Development of an IPK control system and oxygenator model

By
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Development of an IPK control system and oxygenator model

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ABSTRACT

In order to better preserve donor kidney organs during and after procurement, the Universitary Medical Centre Groningen and partners are developing an improved Normothermic Regional Perfusion (NRP) device.
Two main research objectives are to replace blood with a more efficient perfusion medium and to identify biochemical damage markers best indicative of kidney condition.
To aid to this research a custom made Isolated Perfused Kidney (IPK) control system was requested. Furthermore additional research was performed on different oxygenators in order to create a model describing the process of oxygenation within an IPK setup.

The first section of this document contains research investigating the project domain and available hardware solutions currently being used during IPK. Furthermore the oxygenation process and which factors play a role in a membrane oxygenator were investigated.
The second section entails development of an IPK control system and an oxygenator model based on multivariate polynomial regression.

The developed prototype IPK control system was able to pump a kidney at a desired pressure pattern and has been used in a multitude of IPK experiments. The developed model has an R^2 value of 99,88% with no sign of overfit and with every parameter adding a significant contribution.

ACKNOWLEDGEMENTS

The results set forth in this report have been brought about thanks to the effort of several parties.

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DECLARATION

I hereby certify that this report constitutes my own product, that where the language of others is set forth, quotation marks so indicate, and that appropriate credit is given where I have used the language, ideas, expressions or writings of another.

I declare that the report describes original work that has not previously been presented for the award of any other degree of any institution.

Signed,

Ale-Watze Wiegerma

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LIST OF ABREVIATIONS

UMCG	University Medical Centre Groningen
NRP	Normothermic Regional Perfusion
IPK	Isolated Perfused Kidney
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
CS	Cold Storage
NP	Normothermic Perfusion
PWM	Pulse-Width Modulated
PID	Proportional-Integral-Derivative
NMP	Normothermic Machine Perfusion

1 RATIONALE

People may need an organ transplantation if one of their own organs' performance has decreased to such an extent that a person's life has a significant decrease in quality of life or the person is even in a life-threatening situation.

In order to meet the demand for required organs for transplantation, residents of the Netherlands can choose to make their organs available for transplantation when they die. In this way, their organs (such as heart, lungs, liver, intestines, pancreas and kidneys) and tissue (such as cornea, bone, skin, heart valves and blood vessels) [1] can be made available for transplantation.

For years, there has been difference in organ supply and demand as there are not enough donors and the available organs of donors can be of lesser quality due to the conditions in which the donor died and conditions in which the organs were retrieved. Figure 1 shows the waiting list for several organs according to Eurotransplant, an organization facilitating efficient exchange of donor organs between several European countries. It shows that the supply and demand gap is the largest for kidneys.

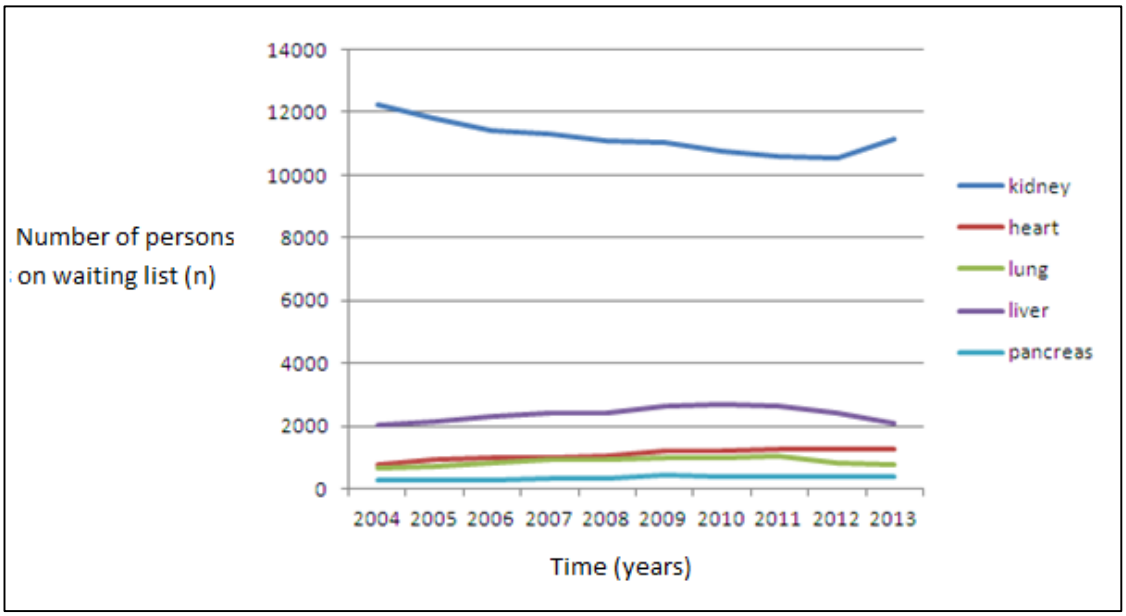

Figure 1 Waiting lists for several organs (Eurotransplant). The waiting list for the kidney organ is significantly higher than for other organs [1].

Figure 2 shows the number of performed transplantations for different organs according to Europlant. It can be concluded that there is a large difference between the number of people waiting to receive a kidney and the people who actually receive one as seen in figure 2.

People who need a kidney transplant can experience a range of symptoms indicating lesser kidney function such as weakness, shortness of breath, lethargy, confusion and abnormal heart rhythms due to an inability to remove potassium from the blood. If the kidneys fail completely patients will even need a dialysis device until transplant [2]. However survival of persons on a waiting list for a kidney transplant cannot be assured. For the United States, 13 persons waiting for a life-saving kidney transplant die each day, whilst over 2014, 3668 people became too sick to receive a kidney transplant [3]

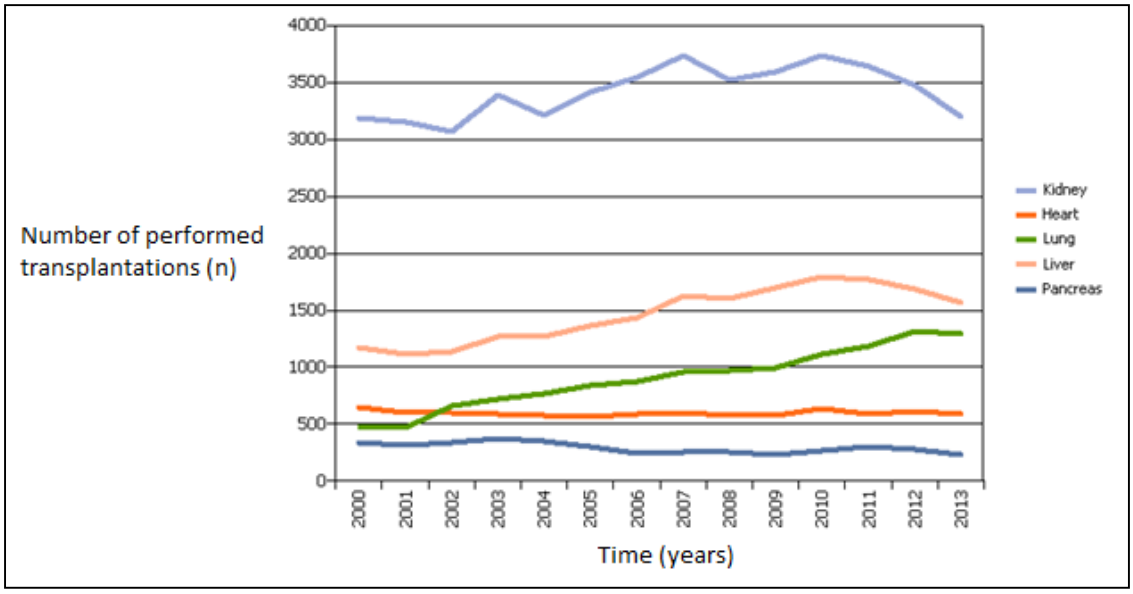

Figure 2: Number of performed transplantation for different organs (eurotransplant) [1]. The number of performed kidney transplantations is much higher than for other organs however there is still a large gap between supply and demand.

When a donor dies, depending on type of death, organs are damaged by warm ischemia. Warm ischemia is insufficient blood supply to organs at normothermic temperatures (37 degrees Celsius).

The UMCG hospital in Groningen in cooperation with several partners, has initiated a project which aims at creating an improved type of normothermic regional perfusion (NRP) device (called donor assist). NRP is a technique used to supply organs with oxygenated blood when the circulatory system of the body has failed. It regionally applies perfusion (pumping of medium through an organ) to the desired organs thereby preserving the organ quality at the place of asystole (heart stop).

The donor assist is used immediately after asystole to regionally restore the abdominal circulation allowing continued supply of oxygen and removal of carbon dioxide in abdominal organs such as the kidneys. It aims at bridging the gap without oxygen provision between asystole and procurement of organs and thereby aiming to reduce organ damage or maybe even improve the organs during this intervention.

A project of such a magnitude has several parties working on different aspects. One of these parties is Leonie Venema who is a PhD candidate employed at the surgical research lab at the UMCG hospital.
Her aim within this project is the development and validation of a new organ perfusion solution able of supplying oxygen, recover organ damage and provide nutrients.
Her second aim is defining biochemical damage markers which can be measured during NRP and used to assess the condition of a kidney.

To aid to her research a custom made Isolated Perfused Kidney (IPK) control system was requested. IPK entails the machine perfusion of an isolated (porcine) kidney. Furthermore in order to drive down the cost of an IPK setup, the use of lower cost membrane oxygenators (a device that enriches

medium with oxygen through diffusion) over a commercially available oxygenator is investigated. In order to assess how well oxygenators perform, a model will be created on the oxygenator function with respects to parameters flow, temperature, gas: medium flow ratio and ventilation ratio.

1.1 Research question

In order to arrive at the desired results from the rationale the following central research question has been defined:

"How can a control system be created for an IPK setup and how can the functionality of an oxygenator be modelled?

With sub-questions:

- How can an IPK setup and control program be created?
- Which measureable parameters during IPK can be useful for assessing kidney condition?
- Which available oxygenator is most suitable to have its function modelled?

2 SITUATIONAL & THEORETICAL ANALYSIS

In this chapter the situational and theoretical analysis of this project is given. The current issues which have been briefly described in the rationale will be given more depth and explanation here.

2.1 Donor organs

This section describes how donor organs, kidneys in particular, are handled after the donor is deceased.

2.1.1 Retrieval of donor organs

When a donor deceases, a retrieval process is initiated to acquire the organs in an optimal way in order to preserve organ quality. There are several types of deaths which need to be considered when donors are concerned.

Two categories of organ donors are discriminated. One is Donation after Brain Death (or DBD). In this situation a patient is brain dead, meaning the patient will never be able to regain consciousness again. However the patient is kept 'alive' as in that heart beat and reanimation is kept on going artificially in order to preserve the condition of the donor organs. This is the more ideal situation where the condition of organs are guaranteed and there is no rush for medical personnel to retrieve the organs.

The second category is Donation after Circulatory Death (or DCD). In this situation the heart has stopped beating resulting in cessation of circulation in the body. This results in a period of warm ischemia (restriction of blood supply) where organs are no longer provided with oxygen, nutrients or removal of waste products. This results in damage to the organs as cells in the tissue will start to swell due to processes such as sodium-potassium pumps not being able to properly work anymore.

DCD is the most harmful category of donor passing and it is subdivided in four categories according to Maastricht classification: Brought in dead, unsuccessful resuscitation, awaiting cardiac arrest and cardiac arrest after brain-stem death [4].

2.1.2 Donor assist

In order to preserve the quality of DCD organs, the donor assist is being developed by organ assist. Organ assist is a company formed by the research of dr.ir. A. van der Plaats who created a hypothermic liver perfusion system for improved preservation in organ transplantation [5] in order to obtain his PhD degree at the UMCG in 2005.
Currently his company is developing the donor assist. The aim of the new donor assist is to be a more compact model able of restoring abdominal circulation in situ as soon as possible to prevent organ damage.
The donor assist is designed to be a light weight portable device which medical personnel can apply at the place of a donor's passing.

2.1.3 Description of the kidney

Kidneys are bean-shaped organs mostly known for their function in clearing waste in the blood in the form of urine. The kidney also maintain pH-levels, water levels and produce hormones used for

different functions in the body (i.e. erythropoietin which stimulates red blood cell production) [6]. The filtering process takes place in the nephrons, the most important unit in the kidney. There are millions of nephrons present in a kidney. A schematic of the kidney is displayed in figure 3.

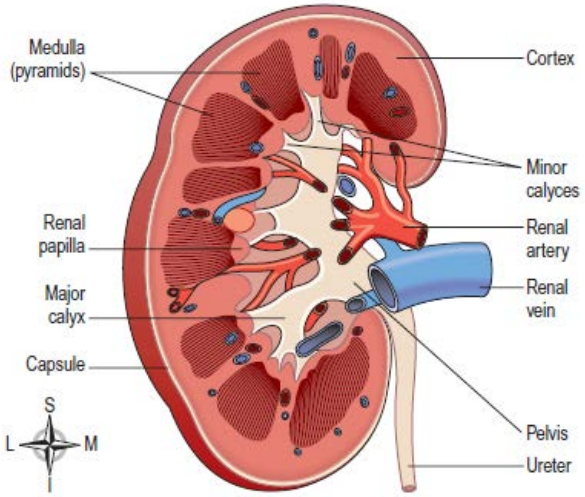

Figure 3: Schematic of the kidney [6]

The kidney filtering process has an active and passive function. The passive function takes place in the renal cortex. Blood enters the kidney and is sent through afferent arterioles. These blood vessels develop into a pattern of a knot known as the porous glomerulus, through which blood is pushed with high pressure. The knot functions to create a higher surface area where fluid containing small waste (in contrast to larger red blood cells and proteins) is squeezed outside of the blood. Roughly a fifth of the fluid going into the glomerulus is squeezed out into the so called Bowman's capsule and results in filtrate. The Bowman's capsule transports the filtrate into the proximal tubule where the active process will take place.

The active process begins in the proximal tubule where an ATP powered active reabsorption process (which needs to be ATP powered to pump against concentration gradient) reabsorbs electrolytes and glucose from the filtrate back into the bloodstream.
However, the active process takes place mostly in the loop of Henle which crosses from the renal cortex into the renal medulla (which is mostly salty to absorb more water).
The loop of Henle can be split in two segments. One half of the loop functions to also reabsorb salts (such as Na+, Cl-, K+) from the filtrate into the medulla. This half is impermeable to water. The other half is permeable to water. Water will be reabsorbed in the bloodstream based on osmosis due to concentration differences after the reabsorption of the salts.
The final part of the nephron is the distal tubule where more reabsorption of electrolytes happens. The filtrate from the distal tubule goes into collection ducts in the medulla where more water absorption can occur. The extent to which this occurs depends on hormones (such as antidiuretic hormone produced by the pituitary gland) influencing the permeability of the collection ducts. This control mechanism allows for reabsorbing more or less water.
Two kidneys produce roughly 180 liters of filtrate per day, of which so much is (actively) reabsorbed that only 1-2 liter of urine is produced. The urine is transported through the ureter to the bladder where it awaits relieve.

2.1.4 Kidney function after transplantation

During a period of warm ischemia, kidneys can become severely damaged depending on the length of the ischemia. The extent to which a kidney is damaged results in a heightened chance of primary non function and delayed graft function (kidney recovery time or delayed onset of kidney functioning after transplantation) [7]. Primary non function means that after the transplantation the kidney refuses to work and kidney functioning can no longer be recovered. Delayed graft function means that the transplanted kidney fails at first but will start to regain functioning as time progresses. According to a clinical study by Reznik et al [8], primary non function and delayed graft function were significantly reduced when using NRP compared to the more conservative method of hypothermic perfusion.

2.2 Normothermic machine perfusion of the kidney

In this section an overview is given on solutions and systems which are currently used in the surgical research lab for normothermic machine perfusion (NMP) of the kidney.

2.2.1 IPK setup

The IPK setup used to perfuse kidneys is displayed in figure 4.

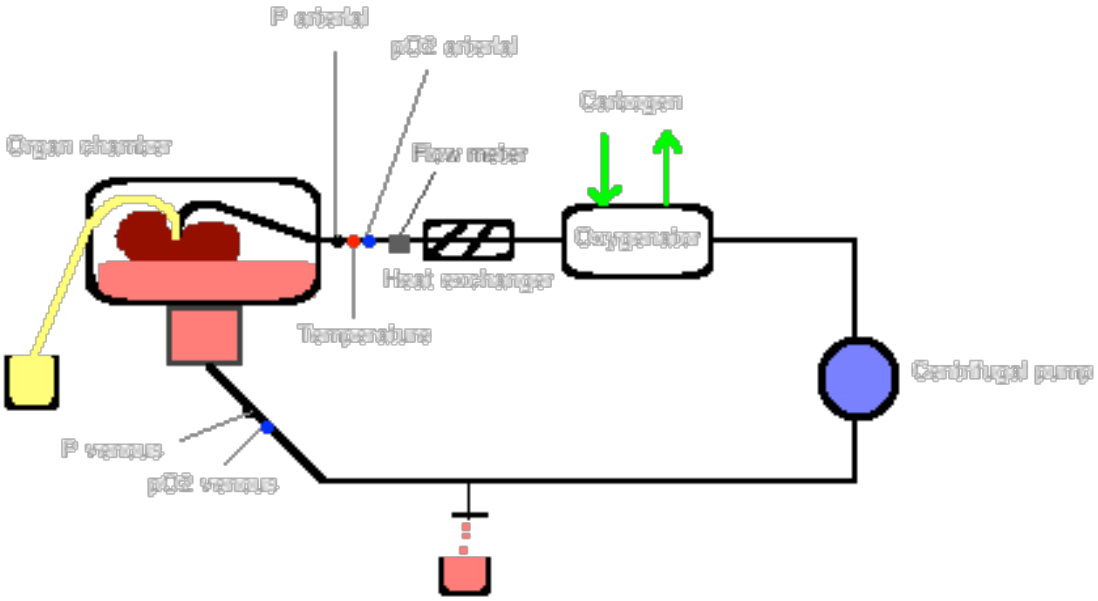

Figure 4: Porcine kidney perfusion setup [9]

The kidney is placed in the organ chamber. Blood is pumped through the system using a centrifugal pump. The blood is oxygenated by the oxygenator before the blood is pumped into the kidney. Various sensors are present to measure temperature, flow, oxygen levels before and after kidney perfusion (to determine oxygen uptake). A heat exchanger is present to warm up the medium to normothermic temperatures. Urine is also collected from the kidney.

2.2.2 IPK parameters of interest

All parameters which are going to be assessed during experiments are listed in appendix A [9]. A few IPK parameters of interest, which could be measured real-time, are shown in table 1.

Parameter	Interest
Hemodynamics	Includes the flow, pressure and resistance of the kidney. At IPK initiation the kidney first needs to open up. Flow gradually increases as the kidney opens up and resistance decreases.
Intra-renal resistance	The measure of resistance of a kidney can be an indicator on kidney quality. It is defined as the pressure over flow.
Renal oxygen extraction	Measuring medium oxygen levels before and after the kidney allows to determine the oxygen uptake of the kidney which can be used as an indicator of kidney quality.

Table 1 IPK parameters of interest

Furthermore the kidney has a pH regulating function which could hypothetically also be measured real-time. When an IPK experiment is started the pH level should remain roughly between 7.35 – 7.45 (physiological levels). If the pH drops below that level and does not recover it can be seen as an indication of decreased renal function.

The condition of the kidney is commonly assessed as the renal function using the parameters urine output (linked to the glomerular filtration rate) creatinine clearance (ability of kidney to clear the creatinine protein from the blood) and fractional excretion of sodium (expression of proximal and distal tubular and loop of Henle injury) [10]. Of these parameters creatinine clearance and fractional excretion of sodium require lab analysis to be determined where urine output could also be determined real-time and could hypothetically be added to the parameters to be determined during perfusion.

Hosgood et al [11] performed an experiment where the control group contained kidneys which had been stored on cold (4 °C) storage (CS) for 24 hours and another group with CS for 23 hours following by 1 hour of normothermic perfusion (NP) at 38 °C in order to test the underlying mechanisms of kidney condition improvement after NP.
They concluded that NP kidneys had significantly lower levels of intrarenal resistance, maintained their acid-base homeostasis, showed higher levels of oxygen consumption and significantly reduced amount of tubular injury (expressed in fractional excretion of sodium).

Harper et al [12] performed an experiment where four groups of kidneys (n=6) were subjected to 7, 15, 25 and 40 minutes of warm ischemic time (time the kidney receives no new blood with oxygen and nutrients at a temperature of 37°C with normal metabolic rate). They showed that creatinine

clearance, urine output, renal hemodynamics and oxygen consumption deteriorated proportionally to longer periods of warm ischemic time. Damage to the kidney and kidney condition was shown to be proportional to the time the kidney received no blood.

2.2.3 Kidney assist

Organ assist has already provided the research group with a device called kidney assist, created to drive an IPK setup. However, this device is battery powered and does not have enough power to increase pressure up to pressures of 120 mmHg. The drawback of this is that common porcine (or human) blood pressure conditions cannot be simulated but a greater drawback is that the kidneys may produce less (or even refuse producing) urine at simulated pressures between 60 and 90. A regular resting heart rate results in blood pressures between 70 and 120 mmHg [13].
An IPK pump drive is desired which can also reach pressures up to 120 mmHg in order to ensure that the kidney can have the systolic and diastolic pressures it is used to in order to properly produce urine.

The kidney assist is designed to provide a sinusoidal pressure alternating between 20% above and below a certain pressure. It was initially designed to provide at an average of 25 mmHg pressure. The kidney assist registers the flow, pressure and temperature and can record these values in a log, which can only be extracted afterwards.
One aim of the project is to create an IPK control system which records and displays data real-time and which is more configurable than a commercial and closed down product such as the Kidney assist.

2.2.4 Available IPK components

Several sensor systems and pump systems were already available and requested by project initiators to be used for this project.

A centrifugal pump alongside a driving circuit was already available. However it was only manually usable and would need to be controlled digitally before it could be used in Labview.
The pump type is Medos Deltastream pumpdrive DP2 which is being driven by a Maxon motor control DEC 50/5 Amplifier.

The flow sensor available is the Transonic Systems TS410, a flow sensor designed especially for isolated perfused organ studies. It has a flow output which can be connected to a data acquisition (DAQ) board.

The pressure sensor was already available from a previous project and works with a Labview program. However the program came in the form of an .exe program meaning that the code behind the program was not available. A challenge for this device is to recreate the code allowing a computer to interface with the sensor.
The hardware inside the box comprised a Labjack U12 DAQ and a Truwave pressure transducer created by Edwards, USA.

The oxygen sensor currently being used are optical oxygen meters called Fibox 4 created by the company Presens, Germany. By settings two of these units before and after the kidney or oxygenator can the kidney oxygen uptake or oxygenation capacity of the oxygenator be determined.

The desired interfacing software is Labview. Labview is a programming environment developed by National Instruments, USA. It is characterized by allowing easy creation of user interfaces. By using Labview an intuitive and easy to use GUI can be developed for medical personnel.
The Labview program will need to have control and measurement of all aspects of the IPK integrated into one program. It will also need to be able to record all sensor values into a file.

2.3 Oxygenation

This section presents a description of the process of oxygenation and the available oxygenators.

2.3.1 Description of an oxygenator

In order to provide the medium (blood or other fluids) with oxygen for the perfused kidney, membrane oxygenators are implemented in the IPK setup. An oxygenator is a device containing a great number of hollow tubes with membrane walls allowing exchange of gasses. Medium is pumped over the tubes and exchange of oxygen from the oxygen rich gas to the oxygen poor medium is done through diffusion. Modelling of this physical process is vital to gaining information on the gas exchange capacity of the used oxygenator [14]. Figure 5 shows the overview of an oxygenator.

Figure 5: Overview of an oxygenator (Medos [15])

2.3.2 Gas diffusion in membrane oxygenators

The fraction of inspired oxygen (FiO_2) ratio relates to the ratio between oxygen and other gasses such as nitrogen in the air blown over the oxygenator. The ventilation ratio is the relation between the amount of gas blown over the oxygenator and the amount of medium pumped over the hollow tubes.

A higher gradient of oxygen in the air mixture and a higher gas to medium ratio will result in a larger difference between oxygen concentration in the medium and the air resulting in higher rates of diffusion.

Fick's laws can be used to describe the process of gas exchange [16].
$V_{gas} = (A/T)\ (D)\ (P1\text{-}P2)$
Where A is the surface area, T = tissue thickness, D = diffusion constant of gas and (P1-P2) is the difference in partial pressure across the interface.

The oxygenation capacity of the oxygenator is determined using two PreSens Fibox 4 oxygen sensors at both the inlet and outlet of the oxygenator. The difference between measurements is the expression for how well the oxygenator is supplying the blood with oxygen. The Fibox 4 can express values for oxygen levels in %O2, hPa, Torr, ppb (µg/L), ppm (mg/L) or µmol/L. Of these parameters, hPa, or hectopascal, was requested to be used.

Temperature has been shown to have an inverse relation with the amount of oxygen which can be taken up by aqueous solutions. As temperature of the medium increases, the oxygen capacity of the medium decreases [17]

2.3.4 Available oxygenators

The oxygenators currently used are suitable for human cardiopulmonary bypass oxygenation. However, a single porcine kidney does not require as much oxygen as a human and because these oxygenators are expensive (roughly 300E for one time use), options need to be investigated for lower cost oxygenators with a lesser capacity for oxygenation [18].
Three different types of oxygenators are to be tested. The control oxygenator (currently being used in IPK) HILITE 1000, HPH Mini and a homemade oxygenator. Their characteristics are displayed in table 2. For one of these oxygenators a model will be created to assess the underlying dynamics of gas exchange in different situations.

	HILITE 1000	**HPH mini**	**Homemade 1 oxygator**
Priming volume	57 ml	14 ml	x
Gas exchange area	3900 cm^2	700 cm^2	1000 cm^2
Fiber material	Polypropylene	Polysulfone	Silicone
Price	€300,-	€145,-	x
Flow range	0 -1 L/min	x	x
Manufacturer	MEDOS, medizintechnik, Stolberg, Germany	Minntech, Minneapolis, USA	

Table 2: 3 oxygenators that are going to be tested. [18]

2.4 Stakeholders

In table 1, direct and indirect stakeholders to this project are displayed.

Level	Stakeholder	Interest
Primary	Student	The project is student's graduation project. In order to graduate, student will have to deliver appropriate results.
Primary	Hanze UAS	University of the student which has several interests in the project. The school has a supervising role for the student. The school's education 'Sensor System Engineering' is in its pilot year and the project will be a test case of how well a student of the program is able to perform in the field and how much of an added value he can deliver using the tools acquired in the program. Furthermore it is in the interest of the school to have a successful project together with the other stakeholders of the project in order to setup/improve a network which future students of the program might be able to use.
Primary	UMCG research group	Current IPK systems are third party product and as such can barely be changed as opposed to open systems. The research group wants to have a custom made control system which can be altered to their liking and which will improve current weaknesses with existing systems (for instance, if the desired pressure in the setup is too high, the current system will no longer create a sinusoid alternating between systolic and diastolic pressure).
Secondary	Patients	There is a waiting list for people who need new kidneys. This project aims to add to research for a new donor assist device, allowing improved handling of donor kidneys after donor passing. This will need to increase the number of kidneys in transplantable condition.
Secondary	Medical staff	Medical staff of the UMCG may have little technical knowledge and as such an intuitive and easy to use system needs to be provided for IPK.
Secondary	Healthcare insurances	Healthcare insurances have to cover their clients' medical bill. Medical devices lowering costs for diagnostic and therapeutic treatments will lower the costs for treatment of clients.

Table 3: Stakeholders to the project

4 CONCEPTUAL MODEL

This section contains the conceptual models for the IPK control system and oxygenator model based on gathered information.

4.1 Concept overview IPK control system

Figure 6 shows the general concept overview for the IPK control system to be created based on wishes of the research group. Medical personnel will be able to use this concept to control how a kidney is pumped in the IPK setup.

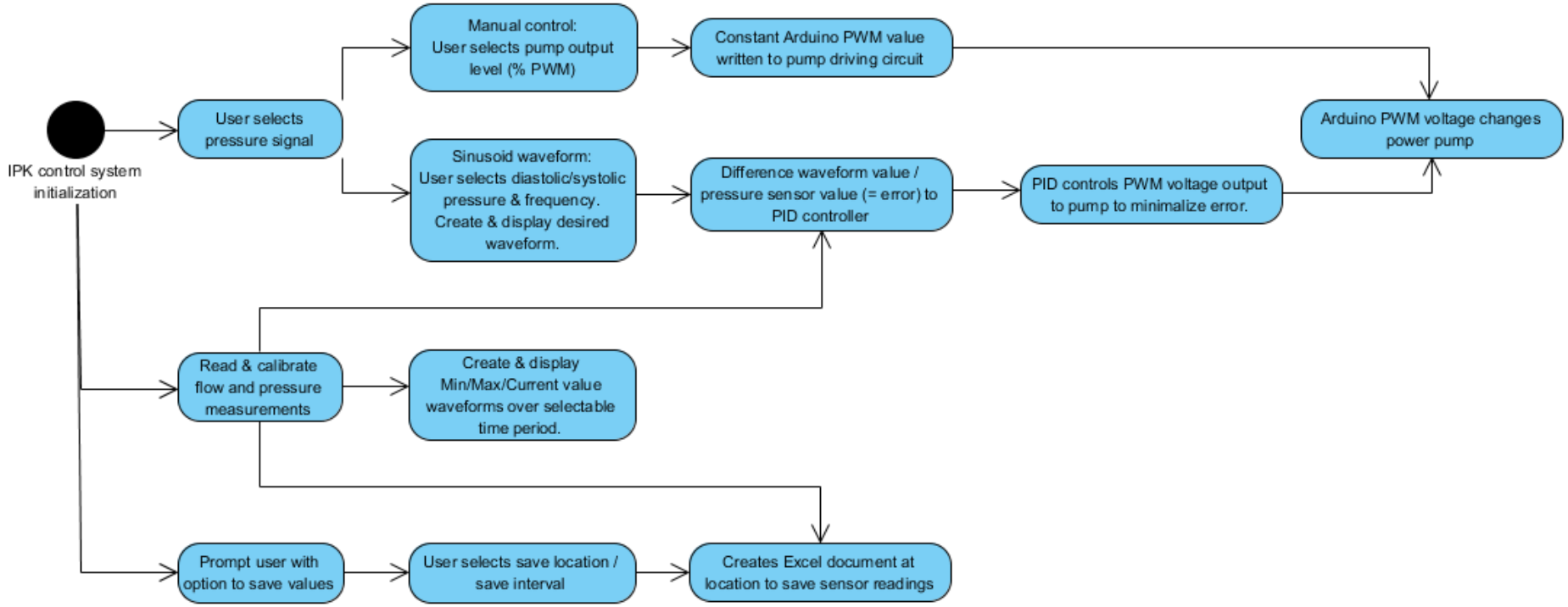

Figure 6: flow chart IPK control system

4.2 Concept overview oxygenator model

In order to describe the underlying dynamics of the process of oxygenation of a medium, one approach is to create a mathematical model based on known literature and relationships between parameters. However, it suited the scope of this project better to take a top down approach instead to create an empirical model covering the gathered data points.
This meant gathering data on the relationship between the oxygenation capacity of an oxygenator and several parameters. A model describing this relationship could then be created using a technique called multivariate polynomial regression resulting in a curve fit of the data.
Initial data showed relation between parameters to be best described with maximum power of n=2. Higher powers result in an s shaped regression curve which did not fit the data.

Initial parameters were chosen to be flow, temperature, concentration of inspired oxygen and ventilation ratio as these are parameters of interest in the process of oxygenation and the research group requested these parameters to be included in the measurements.
Depending on the kidney, different flow patterns can be seen and so the oxygenator needs to be tested for a wide range of flows. Temperature has been shown to have a relation with the uptake of oxygen by a medium (see section 2.3.2). Information on how well the process of oxygenation functions at different temperatures is desired, as machine perfusion is performed at different temperatures.
The concentration of inspired oxygen parameter was included as there is a relation between inspired oxygen concentration and oxygen transfer rate within oxygenators. Furthermore, accessibility between medium and inspired gas is of importance and can be influenced with the ventilation ratio [19].

A general model template describing a relationship between these parameters based on regression is:

$$Y = a_0 \pm a_1 x_1{}^n \pm a_2 x_2{}^n \pm a_3 x_3{}^n \pm a_4 x_4{}^n \pm a_5 x_1 x_4{}^n \pm a_6 x_2 x_4{}^n \pm a_7 x_3 x_4{}^n \pm a_8 x_1 x_3{}^n \pm a_9 x_1 x_2{}^n \pm a_{10} x_2 x_3{}^n + \epsilon$$

Where:
Y = Oxygenation capacity (hPa)
a_x = weight for respective parameter
X_1 = Medium flow (mL/min)
X_2 = Temperature (°C)
X_3 = Concentration of inspired oxygen (% FiO_2)
X_4 = Ventilation ration (gas: medium ratio)
ϵ = error

5 RESEARCH DESIGN

The research design section describes the steps taken to create and test a prototype IPK control system and the experimental setup and protocol used to gather data for the model.
The control system was also used to drive the experimental setup where data was collected on the underlying dynamics describing the oxygenation capacity of an oxygenator.

5.1 IPK Control system

In order to create an IPK control system, several steps were undertaken. The first steps focused on integrating available hardware to have sensors values be read into a laptop running Labview software and have the pump run according to a desired pressure pattern.
The software entailed a Labview program controlling the Labjack U12 and Arduino UNO and providing all functionality as displayed in section 4.1.

5.1.1 Control system hardware

A control system was created to drive the IPK setup at a pressure alternating between a, to be chosen, systolic and diastolic pressure. The system would take sensory input on pressure and flow parameters.

The pump available was a Medos Deltastream pumpdrive DP2 which is driven by a Maxon motor control DEC 50/5 Amplifier. In order to drive the pump using Labview, an Arduino Uno microcontroller was implemented. Using Labview Interface for Arduino toolkit, the Arduino could be controlled in Labview.
The Maxon amplifier speed of the pump setting is controlled using a Pulse-width Modulated (PWM) signal ranging between 0 – 5V coming from the Arduino. The Arduino microcontroller has an 8 bit DAC converter which allows for a resolution of 256 steps which was found to be sufficient.

A Truwave pressure transducer (Edwards, USA) is used to measure the pressure. A Labjack U12 DAQ device was used for sensor data acquisition. A library used to interface the DAQ board with Labview was made readily available by the manufacturer Labjack.

The flow is measured using a Transonic Systems TS410 Flow sensor. The analogue output of this device was captured using the Labjack U12 and could so be read into Labview.

The flow and pressure signals were calibrated in Labview. The flow sensor readout where read at different PWM outputs (resulting in higher flows) with step size 32 up to the maximum of 256. The pressure sensor readout was calibrated by using a clamp to increase pressure within a test setup with incremental steps up to 140 mmHg.

The flow was calibrated versus the TS410 output value where the Truwave pressure sensor was calibrated versus another Truwave sensor/Labview/LabJack implementation which was already calibrated.
The calibration graphs and equations can be seen in figure 7 and 8.

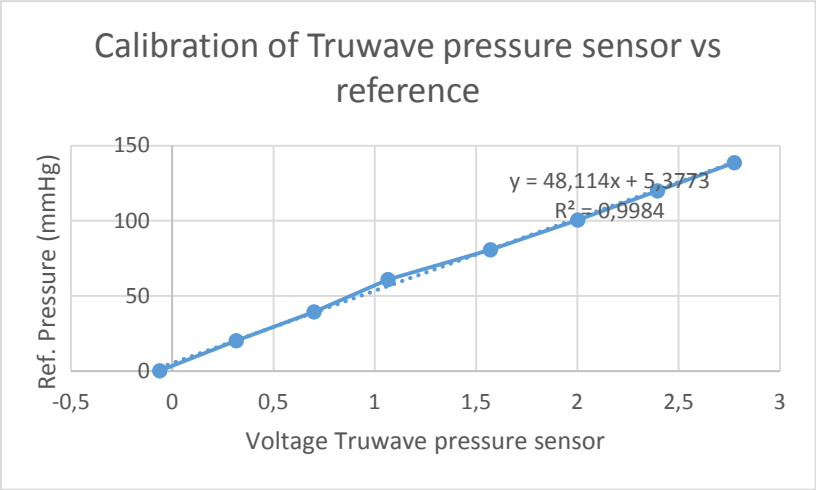

Figure 7: Calibration of Truwave pressure sensor versus reference

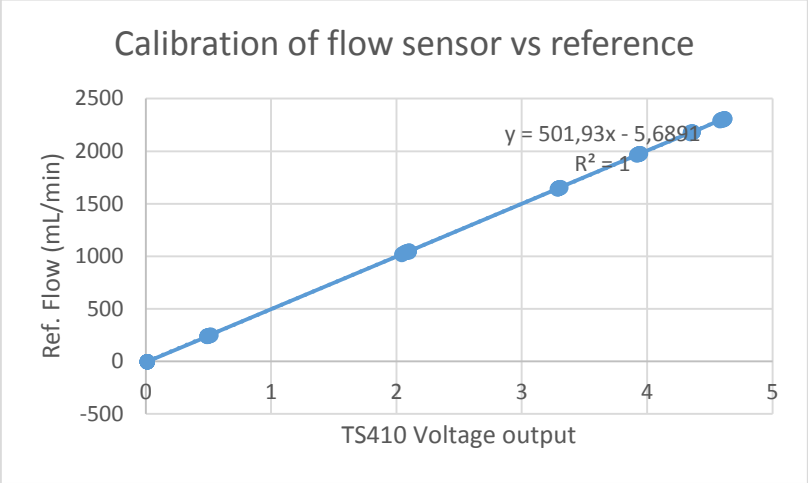

Figure 8: Calibration of flow sensor versus reference

5.1.2 Control system software

The created Labview program displayed the sensor values both real-time and displayed the values over time in a graph in order to show how the kidney behaved over time.
The readings could also be saved to a file where the user could select the time interval between saves.

The user has two options for driving the pump. The first is manual, setting a fixed PWM output. This setting could be used to get the IPK setup ready. The second option is a sinusoid alternating between a selectable systolic and diastolic pressure with a selectable frequency.

The sinusoid was recreated in the IPK system by having the difference between pressure sensor readings and the desired sinusoid be input as the error to a proportional – integral – derivative (PID) controller which would adjust PWM output in order to get the pressure sensor readings to match the desired sinusoid.

5.2 Model

Using the IPK control system and the equipment provided by the UMCG was data gathered on the process of oxygenation. For each parameter was investigated whether its relation to the oxygenation capacity is linear or nonlinear. Using Minitab software was the data analyzed and a curve fitting model created based on multivariate polynomial regression.

5.2.1 Experimental setup

Using the IPK control system and equipment available in the surgical research laboratory an experimental setup could be created for performing measurements on the oxygenators.

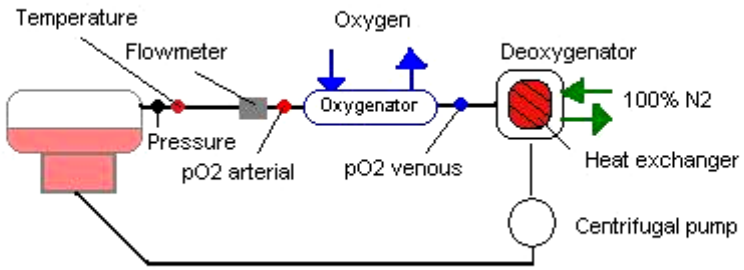

Figure 9: Experimental setup oxygenator tests [18]

Figure 9 shows the experimental setup used for testing the oxygenators. A centrifugal pump, controlled by the IPK control system, is used to pump the medium (1 liter fresh water) through the setup at a certain flow. The water is first pumped through a de-oxygenator. Nitrogen gas is blown over the de-oxygenator resulting in removal of oxygen in the water through diffusion. The de-oxygenator has an integrated heat exchanger. A heated water bath is set at the desired temperature and connected to the de-oxygenator. The water bath then pumps water with a certain temperature through the de-oxygenator, heating up the medium to the desired temperature.
The heated, deoxygenated medium is then pumped towards the oxygenator. Two oxygen sensors (PreSens Fibox 4) are connected, each at one side of the oxygenator. The difference between the two sensors determines the oxygenation capacity of the oxygenator.
2 liter gas tanks containing air and pure oxygen were supplied by the UMCG. Using a respiratory gas blender could a certain mixture/concentration of inspired oxygen be achieved. The gas blender could also be used to set the flow of gas to the oxygenator.
The outlet of the gas blender was connected to the gas inlet of the oxygenator where oxygen diffuses from the oxygen rich gas to the oxygen depleted medium.
Temperature and flow sensors are connected to take measurements. The medium eventually returns to the medium reservoir.

5.2.2 Oxygenator comparison

Initial testing of the HILITE 1000, HPH Mini and homemade oxygenator showed HILITE to have most potential to have its functionality modeled. The HPH mini would leak medium out the gas exhaust

when flows exceeded 200 mL/min. The homemade oxygenator could not handle flows above 300 mL/min. The pressure at the inlet would start increasing up to 300 mmHg when the pump was set higher. The higher resistance resulted in flow actually decreasing.
The HILITE 1000 would perform as expected under all conditions and as such was chosen for data gathering.

5.2.3 Parameters and variations

At least four variations were chosen per parameter in order to be able to determine whether its relation to the oxygenation capacity was linear or quadratic. Variations were kept at a minimum as every increase meant a substantial increase in measurements to be taken which did not fit within the available project timeline.

FiO_2 ratio (in % concentration of oxygen in the air)

- 21
- 50
- 75
- 100

21 % was the minimal setting for FiO_2 ratio and is equal to oxygen concentration in atmospheric air. Other variations were chosen to keep an even spread.

Ventilation ratios (gas flow: liquid flow)

- 0.5 (:1)
- 1 (:1)
- 2 (:1)
- 3 (:1)

Flows (in mL/min):

- 50
- 100
- 200
- 400
- 600

This flow range was chosen based on covering a large spectrum in which a kidney can be pumped during an IPK spectrum.
The variations were chosen to cover a large spectrum of the flow profile of IPK experiment in general which ranges between 50 and 600+ mL/min.

Temperatures (in °C):

- 4
- 15
- 25
- 38

Hypothermic (4 °C) and normothermic (38°C) temperatures are common temperatures at which organs are pumped during machine perfusion experiments. 15 and 25 were chosen as variations in between for a more even spread.

Data collection was done following the protocol described at appendix B.

6 RESULTS

The section will present the results as delivered during the graduation phase.

6.1 IPK Control system

The hardware of the IPK control system was assembled as displayed in figure 10. The final version of the Labview control program during use in an IPK experiment is displayed in figure 11. One can see the distinct flow pattern of a kidney initially opening up to the flow and closing down a bit as time progresses.
So far the IPK control system was used in roughly 15 IPK experiments, each taking 4-6 hours to complete. The system has malfunctioned 2-3 times due to development inefficiencies (such as the operating system restarting for updates during an experiment, aborting the Labview program).
The software program can be found in appendix D.

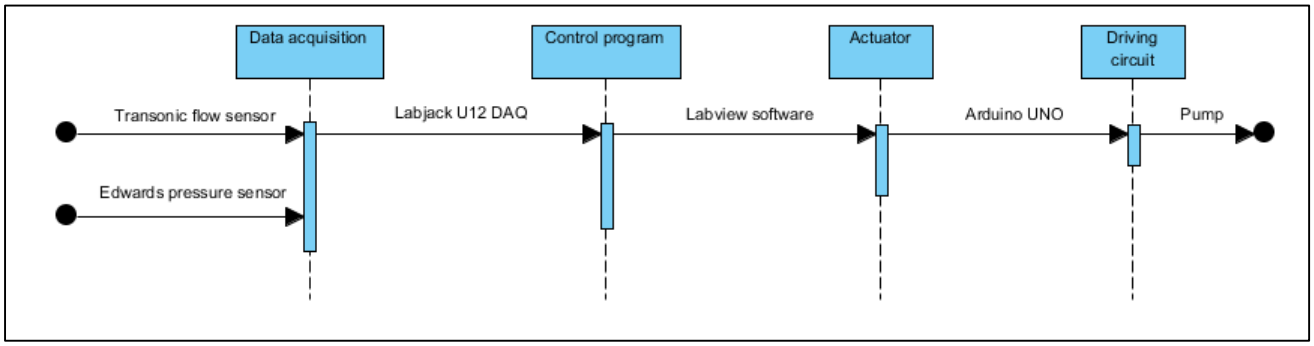

Figure 10: Hardware overview of the IPK control system.

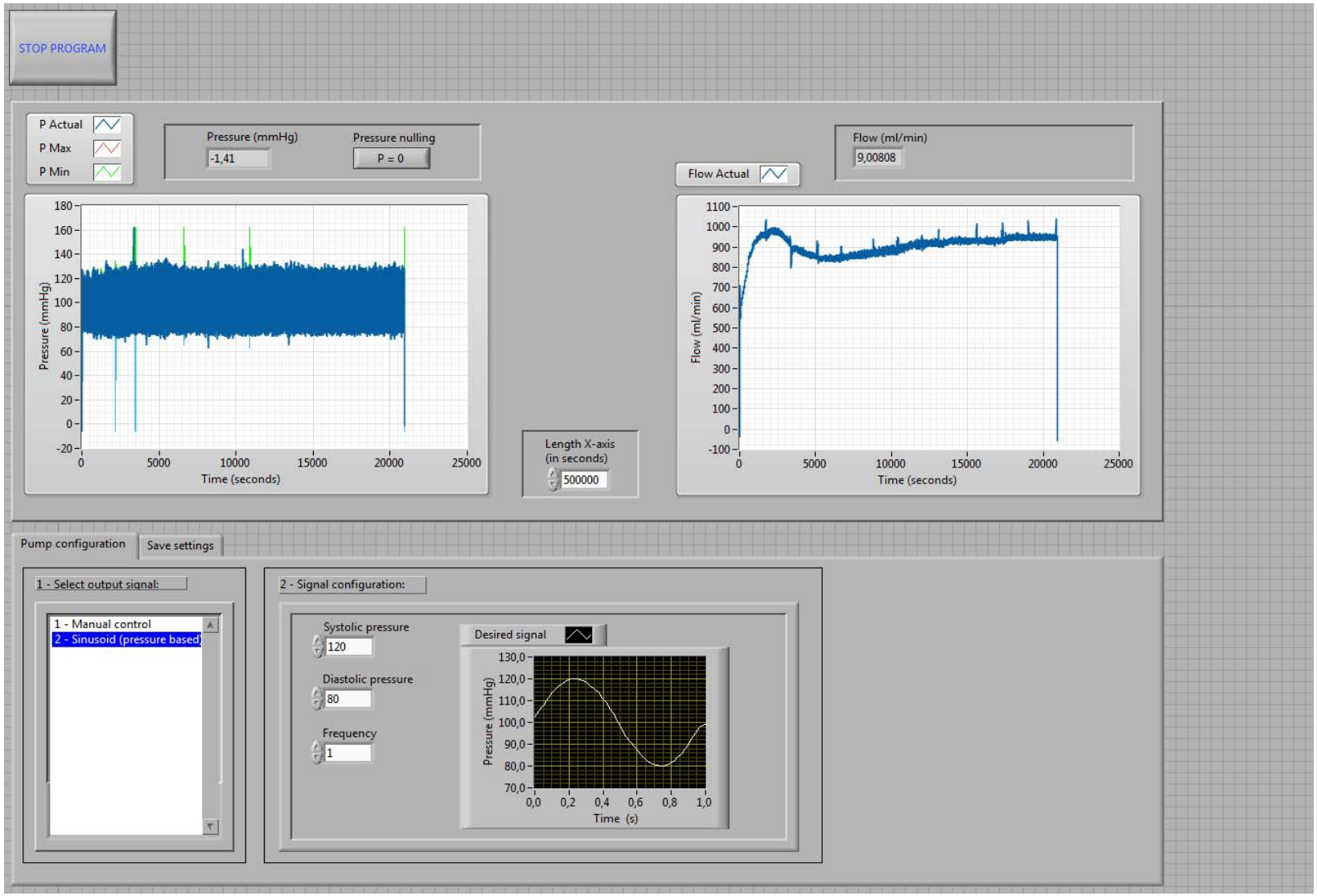

Figure 11: User interface IPK control system during experiment. The user can select a systolic and diastolic pressure and frequency (with corresponding signal displayed at 'desired signal' graph) at which a kidney can be pumped.

6.2 Initial NMP results

This section will present initial results of the kidneys which have been pumped in the IPK setup. The information has been provided by Leonie Venema.

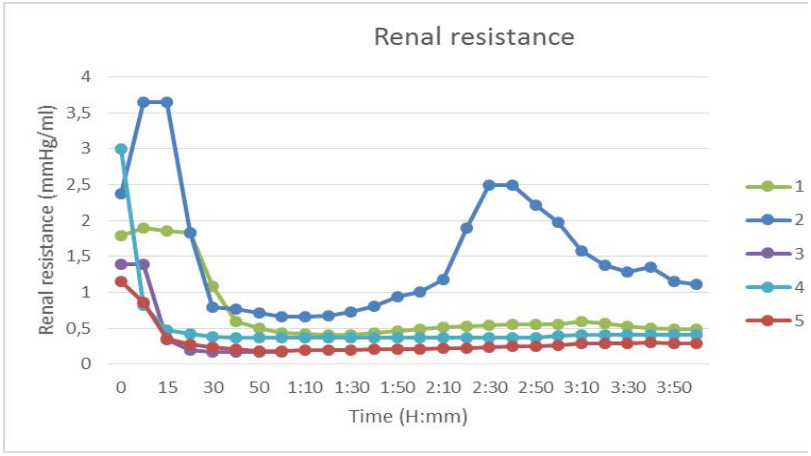

Figure 12: Renal resistance over time.

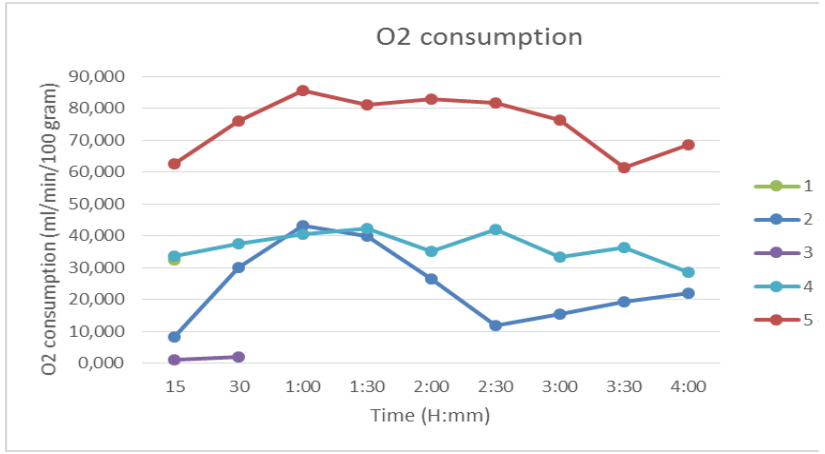

Figure 13: O2 consumption over time.

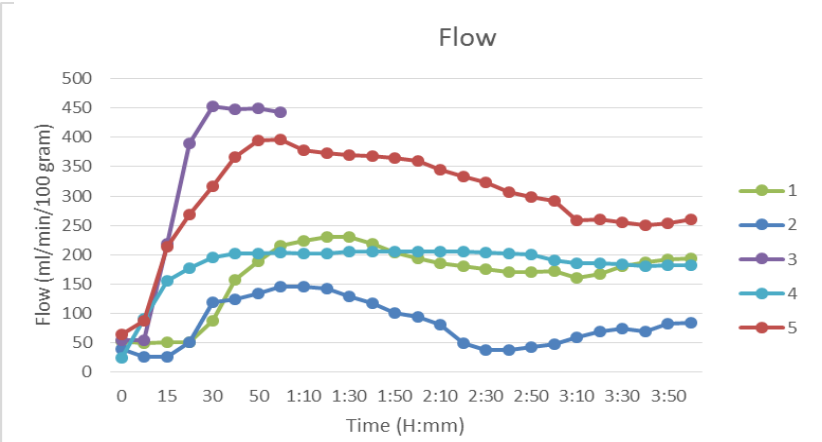

Figure 14: Flow over time.

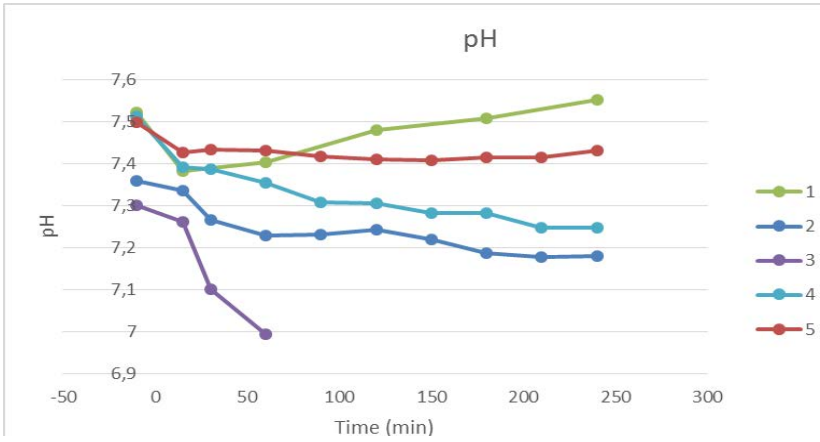

Figure 15: pH over time.

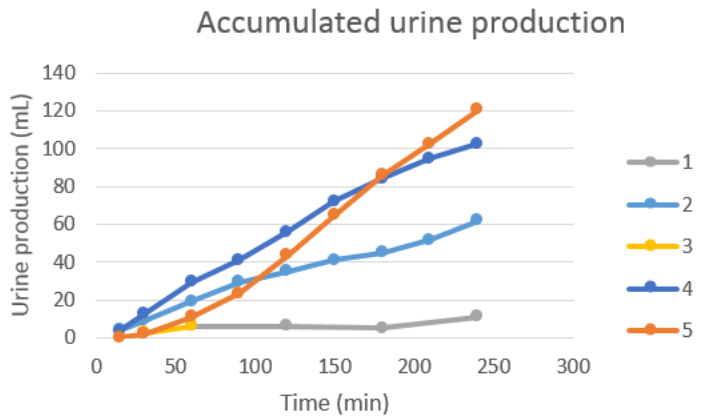

Figure 16: Accumulated urine production over time.

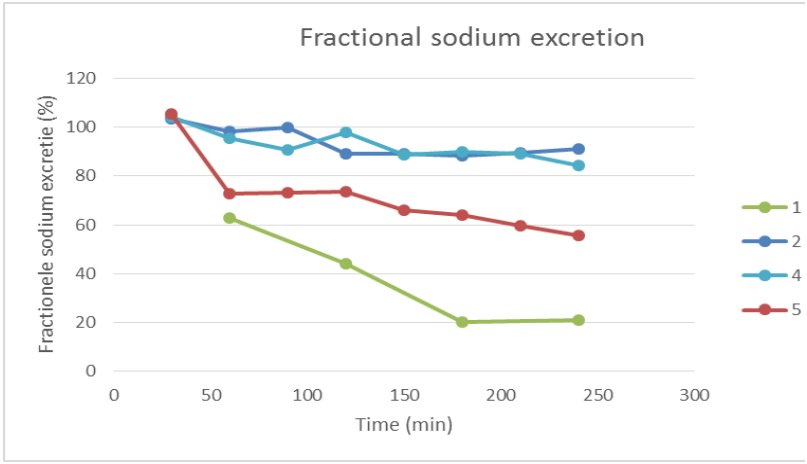

Figure 17: Fractional sodium excretion over time.

Figure 18: Creatinine clearance over time.

Figures 12 through 16 display measurable parameters during IPK whilst figures 17 and 18 show parameters linked to renal function which are determined after the experiment.

Research on the performance of kidneys is still ongoing and conclusions should not be made for as long as machine perfusion of kidney experiments are still being run and results are being processed. So far, newly gained insights have required continued conclusion adjustment.

However, the research so far does seem to agree with literature as kidneys showing higher flow levels, lower renal resistance, a maintained pH level and a higher oxygen consumption also seems to show better creatinine clearance and a better fractional sodium excretion thereby showing promise in being able to determine quality of the kidney based on real-time measurable parameters.

6.3 Model

Alongside development of an IPK control system and initial analysis of NMP results, the measurements for the model were acquired. Roughly 5000 measurements were acquired for the model.
In order to create a model based on multivariate polynomial regression, it first needed to be determined whether each parameter has a linear or quadratic/polynomial relation with the oxygenation capacity. This was done by analysis and comparison of the least mean squared error of the trend lines for a linear and polynomial fit for each parameter.
The trend lines with the best fit are shown in figure 19 through 22.

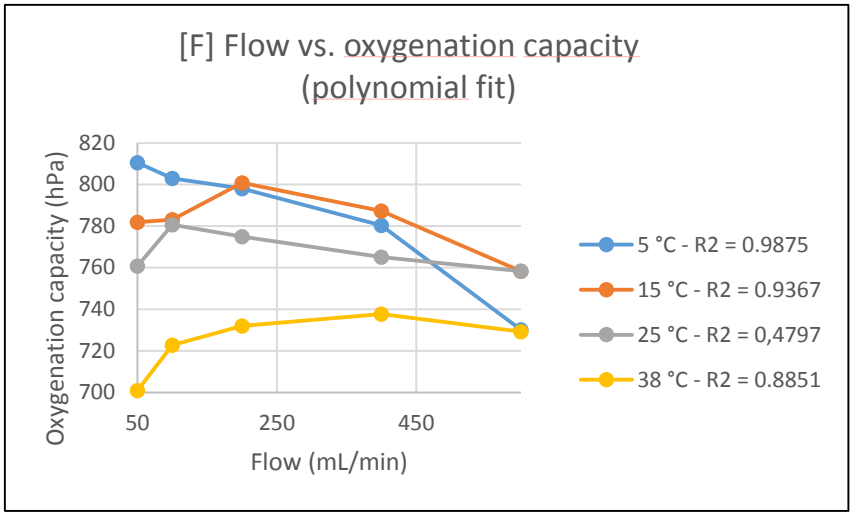

Figure 19 Flow versus the oxygenation capacity for different temperatures with the respective R2 values of the polynomial trend lines.

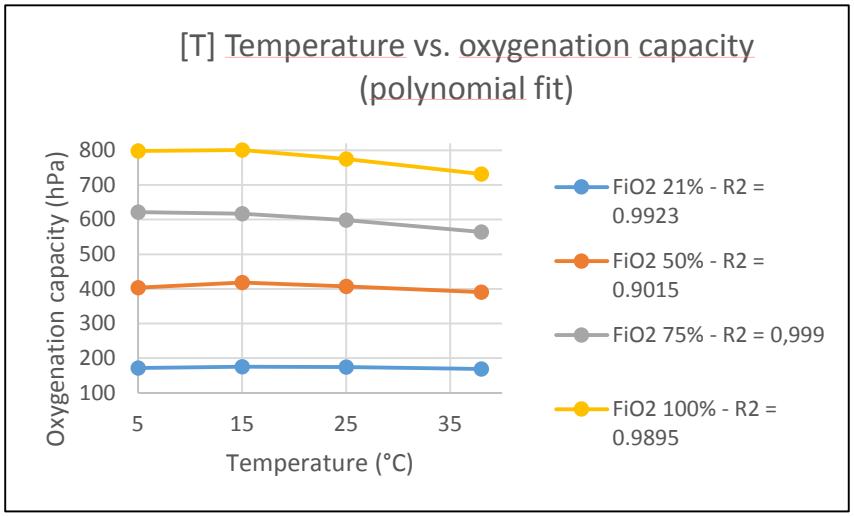

Figure 20: Temperature versus the oxygenation capacity for different FiO2 concentrations with the respective R2 values of the polynomial trend lines.

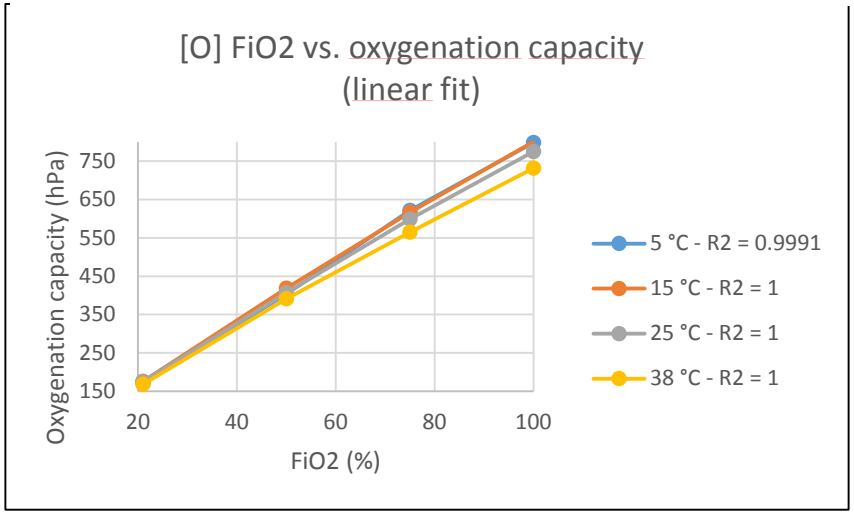

Figure 21: FiO2 versus the oxygenation capacity for different temperatures with the respective R2 values of the linear trend lines.

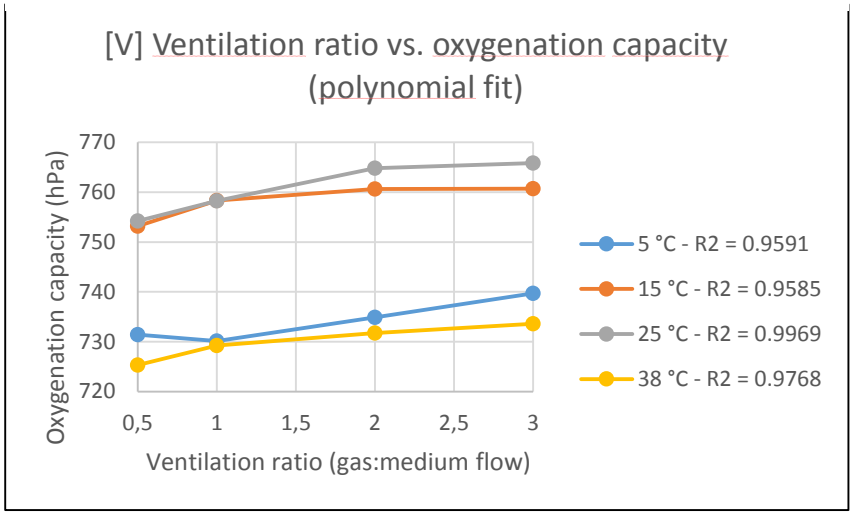

Figure 22: Ventilation ratio versus the oxygenation capacity for different temperatures with the respective R2 values of the polynomial trend lines.

A Pearson correlation test was used the relationship between parameters and the oxygenation capacity.

Correlation: F; T; O; V; OC

	F	T	O	V
T	0,004			
	0,895			
O	−0,001	0,000		
	0,983	0,999		
V	−0,072	0,001	−0,000	
	0,030	0,984	1,000	
OC	−0,033	−0,045	0,989	−0,002
	0,318	0,171	0,000	0,954

Cell Contents: Pearson correlation
P-Value

Figure 23: Correlation between parameters and the oxygenation capacity.

In figure 23, the upper number, ranging between -1 and 1 shows the strength and direction of the relationship between two parameters. Zero means no correlation. The lower number is the p-value indication how significant the relation is (where p-value < 0.05 is significant).
One can see that the parameters have little to no correlation with each other (independent). Furthermore, it can be seen that there is a strong positive correlation between concentration of inspired oxygen and oxygenation capacity.

Initial results further showed two outliers in the data, determined to be due to clerical errors. These were removed before assessing the residual plots. An initial standardized residual versus fits plot showed that there was a missing exponential to explain the curvature which can be seen in figure 24.

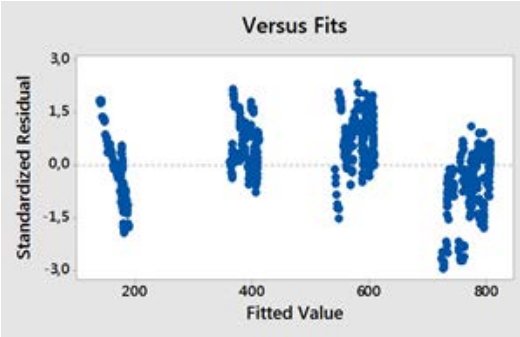

Figure 24: Standardized residual versus fitted value.

Based on figure 24, it was decided to also let the parameter concentration of inspired oxygen (O) be polynomial. This removed the curvature from the plot and also improved the R^2 value of the model.

Using a regression model function for multivariate polynomial regression in Minitab [20] was an initial model determined to be:

$$\begin{aligned} OC = {} & -23.33 + 0.03062 * F + 1.891 * T + 9.0843 * O + 0.35 * V + 0.003147 * F * T - 0.000367 * F \\ & * O + 0.00590 * F * V - 0.021671 * T * O - 0.0528 * T * V - 0.00619 * O * V \\ & - 0.000165 * F^2 - 0.05127 * T^2 - 0.008281 * O^2 - 0.184 * V^2 \end{aligned}$$

Where:

OC = oxygenation capacity (in hPa)
F = flow (in mL/min)
T = temperature (in degrees Celsius)
O = concentration of inspired oxygen (in %)
V = ventilation ratio (gas: medium flow in mL/min)

Residual plots were used to further analyze the model. The normal probability plot should roughly follow a straight line to indicate normal distribution.
For a residual plot versus fits the points should fit randomly on both sides of zero with no distinguishable pattern to indicate residuals randomly distributed and having constant variance.
The histogram can be used to check for a long tail in one direction (indicating skewness) or a bar that is far away from the other bars (indicating outliers).
The standardized residual versus order plot can be used to determine whether the residuals are independent of each other. Preferably, points fall on either side of zero with no distinguishable pattern. Distinguishable patterns can indicate that residuals are correlated [21].

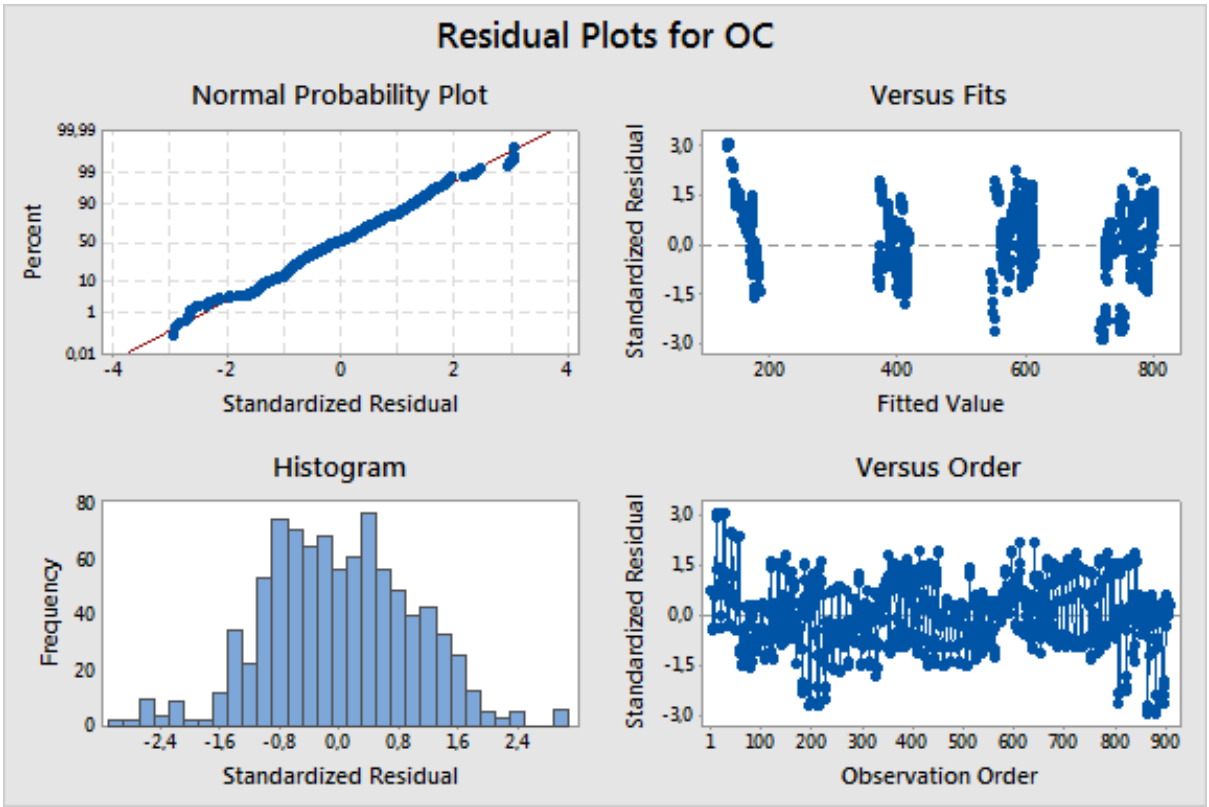

Figure 25: Residual plots for oxygenation capacity

In figure 25 can be seen that the residual plots fit these conditions thereby confirming that least squares assumptions [22] for ensuring most precise estimates, are being met:

- Residuals have a mean of zero.
- All predictors are uncorrelated with the residuals.
- Residuals are not correlated with each other.
- Residuals have a constant variance.
- No predictor variable is perfectly correlated (r=1) with a different predictor variable.
- Residuals are normally distributed.

In order to assess the relative contributions of the different terms to the model, the p-values for the constants are considered. Coefficients with a P-value lower than 0.05 are considered a significant addition to the model.

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-23,33	2,74	-8,52	0,000	
F	0,03062	0,00723	4,24	0,000	33,95
T	1,891	0,113	16,75	0,000	30,02
O	9,0843	0,0506	179,66	0,000	34,81
V	0,35	1,67	0,21	0,832	39,64
F*T	0,003147	0,000101	31,08	0,000	5,88
F*O	-0,000367	0,000042	-8,67	0,000	7,33
F*V	0,00590	0,00133	4,44	0,000	6,03
T*O	-0,021671	0,000701	-30,92	0,000	8,25
T*V	-0,0528	0,0218	-2,43	0,015	7,09
O*V	0,00619	0,00906	0,68	0,495	8,58
F*F	-0,000165	0,000009	-19,22	0,000	21,81
T*T	-0,05127	0,00206	-24,91	0,000	20,08
V*V	-0,184	0,392	-0,47	0,638	28,75
O*O	-0,008281	0,000359	-23,08	0,000	26,47

Figure 26: Characteristics of all coefficients.

From figure 26 can be seen that every coefficient except V, O * V and V^2 are significant additions to the model. It would seem that the ventilation ratio has relatively little impact on the oxygenation capacity of an oxygenator and as such one could decide to leave out the parameter ventilation ratio when controlling the process of oxygenation. These coefficients could be removed (without significant changes in residual plots or R^2 values) as they are not significant contributions, resulting in the following final model equation:

$$\begin{aligned} OC = {} & -23{,}41 + 0{,}03024 * F + 1{,}888 * T + 9{,}0953 * O + 0{,}003147 * F * T - 0{,}000369 * F * O + 0{,}00603 \\ & * F * V - 0{,}021671 * T * O - 0{,}0513 * T * V - 0{,}000165 * F^2 - 0{,}05127 * T^2 \\ & - 0{,}008281 * O^2 \end{aligned}$$

Where:

OC = oxygenation capacity (in hPa)
F = flow (in mL/min)
T = temperature (in degrees Celsius)
O = concentration of inspired oxygen (in %)
V = ventilation ratio (gas: medium flow in mL/min)

As can be seen in figure 27 all parameters in this model now have a significant contribution. These are the parameters which are most important in order to control oxygenation.

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-23,41	2,01	-11,63	0,000	
F	0,03024	0,00692	4,37	0,000	31,17
T	1,888	0,110	17,24	0,000	28,35
O	9,0953	0,0479	189,94	0,000	31,30
F*T	0,003147	0,000101	31,16	0,000	5,86
F*O	-0,000369	0,000042	-8,76	0,000	7,29
F*V	0,00603	0,00107	5,64	0,000	3,92
T*O	-0,021671	0,000700	-30,96	0,000	8,25
T*V	-0,0513	0,0156	-3,28	0,001	3,67
F*F	-0,000165	0,000009	-19,32	0,000	21,54
T*T	-0,05127	0,00206	-24,94	0,000	20,08
O*O	-0,008281	0,000358	-23,11	0,000	26,47

Figure 27: Characteristics of all significant coefficients.

The final model showed an R^2 value of 99.89% with p-values for each parameter lower than or equal to 0.001 meaning every parameter had a significant contribution. Model curve passing through the origin could be desired for a model describing a physical phenomenon, however this is not the case due to the offset constant.

One danger of modelling is over fitting. This generally happens when one attempts to estimate too many parameters from a too small sample size. In order to create a model one needs a minimum of 10-20 observations per parameters [23]. There are 14 parameters (including interaction effects and squared terms), requiring a rough minimum of 140-280 data samples. This model encompassed a total of 5000 values.
In order to detect overfit models cross-validation is used to see how well the model predicts other data points. Minitab includes a cross-validation tool with the predicted R^2 value. The predicted R^2 value is determined by removing each observation from the data set and then determining how well the model estimates the missing observation. The predicted R^2 value was 99.88% meaning that there is no indication of overfit.

In addition, a random subset of the data was selected to see how the model would predict the values, as can be seen in table 4.

#	Flow (mL/min)	Temperature (° Celsius)	Concentration of inspired oxygen (%)	Ventilation ratio (gas: medium)	Measured Oxygenation Capacity (hPa)	Predicted Oxygenation Capacity (hPa)	Error (in hPa)	Error (in %)
1	202	5,1	21	0,5	168,73	171,44	2,71	1,58
2	401	15,3	21	3	176,14	179,39	4,41	2,44
3	50	14,7	75	2	599,23	604,36	6,83	1,12
4	54	38,7	21	1	157,22	148,21	-8,96	-6,04
5	607	38,2	21	2	167,51	172,74	5,58	3,22
6	202	24,8	75	3	602,11	593,77	-5,58	-0,93
7	201	24,6	50	3	407,33	408,50	3,19	0,77
8	399	25,1	100	0,5	763,06	766,21	4,09	0,53
9	399	5,5	21	3	167,91	165,11	-1,65	-0,99
10	103	14,66	100	1	785,33	788,97	5,05	0,63

Table 4: Random subset of data was selected to further test the model.

The overall error expressed as a percentage between the measured and predicted oxygenation capacity was determined 99.60 %.

Using this model can be determined that the concentration of inspired oxygen is the biggest influence on the process of oxygenation. Ventilation ratio was the factor least contributive to oxygenation.
Using this model one can determine how well the HILITE 1000 oxygenator can perform under different conditions.

7 CONCLUSION

An IPK Control system could be created using available hardware components, an Arduino UNO microcontroller and a laptop running Labview software. The system could drive an IPK setup with a sinusoidal pressure pattern alternating between a selectable systolic pressure, diastolic pressure and frequency. It collects sensor data from multiple sensors which it stores to an excel file. Some bugs need to be resolved and finishing touches need to be made before the medical personnel can use it fully.

Current machine perfusion experiment results suggest that kidneys with higher flow levels, lower renal resistance, a maintained pH level and a higher oxygen consumption can be linked to better creatinine clearance and a better fractional sodium excretion. These parameters show promise to be used as measurable parameters for assessing kidney condition.
However, more research will need to be performed before definitive conclusions can be drawn on the usability of these parameters for assessing kidney condition.

An empirical model, using multivariate polynomial regression was created for the HILITE 1000 oxygenator which showed most suitable to have its functionality modelled. Parameters with a non-significant contribution were mostly related to the ventilation ratio and removed from the model. The final model showed an R^2 value of 99.89% with no indication of overfit. Each parameter had a significant contribution (p-values =< 0.001).
Using this model could be determined how well the HILITE 1000 performs under different conditions and which parameters contribute most to the process of oxygenation.

7.1 Discussion

The current version of the IPK control system still has a few bugs which need to be addressed. The biggest ones are the program not properly initiating the Labjack U12 causing no measurements for pressure to be taken initially. Furthermore the program tends to run increasingly slower as program runs for hours resulting in the desired frequency not being met.

Several aspects of the model data gathering are up for discussion. Foremost, a process in which 5000 measurements are taken manually is bound to have some minor influence by sheer human error as it is a tedious and repetitive process. Efforts were made to keep these influences at a minimum (such as by partially repeating steps which were performed erroneously).
The gas blender settings for FiO_2 could be set rather precisely however its control mechanism for gas flow was tedious to get right due to its imprecise method of control. Furthermore it had shown to occasionally (minor) drift resulting in the gas flow to possibly deviate from the initial setting by a small margin over time. It was aimed to keep these deviations from occurring but there is a chance irregularities have not been excluded for the whole duration of data gathering.
The control mechanism also had no setting for 50 mL/min as the lowest indicated setting was 100 mL/min. In order to still reach 50 mL/min (which could not be excluded as it is an interesting variation for IPK), the gas flow was opened with the smallest difference.
Furthermore it was difficult to keep lower medium temperatures such as 5 °C stable, especially at low medium flow rates (resulting in a less effective heat exchange between water bath and medium in the de-oxygenator), as the environment temperature was 21 °C. In order to keep the water bath at 5 °C, ice needed to be added continuously for the duration of data gathering. As a result the oxygen measurements are also not kept stable as the measurements are influenced by the medium temperature. The fluctuations in these measurements are most present at lower temperatures where temperature swings were most frequent. For the model, these fluctuations are of lesser influence as the oxygen measurements were correlated with corresponding medium temperature at the respective times of measurement.

7.2 Future recommendations

A future recommendation for the IPK control system is to simulate an arterial waveform instead of a pulsatile sinusoid. An arterial waveform customary for the body would be a more truthful pressure pattern to pump the kidney with.
Furthermore, the addition of sensors for parameters such as temperature and pH could be investigated.
The current model created was a curve fit equation fitting the gathered data. However, a model built from existing knowledge on the relations of the physics behind the process of oxygenation could be investigated alternatively.

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APPENDIX A: Measured parameters during IPK analysis

Overview markers

Pre-perfusion:

- Weight
- Cortical biopt (ATP and histology)

Bloodgas:

- **Arterieel**
  - pH
  - pO_s
  - pCO_2
  - HCO_3-
  - Base excess
  - Lactate
- **Veneus**
  - pO_2

Perfusion parameters:

- Druk (mmHg)
- Flow (mL/min)
- Temperature perfusate (◦C)
- Intrarenale resistance
- Renal oxygen extraction

Renal function:

- Urine output (ml/h)
- Serum creatinine levels (umol/l)
- Urine creatinine levels
- Creatinine clearance (ml/min/100g) -> Glomerular filtration rate GFR

Tubular function:

- Filtration fraction **FF** (GFR/(renal blood flow x (100-Ht)))
- Fractional Na excretion (FEN_a = $[Na^+]_{urine}$ x creat.concentratie plasma / $[Na^+]_{plasma}$ x creat.concentratie urine
- N-acetylglucosamine **NAG**
- Alanine aminopeptidase **AAP**

Biochemical damage markers:

- Aspartaat transaminase **AST**
- Lactaat dehydrogenase **LDH**
- Ureum
- Serum Neutrophil Gelatinase-associated Lipocalin **sNGAL**
- Serum Cystatin C **sCysC**

APPENDIX B: Protocol data gathering

Use the HILITE 1000.

1. Turn off O2 supply, N2 flow until veneus oxygen measurement =< 10 hPa. Use high N2 flow (>1.5L/min) and medium flow (>0.5L/min). Continue using high N2 flow during experiment to minimalize oxygen content medium before oxygenator.
2. Temperature of the waterbath is set on 5° C.
3. The gas:medium flow ratio is set to 1:1 (FiO_2 The experiment starts with a flow rate of 50 ml/min with a FiO_2 of 0.21 and a gas: bloodflow ratio of 0.5:1 for 1.5 minutes. pO_2 at the inlet and outlet is measured and written down every 30 seconds.
4. Increase the flow rate to 100 ml/min with a FiO_2 of 0.21 and a gas:medium flow ratio of 0.5:1 for 2.5 minutes. pO_2 at the inlet and outlet is measured and written down every 30 seconds.
5. Increase the flow rate to 200 ml/min with a FiO_2 of 0.21 and a gas:medium flow ratio of 0.5:1 for 2.5 minutes. pO_2 at the inlet and outlet is measured and written down every 30 seconds.
6. Increase the flow rate to 400 ml/min with a FiO_2 of 0.21 and a gas:medium flow ratio of 0.5:1 for 2.5 minutes. pO_2 at the inlet and outlet is measured and written down every 30 seconds.
7. Increase the flow rate to 600 ml/min with a FiO_2 of 0.21 and a gas:medium flow ratio of 0.5:1 for 2.5 minutes. pO_2 at the inlet and outlet is measured and written down every 30 seconds.
8. The gas:medium flow ratio is set to 2:1 (FiO_2 of 21%) and steps 3 – 7 are repeated.
9. The gas:medium flow ratio is set to 3:1 (FiO_2 of 21%) and steps 3 – 7 are repeated.
10. The gas:medium flow ratio is set to 0.5:1 (FiO_2 of 50%) and steps 3 – 7 are repeated.
11. The gas:medium flow ratio is set to 1:1 (FiO_2 of 50%) and steps 3 – 7 are repeated.
12. The gas:medium flow ratio is set to 2:1 (FiO_2 of 50%) and steps 3 – 7 are repeated.
13. The gas:medium flow ratio is set to 3:1 (FiO_2 of 50%) and steps 3 – 7 are repeated.
14. The gas:medium flow ratio is set to 0.5:1 (FiO_2 of 75%) and steps 3 – 7 are repeated.
15. The gas:medium flow ratio is set to 1:1 (FiO_2 of 75%) and steps 3 – 7 are repeated.
16. The gas:medium flow ratio is set to 2:1 (FiO_2 of 75%) and steps 3 – 7 are repeated.
17. The gas:medium flow ratio is set to 3:1 (FiO_2 of 75%) and steps 3 – 7 are repeated.
18. The gas:medium flow ratio is set to 0.5:1 (FiO_2 of 100%) and steps 3 – 7 are repeated.
19. The gas:medium flow ratio is set to 1:1 (FiO_2 of 100%) and steps 3 – 7 are repeated.
20. The gas:medium flow ratio is set to 2:1 (FiO_2 of 100%) and steps 3 – 7 are repeated.
21. The gas:medium flow ratio is set to 3:1 (FiO_2 of 100%) and steps 3 – 7 are repeated.
22. Set bath temperature to 15 C°. Repeat step 3-23.
23. Set bath temperature to 25 C°. Repeat step 3-23.
24. Set bath temperature to 38 C°. Repeat step 3-23.

APPENDIX C: Protocol oxygenator testing

Start with the HILITE 1000 oxygenator.

1. Turn off O2 supply, N2 flow until veneus oxygen measurement =< 10 hPa. Use high N2 flow (>1.5L/min) and medium flow (>0.5L/min). Continue using high N2 flow during experiment to minimalize oxygen content medium before oxygenator.
2. Temperature of the waterbath is set on 4° C.
3. The experiment starts with a flow rate of **50 ml/min** with a FiO_2 of 0.21 and a gas:medium flow ratio of 0.5:1 for 2.5 minutes. pO_2 at the inlet and outlet is measured and written down every 30 seconds.
4. Increase the flow rate to **100 ml/min** with a FiO_2 of 0.21 and a gas:medium flow ratio of 0.5:1 for 2.5 minutes. pO_2 at the inlet and outlet is measured and written down every 30 seconds.
5. Increase the flow rate to **200 ml/min** with a FiO_2 of 0.21 and a gas:medium flow ratio of 0.5:1 for 2.5 minutes. pO_2 at the inlet and outlet is measured and written down 30 seconds.
6. Increase the flow rate to **300 ml/min** with a FiO_2 of 0.21 and a gas:medium flow ratio of 0.5:1 for 2.5 minutes. pO_2 at the inlet and outlet is measured and written down 30 seconds.
7. Increase the flow rate to **400 ml/min** with a FiO_2 of 0.21 and a gas:medium flow ratio of 0.5:1 for 2.5 minutes. pO_2 at the inlet and outlet is measured and written down 30 seconds.
8. Increase the flow rate to **500 ml/min** with a FiO_2 of 0.21 and a gas:medium flow ratio of 0.5:1 for 2.5 minutes. pO_2 at the inlet and outlet is measured and written down 30 seconds.
9. Increase the flow rate to **600 ml/min** with a FiO_2 of 0.21 and a gas:medium flow ratio of 0.5:1 for 2.5 minutes. pO_2 at the inlet and outlet is measured and written down 30 seconds.
10. The gas:medium flow ratio is set to 1:1 (FiO_2 of 0.21) and steps 3 – 9 are repeated.
11. The gas:medium flow ratio is set to 2:1 (FiO_2 of 0.21) and steps 3 – 9 are repeated.
12. The gas:medium flow ratio is set to 0.5:1 (FiO_2 of 0.5) and steps 3 – 9 are repeated.
13. The gas:medium flow ratio is set to 1:1 (FiO_2 of 0.5) and steps 3 – 9 are repeated.
14. The gas:medium flow ratio is set to 2:1 (FiO_2 of 0.5) and steps 3 – 9 are repeated.
15. The gas:medium flow ratio is set to 0.5:1 (FiO_2 of 1) and steps 3 – 9 are repeated.
16. The gas:medium flow ratio is set to 1:1 (FiO_2 of 1) and steps 3 – 9 are repeated.
17. The gas:medium flow ratio is set to 2:1 (FiO_2 of 1) and steps 3 – 9 are repeated.
18. Temperature of the waterbath is set on 21° C. Flow rate is increased to 1 – 1,5 L/min to increase the temperature of the perfusate to 20 - 22° C. Afterwards flow will be set on 50 ml/min and steps 3 – 17 are repeated.
19. Temperature of the waterbath is set on 38° C. Flow rate is increased to 1 – 1,5 L/min to increase the temperature of the perfusate to 37 - 38° C. Afterwards flow will be set on 50 ml/min and steps 3 – 17 are repeated.
20. Repeat step 1-19 for the HPH mini oxygenator
21. Repeat step 1-19 for the homemade oxygenator

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APPENDIX D: Files

Labview program:

IPK Control V0.32 Stable.vi