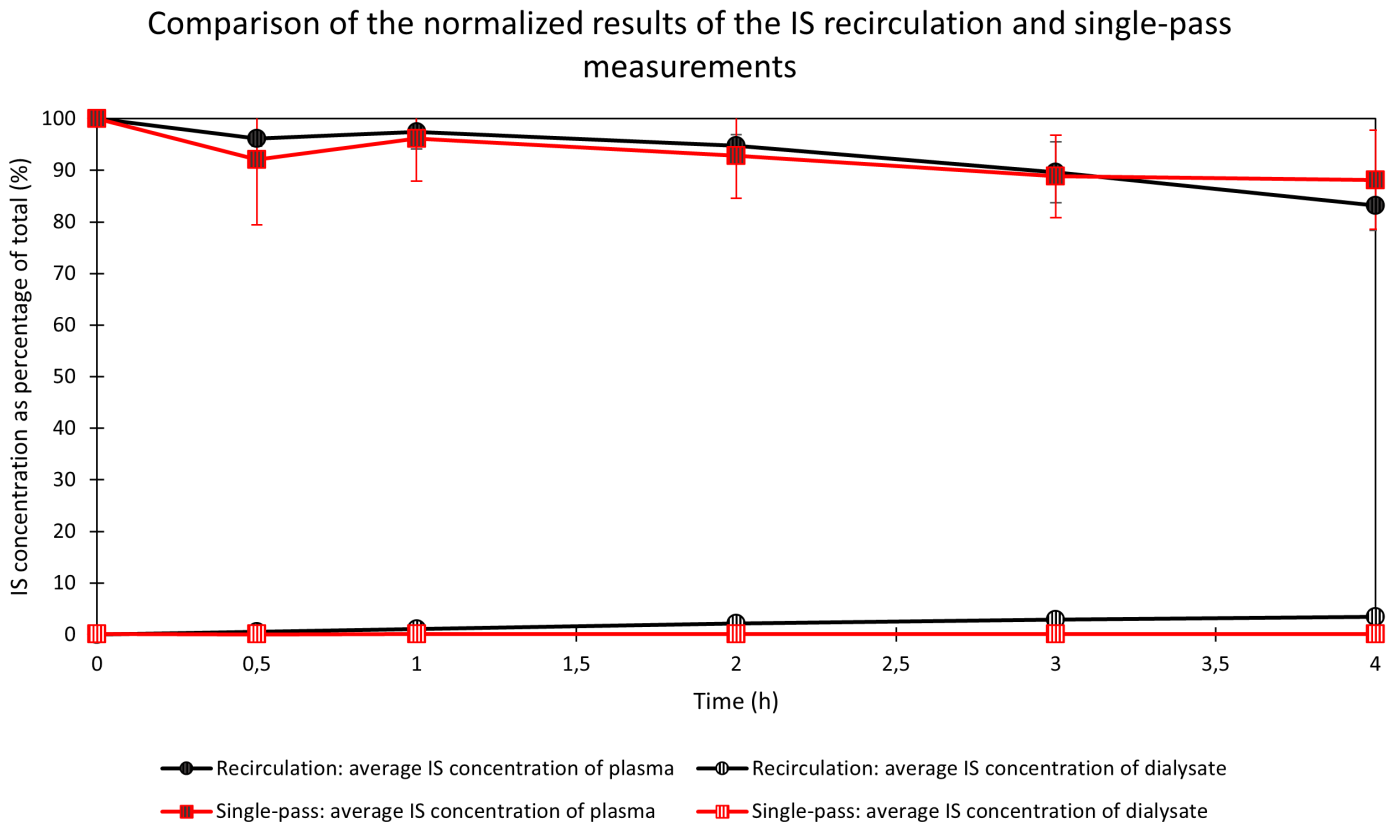


Figure 40: Comparison of the recirculation (n=3) and single-pass (n=3) results of the IS measurements.

The total removal of IS from the plasma by the recirculation and single-pass measurement set-ups is also compared. An average of 16 percent of IS per square meter is removed by the recirculation set-up after four hours. By the single-pass set-up an average of 12 percent IS per square meter is removed. Compared to previous studies (Table 8) the total amount of IS removed by this study is similar to results of previous studies. However, due to a large variation in the obtained results of the single-pass measurements the standard deviation of the single-pass calculations is very high. This large variation was partly caused by some peaks in the IS concentration measurements which could not be logically explained. The large standard deviation makes the results of the calculations not reliable. More single-pass measurements with the toxin IS should be carried out to give a reliable conclusion about the difference in total removal between the two set-ups.

3.4.8 Free fraction of IS

The free fraction of IS is also calculated from the results. From literature it is known that IS has a free fraction of approximately 10 percent. From the results of the IS recirculation measurements an average free fraction of 15 $\pm$ 7 percent is calculated. From the results of the IS single-pass measurements an average free fraction of 11 $\pm$ 3 percent is calculated.

3.4.9 IS recirculation compared to HA recirculation: total removal

To clearly see the difference between the total removal of HA and IS by the recirculation measurements, the recirculation results of the two toxins are plotted together. This can be seen in Figure 41. In the figure can be clearly seen that the total removal of HA as percentage of the total is significantly higher compared to the total removal of IS as percentage of the total. This can be explained by the higher free fraction of

HA compared to IS, which makes HA easier to remove.

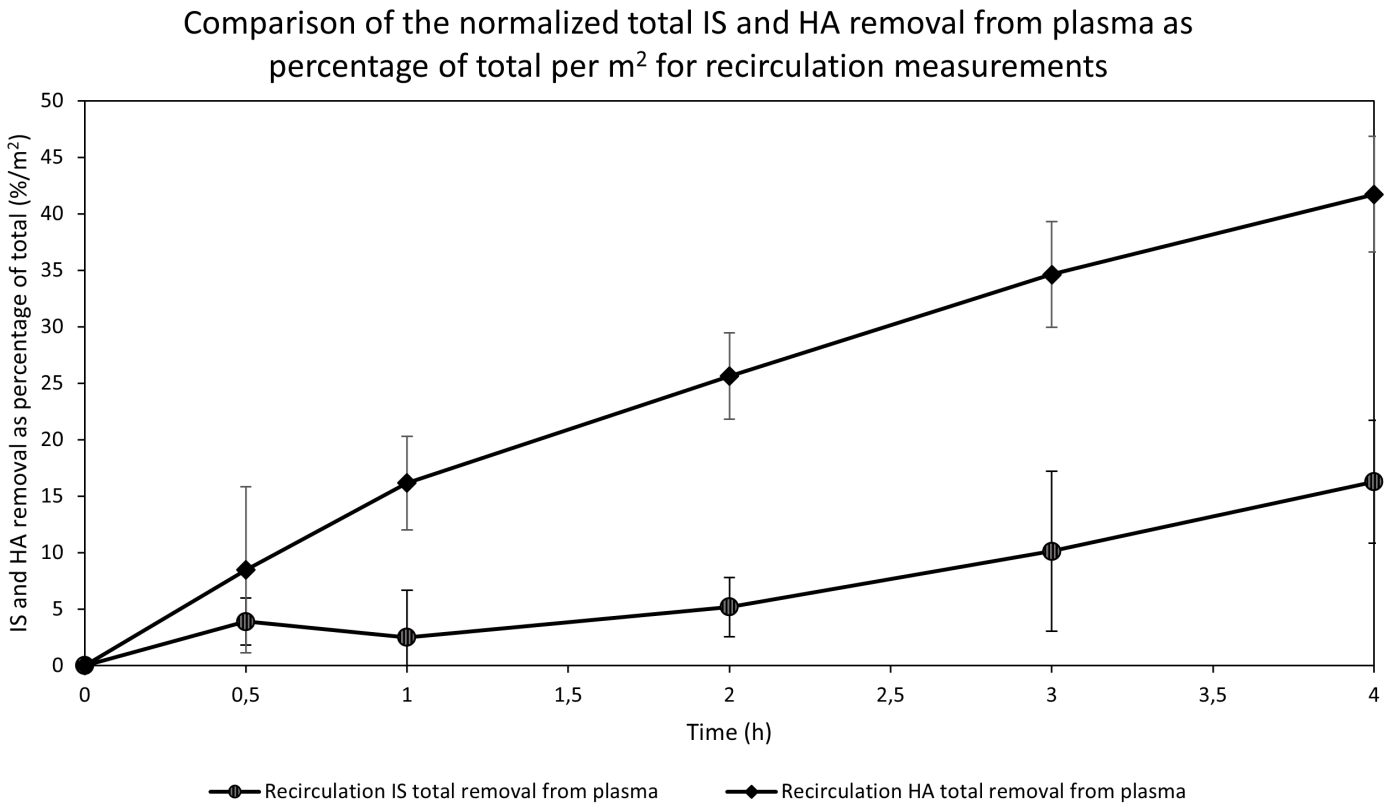


Figure 41: Comparison of the recirculation results of the total removal of HA (n=3) and IS (n=3) from plasma as percentage of the total amount.

3.5 Further discussion points

There are some factors which could have possibly influenced the results of the recirculation and single-pass measurements. First of all, the concentration of the creatinine, IS and HA toxins in the plasma that has been used was not zero before adding the toxins. This means the final concentration of the toxins in the plasma was often higher than the desired concentration. Also, for the creatinine measurements plasma of two different donors has been used. Considering the fact that the plasma of each patient has slightly different components, the varying composition of the plasma could have influenced the results. For the IS and HA measurements plasma of only one patient has been used, so for these measurements this was not the case. One other factor which could have influenced the results of the protein-bound toxins is the choice of the Fresenius FX1000 fibres for the experiments. These fibers are designed for the removal of water-soluble toxins like creatinine and are therefore not specifically designed for the protein-bound toxins HA and IS. This could have influenced the removal efficiency for these toxins. As a final point, one other factor that could have influenced the measurements is the presence of air bubbles in the tubes or module during the measurement. Air bubbles inside the module could reduce the amount of dialysate that is in contact with the fiber and therefore decrease the removal of toxins. Air bubbles were sometimes visible during the creatinine, HA and IS measurements. However, by moving the tubes the air bubbles have been removed when visible.

4 Conclusion

In this study the difference between the uremic toxin removal of single-pass and recirculation dialysis experiments has been investigated. Several recirculation and single-pass haemodialysis measurements have been successfully carried out using the Convergence set-up and in-house made modules. The measurements have been done with three different toxins, to be able to see the influence of the specific characteristics of the toxins on the removal efficiency. From the obtained results the total removal, dialysance or clearance and the concentration decrease over time have been calculated.

4.1 The creatinine measurements

From the creatinine measurement results can be concluded that the single-pass measurements set-up is more efficient in removing creatinine from the plasma under the specified conditions, compared to the recirculation set-up. The difference in the decrease of creatinine in the plasma between the two set-ups amounted to a 12 percent higher removal by the single-pass set-up. This difference was significant. The difference between the total removal of the two set-ups amounted to an 11 percent higher removal by the single-pass set-up. This difference was also significant. The biggest difference in removal was noticeable after 2/3 hours. Before this time the two set-ups had almost similar results. For shorter measurements (of less than approximately 2 hours), it is therefore not needed to use a single-pass set-up instead of a recirculating set up because it will not lead to a higher removal according to the results of this thesis. For longer measurements (of approximately more than 2 hours) the use of a single-pass set-up is advised, since this will lead to a more efficient creatinine removal.

4.2 The protein-bound toxin measurements

From the hippuric acid measurement results can be concluded that under these circumstances the single-pass set-up and recirculation set-up have similar outcomes. This conclusion is mostly based on the results of the HA concentration in the plasma and dialysate over the period of four hours. These results did not show a significant difference between the two set-ups. The results of the total removal also showed no significant difference between the set-ups, however due to the large standard deviation in the calculation of the total removal these results were less reliable. In conclusion, the results of this thesis showed no improved removal of HA by the single-pass set-up compared to the recirculation set-up.

From the indoxyl sulfate measurement results can be concluded that under these circumstances the single-pass set-up and recirculation set-up have similar outcomes. The results of the IS concentrations in the plasma and dialysate showed no significant difference in the IS concentrations after four hours between the two set-ups. The results of the total removal calculations were not taken into account, because of the high standard deviation of the single-pass calculations. From the IS results can be concluded that the single-pass set-up showed no improved removal of IS from the plasma, when compared to the recirculation set-up However, the relatively high standard deviation of the single-pass calculations makes these results less reliable.

5 Outlook

In future work several additional experiments could be done, to further improve the knowledge of the difference between single-pass and recirculation dialysis experiments. New types of measurements could be carried out using a slightly different set-up, different toxins or blood instead of plasma.

Recirculation and single-pass measurements using creatinine have been successfully carried out in this study. The results of this study showed that the single-pass measurements have been more effective in removing creatinine from the plasma. The single-pass measurements have been compared to recirculation measurements using 100 ml of dialysate volume. To determine the volume for which the single-pass and recirculation systems have similar removal efficiency, more creatinine recirculating measurements could be carried out using different volumes of dialysate solution. In the single-pass measurements a total of 2.4 L of dialysate is used in four hours. Recirculation measurements could be carried out using a dialysate volume that is closer to this value. For example with a dialysate volume of 200 ml, 500 ml and 1 L. It would be interesting to see if the increase of dialysate volume could increase the concentration gradient between the plasma and dialysate by a sufficient amount, to make the recirculation set-up as effective as the single-pass set-up.

In addition to this, the single-pass measurements which have been carried out in this experiment could be repeated using a longer time period for the measurement. The results of this study show that for the creatinine measurements the single-pass set-up still has a decrease of creatinine in the plasma between 3 and 4 hours after starting the experiment. Increasing the time-period of the measurements, for example to 8 or 24 hours, could show an even more effective removal of creatinine by the single-pass set-up. This could also show an even bigger difference between the recirculation and single-pass set-ups, considering the fact that the recirculation set-up showed a decreased removal of creatinine form the plasma after 3 hours in this study. Probably due to the reduced concentration gradient.

Future work could also focus on the experiments done in this study with the use of full human blood instead of human plasma. This would create an experiment which is closer to the real haemodialysis treatment situation at the hospital. Doing the experiments with blood would give information about the effect the blood cells inside the blood would have on the removal efficiency of the single-pass and recirculation set-ups. This would also make it easier to more accurately compare the results of the Convergence set-up with in vivo studies which are carried out at the hospital.

Next to this, the recirculation and single-pass measurements could also be carried out using more different toxins. For example with the middle-molecule uremic toxin β-2-microglobulin, which is difficult to remove during current haemodialysis therapy. This information would be useful for future experiments at the University of Twente which use this type of toxin and the Convergence set-up. These experiments could then be carried out using the single-pass set-up, if this set-up is indeed shown to be more effective.

Another interesting possibility for future work is repeating the single-pass and haemodialysis experiments with a different type of fiber. For the experiment of this thesis the high-flux hemodiafiltration FX1000 fibers have been used, but it would be interesting to see the influence of the use of low-flux fibers on the results. Considering the fact that a low-flux fiber with a lower clearance will probably remove less toxins from the plasma, this measurement could maintain a higher concentration gradient between the plasma and dialysate. The higher concentration gradient in the recirculation measurements could reduce the difference in removal between the two set-ups. It would be interesting to see the influence of another fiber, also to be able to give an accurate advise about which set-up should be used in combination with a low-flux fiber when using the Convergence for future research.

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