An Optical Transmission Method for Continuous Measurement of Blood Volume Changes during Haemodialysis

Diploma Paper
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Abstract

The purpose with this thesis was to design and evaluate a device for continuous monitoring of blood volume changes during haemodialysis. Because of a reciprocal relationship between blood volume and haemoglobin concentration an optical transmission method was used on the blood tube to measure how dense the blood was. Bovine blood has been used as a substitute for human blood.

Light propagating through the blood will not only be affected by absorption by the haemoglobin in the red blood cells but also by scattering to the cell membranes. Therefore, other properties than the optical path length, oxygen saturation and different haemoglobin concentrations must be taken into consideration. Parameters that have been examined that might influence the transmitted light are osmolality, blood flow and degree of haemolysis, which can change the shape, the distribution and size of the red blood cells. Different light sources and opto holders have been examined to minimise the scattering effects. A few scattering theories have been investigated and multiple-scattering simulations have been performed using a Monte Carlo program.

The experimental results display a non-linear dependence between the transmitted light and the haemoglobin concentration, which implicates the need for absolute measurements. The function is an exponential expression with a second ordered polynomial in the exponent. This curvature is also supported by the theories examined. To achieve accurate results, the emitted wavelength has to be located close to an isobestic point to avoid a dependence of the oxygen saturation. Investigated isobestic points are 800 nm and 548 nm. To reduce changes in transmitted light due to blood flow variations during dialysis, the blood flow has to be measured and compensated for. During a haemodialysis session the osmolality changes are small and will cause only small errors in the transmitted light.
Preface

Haemodialysis treatments have been simulated and different parameters have been investigated at Gambro Lundia AB. Our co-workers Per-Ola Wictor and Björn Pettersson have among other things designed the experimental set-up which is described more thoroughly in their report, An In Vivo Method for Continuous Measurement of Blood Volume Changes during Haemodialysis.

We wish to extend our thanks to several people who were instrumental in the production of this report and the various experiments it describes.
We would first like to thank Thomas Hertz who was our instructor at Gambro Lundia AB, for giving us this exciting project. He was a source of support and help as well as of infading enthusiasm throughout the entirety of this project. We are also grateful to the personnel at Gambro Lundia AB, for their hospitality and for taking the time with us and our problems.
We would like to thank Stefan Andersson-Engels, our instructor at the Division of Atomic Physics at Lund Institute of Technology and the personnel at the division.
At last we wish to thank our co-workers Per-Ola Wictor and Björn Pettersson for their pleasant co-operation.
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1. Introduction

Haemodialysis treatment comprises the removal of considerable amounts of fluid from the circulating blood volume of over-hydrated patients.

When suffering from renal failure water is accumulated in the body. During dialysis fluid is drawn from the blood, which is compensated for through refill from the overhydrated interstitium\(^1\). Therefore haemodialysis has an acute impact on the body’s fluid balance, in part due to the rapid change in circulating blood volume. When the fluid removal rate is more rapid than the body’s refilling rate, the intravascular\(^2\) blood volume decreases. The resulting fluid imbalance has been linked to the complications of hypovolemia-induced hypotension, a diminished blood volume results in a dangerously low blood pressure. Therefore the relevance of the continuous monitoring of blood volume during dialysis. This would be a means to detect hypovolemia at an early stage.

There have been several suggestions for methods of monitoring blood volume changes during haemodialysis. Changes in blood density, blood viscosity or plasma protein concentration have been used as parameters [1]. Most of these methods share the disadvantage that a blood sample is needed for analysis or that they are expensive.

The use of simple, continuous and non-invasive\(^3\) measuring techniques would have many advantages. Most importantly, discreet blood sampling provides relevant data only at the time the blood sample is drawn. Furthermore, invasive techniques are painful and have associated risks.

The total number of erythrocytes, the red blood cells, and the amount of haemoglobin, the red pigment of the blood, have been found to remain constant during dialysis provided no bleeding occurs [2]. Therefore, there is a reciprocal relationship between haemoglobin concentration and blood volume.

\[
BV(0) = \frac{Hb(t)}{Hb(0) \times BV(t)}
\]

where \(BV(0)\) and \(Hb(0)\) are the blood volume and the haemoglobin concentration at \(t = 0\). \(BV(t)\) and \(Hb(t)\) are the blood volume and the haemoglobin concentration at \(t\) [1].

Measurement of haemoglobin concentration to detect blood volume changes are preferable to the measurement of hematocrit\(^4\) because changes of the red cell volume might occur during dialysis due to osmotic\(^5\) changes.

We have investigated the possibility of continuously measuring the haemoglobin concentration in whole blood by means of an optical transmission technique.

\(^1\) Interstitium is the space between the cells.
\(^2\) Intravascular means the inside the blood vessels.
\(^3\) Non-invasive measuring means not involving puncture or penetration of the body.
\(^4\) Hematocrit, Hct, is the percentage of red blood cells to total blood volume.
\(^5\) Osmosis is movement of water through a membrane from a higher to a lower water concentration area.
Red cell volume changes during dialysis is the reason why we have worked with transmitted and not reflected light. The mean cellular volume, MCV of the erythrocytes is linearly proportional to the reflected light [1].
2. Theory

2.1 Background dialysis/blood

2.1.1 Functions of the Kidney
The kidney controls several important functions and processes in the body. It is responsible for the removal of waste products and excess fluids. The kidney also regulates the acid-base balance, the red blood cell production and the blood pressure.

2.1.2 Uraemia
When a kidney fails, urine production is reduced and the components of urine, that is water and waste products, accumulate in the body. As the renal function deteriorates, disorders will develop in most body systems; a syndrome that is called uraemia. Common symptoms are fatigue, anorexia, nausea and skin itching. If left untreated, uraemia will lead to death.

2.1.3 Dialysis/Transplantation
Before 1960 all patients suffering from chronic renal failure died from uraemia. During the last forty years, different therapies have turned out satisfactorily. When only 5% of the kidneys function remains, it will be necessary to start dialysis treatment, either with haemodialysis or peritoneal dialysis, or to provide a new kidney by transplantation [3].

2.1.4 Haemodialysis
In haemodialysis treatment, which is the most common form of dialysis, the blood is purified outside the body, extracorporeally, by an artificial kidney, the dialyzer. As is shown in Figure 1, [4], the blood flows on one side of a thin membrane and dialysate fluid on the other.
The membrane is semipermeable, thus permitting the passage of water and solutes up to a certain size. Two separate processes take place simultaneously.

- Removal of solutes from the blood into the dialysis fluid occur by **diffusion** through the thin membrane. Diffusion is movement of solutes from an area of higher solute concentration to an area of lower solute concentration, see Figure 2, [4].
- Removal of plasma water and solutes occur by applying a hydrostatic pressure difference across the membrane. This process is referred to as **ultrafiltration**, see Figure 2, [4]. The pressure gradient is called transmembrane pressure (TMP). In haemodialysis the combination of both positive (on the blood side) and negative pressure (on the dialysis fluid side) make up the total pressure gradient over the membrane.
Typically, haemodialysis is performed three times per week for 3-5 hours per session.

2.1.5 Dialysate Fluid; Acetat-Bicarbonate

By varying the composition of the dialysis fluid, solutes can be removed or added to the blood through diffusion. Waste products should be removed as efficiently as possible from the blood and are therefore not included in the dialysis fluid at all. In haemodialysis patients the electrolyte balance is disturbed and needs to be normalised. The dialysis fluid should therefore contain normal physiological electrolyte concentrations. The bicarbonate concentration in the blood is normally low in uremic patients and the concentration in the dialysis fluid should be higher than in normal blood.

Because of a lower frequency of hypotension, the use of bicarbonate dialysate in haemodialysis treatment is more common than the use of acetate dialysate. According to de Vries et al 1992 [5], the distribution space of sodium bicarbonate is smaller than for sodium acetate. This results in a higher plasma sodium level during bicarbonate
therapy which provides a more rapid plasma refilling rate, PRR. A lower blood pH during acetate dialysis may also affect the PRR [5].

2.1.6 Complications of Dialysis
During dialysis fluid is drawn from the blood, which is compensated for by refilling from the over-hydrated interstitium. When the fluid removal rate is more rapid than the body's plasma refilling rate, the intravascular blood volume decreases. This can result in a decrease in blood volume of up to 20 per cent [1]. This resulting fluid imbalance causes complications of hypotension, loss of consciousness, nausea, headache, vomiting, dizziness and cramps experienced by patients both during and after treatment [6]. Hypotension is a major complication and an important cause is the incorrect estimation of the post-dialytic dry weight of the patient, too much fluid is drawn from the patient. Ideal dry weight is defined as "the weight at the end of a dialysis treatment below which the patient, more often than not, will develop symptoms of hypotension" [7]. Approximately one out of three haemodialysis sessions is complicated by hypotension [8].

2.1.7 Blood
Blood composes approx. 8% of the body weight, i.e. 5-6 l in a 70 kg male [3]. By centrifugation it is possible to separate blood into its components, blood cells and plasma. The packed cell volume is approximately 50% of the total blood volume [9].

The blood cells are of three different types:

**The red blood cells**, (RBC), the erythrocytes, are the most numerous (5 billion/ml) [3]. Erythrocytes are ellipsoidal cells, each being a disk with a diameter of 7 μm and a thickness of 2 μm [9]. They are encapsulated by a thin membrane containing an intracellular fluid composed of various salt ions, protein molecules and water molecules. They contain the protein haemoglobin, the red pigment of the blood, which binds oxygen and carbon dioxide. Normal red blood cells measure 90 μm^3 in volume [10].

**The white blood cells**, the leukocytes and lymphocytes (7 million/ml) constitute an important part of the immune defence of the body [3].

**The platelets**, the thrombocytes, are the smallest blood cells (300 million/ml). They are important in clot formation and are activated by a vessel injury [3].

**The plasma** contains dissolved salts and proteins, but the main part is water (90%) [3]. In most cases, the salts and the proteins in the plasma differ in composition and concentration from those in the intracellular fluid. The different salt concentrations are of great importance in controlling the volumes of the different body compartments. Normal plasma salt concentration is Na^+ 142 mmol/l, K^+ 3.9 mmol/l, Ca^{2+} 1.3 mmol/l, Mg^{2+} 0.6 mmol/l, Cl^{-}101 mmol/l and HCO_3^{-} 24 mmol/l, [11].
Sodium, $\text{Na}^+$; is the most important electrolyte for the maintenance of osmotic pressure\(^6\) and the volume in the extracellular compartment. Any change in the $\text{Na}^+$ concentration in the plasma will automatically result in a change of the osmotic pressure in the extracellular compartment and lead to volume changes, resulting in either hypovolemia\(^7\) or hypervolemia\(^8\).

### 2.1.8 Haemoglobin

Haemoglobin is contained inside the red blood cells and represents approximately 15 per cent by weight of blood \([12]\). Normal haemoglobin concentration is 132-166 g/l for men and 116-149 g/l for women \([11]\). Haemoglobin is in the form of a tetramer (Hb\(_4\)) with each Hb containing one iron (Fe) atom. The Fe in the haemoglobin exists as Fe\(^{2+}\).

The dominating haemoglobin derivatives in the body are **deoxygenated/reduced haemoglobin**, HbR and **oxyhaemoglobin**, Hb\(_O_2\). Oxyhaemoglobin is responsible for the oxygen transport in the blood. Oxygen is transported in the blood in two distinct states. Under normal physiological conditions, about 98 per cent of the O\(_2\) present in the blood is combined with Hb inside the red blood cells. The remaining 2 per cent is dissolved in the plasma. In the lungs, deoxygenated haemoglobin combines with oxygen;

$$\text{HbR} + \text{O}_2 \Leftrightarrow \text{HbO}_2$$ \hspace{1cm} (2)

Oxyhaemoglobin gives off the oxygen in the muscles where it is stored until it is required for metabolic action. The reduced haemoglobin then uses certain amino groups to bind carbon dioxide and carry it back to the lungs, where it is excreted through expiration \([13]\).

The Oxygen Saturation is defined as:

$$O_2\text{sat} = \frac{c[HbO_2]}{c[HbO_2] + c[HbR]} \times 100$$ \hspace{1cm} (3)

where $c$ denotes the concentration of a given component. The oxygen saturation is an important parameter, e.g. when dealing with patients with respiratory disorders.

There also exist a number of **dyshaemoglobins** which are inactive as oxygen carriers:

---

\(^6\) Osmotic pressure means the hydrostatic pressure needed to prevent the flow of water induced by the osmolarity gradient.

\(^7\) Hypovolemia means too small volume of the blood in the circulatory system.

\(^8\) Hypervolemia means too large volume of the blood in the circulatory system.
Carboxyhaemoglobin - HbCO
The affinity of the iron atom (Fe$^{2+}$) for CO is about 200 times as great as for O$_2$ [14]. This means that HbCO will be formed whenever CO is present. For a smoker this binding can occupy as much as 5% of the total haemoglobin concentration. [15] Normally, HbCO constitutes 0.40-0.70%.

Methaemoglobin (Hemiglobin) - MetHb
In this dyshaemoglobin the iron atom is in the state of Fe$^{3+}$. Up to 1% of the haemoglobin is in the form of methaemoglobin [11]. Smoking can cause a slightly higher MetHb concentration.

Other haemoglobin compounds, such as sulphaemoglobin, SHb, carboxysulphaemoglobin, SHbCO, and cyanmethaemoglobin, HiCN, are of no practical interest here.
2.2 Optical Properties of Blood

A light beam directed towards a sample of whole blood will interact with the blood in three ways. Part of the light is transmitted through the blood, another part is absorbed by haemoglobin and the last part undergoes scattering, the latter being principally due to reflection and refraction of light by red cells which is caused by different refraction index in the plasma and in the blood cells. Scattering by leukocytes and platelets is assumed to be negligible.

2.2.1 Absorption

For the case of no cells in the solution, but only a homogeneously spread haemoglobin solution, no scattering will occur and the light will only be influenced by absorption. The absorption of light depends upon the composition of the medium, the wavelength of the light and the optical path length. The intensity, $I$, remaining in the beam after travelling a distance $L$ through a medium containing a number of absorbing species is given by Beer’s law

$$I = I_0 e^{-(\varepsilon_1c_1 + \varepsilon_2c_2 + \ldots + \varepsilon_nc_n) L}$$  \hspace{1cm} (4)$$

[16], where $I$ is the incident power, $\varepsilon_n$ is the extinction coefficient of species $n$ and $c_n$ is its concentration. The extinction coefficient is a function of the wavelength and is defined as the absorption coefficient multiplied with the concentration. The major absorbing components of the blood are HbR, HbO₂, MetHb and HbCO [11]. Their absorption spectra differ and the wavelengths at which the absorption coefficients are the same for two or more of the compounds are called isobestic points. In Diagram 1, [17], and 2, [18], the absorption coefficients are plotted versus the wavelength.
Diagram 1. Absorption spectra of oxyhaemoglobin (---), deoxygenated haemoglobin (--), carboxyhaemoglobin (-) and hemoglobin (—.—).}

Diagram 2. Absorption spectra of HbO₂ and HbR

2.2.2 Isobestic Points

At the isobestic wavelengths light absorption will not be affected by conversion between the different haemoglobin derivatives, but will depend upon the sum of the concentrations of the components

\[ I = I_0 e^{-\varepsilon c L} \]  

(5)
where $\varepsilon$ is equal to the extinction coefficient at the isobestic wavelength, and $c$ is the sum of the concentrations [16].

Two useful isobestic points for HbR and HbO$_2$ are 800 nm in the near infrared and 548 nm in the green spectrum, see Diagram 1 and 2. 548 nm is also an isobestic point for HbCO.

It has been some confusion in the literature concerning the exact location of the isobestic point of HbR and HbO$_2$ in the near infrared, but “when de-oxygenation is achieved by tonometry with an oxygen-free gas mixture, the isobestic point is always found almost exactly at $\lambda$=800 nm” [19].

The advantage of using near infrared radiation is that the absorption curves intersects at a small angle. Therefore, a small offset in wavelength will not influence the transmitted light if the oxygen saturation slightly differs during dialysis. The extinction coefficient at 800 nm is 0.20 L/(mmol*cm) [20].

At 548 nm there is a triple isobestic point for HbR, HbO$_2$ and HbCO [12]. At this wavelength there is no dependence on the concentration of HbCO, which is an advantage if the blood has a high HbCO concentration. Unfortunately, the absorption curves are rather steep here. If one tries to measure the haemoglobin concentration at this wavelength with a broad spectrum light source, which does not only emit at this wavelength, the reading will result in a large error if the oxygen saturation varies.

The light extinction coefficient of blood in the range of 510-590 nm is over 10 times larger than that for red and infrared light [21]. It is 12.2 L/(mmol*cm) at 548 nm [17]. In Table 1 below wavelengths with corresponding extinction coefficients are viewed.

<table>
<thead>
<tr>
<th>$\lambda$ (nm)</th>
<th>$\varepsilon$ Hb</th>
<th>$\varepsilon$ HbO$_2$</th>
<th>$\varepsilon$ HbCO</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>0.20</td>
<td>0.20</td>
<td>0.01</td>
<td>[20]</td>
</tr>
<tr>
<td>550</td>
<td>12.97</td>
<td>12.01</td>
<td>12.11</td>
<td>[20]</td>
</tr>
<tr>
<td>548.6</td>
<td>12.33</td>
<td>12.33</td>
<td>-</td>
<td>[21]</td>
</tr>
<tr>
<td>548.2</td>
<td>12.23</td>
<td>-</td>
<td>12.23</td>
<td>[17]</td>
</tr>
<tr>
<td>548</td>
<td>12.4</td>
<td>12.4</td>
<td>12.4</td>
<td>[14]</td>
</tr>
<tr>
<td>549.7</td>
<td>-</td>
<td>11.99</td>
<td>11.99</td>
<td>[17]</td>
</tr>
</tbody>
</table>

Table 1. Wavelengths with corresponding extinction coefficients close to the isobestic points.

2.2.3 Scattering

The propagation of light through a scattering and absorbing media such as whole blood is of a very complicated nature. The difficulty is that in a thick layer or with an increasing density of the scattering particles, light that is scattered once will be scattered repeatedly. All these multiple scattering interfere with each other and it becomes extremely difficult to account for the contribution of each scattering particle. Whole blood is a highly scattering medium. The packed cell volume is about one half of the total blood volume [9]. The particle density is thus very high and this means that even for thin samples there will be multiple scattering. The optical density
increases more than twenty times compared with absorption only [22]. Single scattering may be assumed only in thin samples of about 50 \( \mu \)m [9].

2.2.4 Scattering Theories

A lot of theories have been presented with the aim to model the complicated nature of multiple scattering. Some theories, such as the diffusion theory and Twersky’s wave-scattering theory, are analytical. Others are empirically developed like Janssen’s and Andersson and Sekelj’s theories. Finally there are models built on the Monte-Carlo simulation method.

2.2.4.1 Twersky’s Multiple-Scattering Theory

One theory to model multiple scattering was presented by TWERSKY in 1970. He developed a theory based on summation of the amplitudes of scattered electromagnetic waves. The theory has its origins in the wave equation, and treats light scattering by particles that are large compared to the wavelength of the light. The scattering function depends on the wavelength as well as on the shape, dimensions, spatial distribution and orientation of the scattering particles. This method imposes fairly strong restrictions on the geometry and for thick layers of blood, the multiple-scattering theory of Twersky was found to be insufficient [23].

Twersky’s equations solved for rectangular cross section can be expressed in terms of coherent and incoherent light.

\[
T = C + I(\theta)
\]  

(6)

where \( T \) is the transmitted light, \( C \) is the coherent flux and \( I \) is the incoherent flux [22]. The coherent flux, \( C \), decreases exponentially with the path length, \( d \), and can be expressed as follows:

\[
C = e^{-(\sigma_a + \sigma_s \cdot (1-c*V)) \cdot c*d}
\]  

(7)

where \( \sigma_a \) and \( \sigma_s \) are the absorption and scattering coefficients, \( V \) is the particle volume and \( c \) is the concentration of particles[22]. It is the factor \((1-cV)\) that differs from the Beer-Lambert law and if the term \(cV\) is small, equation (7) becomes identical with Beer-Lambert’s law.

The incoherent flux, \( I(\theta) \), is the light that has been scattered by particles and may be measured in any direction. It is the angle \( \theta = 0 \) that is of interest in our application, and the equation is given by:

\[
I(\theta = 0) = K \cdot \left\{ e^{-\sigma_a \cdot c*d} - e^{-(\sigma_a + (1-c*V) \cdot \sigma_s) \cdot c*d} \right\}
\]  

(8)

\( K \) is a parameter that is dependent on the detector and the way the red blood cells are oriented to the light beam [22].
If we consider Equation (8) and rearrange the terms:

\[ T(0 = 0) = e^{-a} \left\{ e^{a(c+d)} + K \left[ 1 - e^{-a(c+d)} \right] \right\} = T_{abs} \ast T_{sca} \]  

(9)

Twerskys model leads to the result that scattering and absorption effects can be considered separately. This assumption, however, has been verified by experiments using very thin blood samples only [23]. For thick blood samples, there will not exist any photons that has not been scattered. \( T_{abs} \) approaches zero and the model collapses. All these disadvantages reduce the use of the multiple-scattering theory to problems of forward transmission in thin layers only.

### 2.2.4.2 The Diffusion Theory

A different approach to the multiple scattering problem is the transport theory. Here absorption and back-scattering of light in a blood sample are treated as obstacles to a flux of propagating photons which is penetrating the sample. This implies summation of light intensities rather than amplitudes in calculating the intensity distribution of the radiation after passage through the sample.

In the extreme case of total loss of the initial distribution of intensities, the transport theory collapses into **diffusion theory**. This approach assumes all incident, reflected and transmitted light diffuses almost isotropically.

Analytical solution for the reflectance and transmittance under certain boundary conditions can be relatively easily derived from the equation of light diffusion. But the theoretical results agree well with the experimental data only when the mean free path for scattering is negligible compared to the thickness of the blood layer and it is not valid near boundaries.[23] The accuracy of this model decreases therefore with decreasing thickness of the blood sample. For very thin layers of blood (<0.4 mm) experiments show the diffusion model to be inapplicable.

Unlike Twerskys wave superposition model, diffusion theory does not provide any prescription of how to express the single-scattering cross-section of particles in terms of their geometry and condition. This is not a serious limitation as long as diffusion is isotropic. Even if scattering is non-isotropic, diffusion theory may still be applied, provided that any initially definite direction of the scattered photon is obliterated. As scattering by a single RBC is not in fact isotropic contrary to the assumption made by the diffusion model, the flux becomes totally randomised only after numerous single scattering events.[23]

The diffusion theory is built on the diffusion equation for photons, and is in its most general form given by

\[ F = -D V p(x, y, z) \]  

(10)

[24]. Here \( p(x, y, z) \) is the photon density, \( D \) is the diffusion constant and \( F \) the photon flux at any point \((x, y, z)\) within the blood. The photon diffusion theory is only valid
when the scattering coefficient is much larger than the absorption coefficient, \( \sigma_s \gg \sigma_a \). [21]

### 2.2.4.3 Janssen's Theory

Anderson and Sekelj (1965) measured the transmittance of whole and haemolysed blood of various haemoglobin concentrations at a wavelength of 805 nm. Janssen [9] analysed their data and based on the diffusion theory, a scattering factor \( \rho \) was then calculated assuming the form

\[
I = I_0 e^{-(\varepsilon c + \rho)L}
\]

where \( I \) is the intensity remaining in the beam after travelling a distance \( L \) through a medium containing a number of absorbing species with extinction coefficient \( \varepsilon \). \( c \) is its concentration.

Janssen found that the scattering factor depended upon the haemoglobin concentration as follows:

\[
\rho = a c e^{-b c}
\]

where \( a \) depends on such parameters as the shape and the volume of a single red blood cell and \( b \) depends on the cell count per unit haemoglobin concentration. Janssen, [9], assumed that scattering due to cells and particles other than erythrocytes is negligible. For high haemoglobin concentrations and thus a high optical densities, the demand of a diffuse light source result in that no light will be transmitted through the sample.

### 2.2.4.4 Andersson's & Sekelj's Theory

Andersson and Sekelj [25] empirically determined a formula for haemoglobin measurement. They looked at transmitted light from whole blood, flowing through a cuvette at 805 nm. The cuvette had a rectangular cross section and they performed measurements on blood depths of 0.7 mm and 0.35 mm. During the experiments, both oxygen saturation and haemoglobin concentration were varied.

The scattering effects were described with a non-linear extinction coefficient \( \varepsilon' \). It was found that \( \varepsilon' \) of whole blood, at a constant depth, decreased as the haemoglobin concentration increased:

\[
\varepsilon' = \frac{1}{a c + b}
\]

Where \( a \) and \( b \) are constants depending on the geometrical and optical condition of the system, and \( c \) is the haemoglobin concentration. The extinction coefficient, \( \varepsilon' \), was then applied to Beer-Lamberts law.
\[ I = I_0 e^{-\mu'c*L} \] (14)

Experiments which confirms this formula have only been found in [25]. Measurements have been performed on thin samples only.

2.2.4.5 Monte Carlo Simulation

The Monte Carlo simulation is a numerical method built on a stochastic model. This model simulates a physical quantity using a number of independent samples.

As a light propagation model, Monte-Carlo describes photon-blood interaction when scattering occurs. However, the method is statistical and to get a reliable result a large number of calculations have to be done. Monte-Carlo does not treat the photon as a wave phenomenon, and ignores phase and polarisation, but predicts the radiant energy transport. The simulations are based on the optical properties \( \sigma_a \), the absorption coefficient, \( \sigma_s \), the scattering coefficient and the \( g \), the anisotropy factor, see Chapter 4.
2.3 Parameters that Might Influence Transmitted Light Through Blood

2.3.1 Osmolality

Osmolality indicates the osmotic activity in a solution. In a diluted solution, e.g. body fluids, the osmolality is the concentration of all sorts of free particles per unit water weight. For example: 1 mmol NaCl is 2 mosmol Na\(^+\) Cl\(^-\).

Due to the high permeability for water in the cell membranes, the intracellular osmolality is equal to the extracellular. If a difference in osmolality occur between the intra- and extracellular fluids, water is rapidly displaced through the membranes and the difference is compensated for. If osmolality in e.g. the extracellular fluid increases, water will be drawn out of the cells until equilibrium is reached. The cellular volume decreases. Since scattering depends on the size of the particle, this will affect the propagation of light through the blood. If the cellular volume decreases, this would change the scattering coefficient.

The main determinants of plasma osmolality are the urea, glucose and sodium concentrations. During routinely performed haemodialysis, the only clinically important changes are those in the urea and the sodium [5]. The urea decrease does not influence the mean cellular volume because the molecules are so small that they can penetrate the cell membranes freely. The changes in plasma sodium concentration can be influenced by variation of dialysate sodium. It has been suggested that the optimal concentration is around 145 mmol/litre [5].

The mean osmolality change in 37 dialyses according to Ishihara et al [26] was 7.2 mosmol, which was estimated by the decrease in sodium and potassium concentration during haemodialysis. This caused a cell volume increase of 1.3%. Therefore the cell volume change due to the change in plasma osmolality will be small in the case of dialysis. According to Mann et al [27] the sodium gradient between plasma water and dialysis fluid may change up to ±5 mmol/l during dialysis.

2.3.2 Temperature

During dialysis the temperature in the blood will not change much. This is due to that the dialysate fluid which is warmed up in the dialyzer also holds a temperature round 37°C. According to de Vries et al [28] no influence of the optical properties of the blood was detected when changing the temperature in the interval 20-40°C and using reflected light.

2.3.3 Blood Flow

The shape, the orientation and the distribution of the red blood cells depends on the blood flow. A turbulent flow gives a random orientation, while in a laminar flow the cells are orientated along the mean direction of the flow. This will affects the
measured scattering and absorption coefficients. In a laminar flow the red blood cells will gather in the middle of the blood tube while in the outer edges there will be plasma [29]. This might result in that the incident light uses the plasma as a light guide and the detector registers an erroneous signal.

A turbulent flow would then be preferable to avoid that the transmitted light is dependent upon the blood flow, unfortunately the turbulence might have a haemolysing affect on the red blood cells.

When there is no flow at all the erythrocytes tend to settle down and stick to each other, which reduces the scattering coefficient significantly [30].

At wavelengths in the green spectrum, the light transmission shows less dependence on the blood flow compared to a wavelength in the infrared spectrum [29].

### 2.3.4 The Degree of Haemolysis

Haemolysis is the destruction of red blood cells, the haemoglobin becomes freely dissolved, and scattering will no longer occur.

The linear relationship between the cell-count and the total haemoglobin concentration is spoiled by the occurrence of haemolysis. Thus scattering is a function of the cell-count only or on both the haemoglobin concentration and the degree of haemolysis.

In the case of an increasing haemolysis at a constant haemoglobin concentration the penetration of light into the blood sample will increase due to loss of scattering cells.

When using a dialysate containing a low sodium concentration water is drawn into the cells. These are changing to more spherical shapes, until the volume increase is too large and the membrane bursts.

At a cell volume change of 4%, the haemolysis rate is about 2% [31].
3. Transmission Experiments

3.1 Material & Methods

3.1.1 AK 100 Preparations
The Gambro AK 100 dialysis machine was used to perform our experiments. The dialyzer comprises two monitors, the blood and the fluid monitor. The blood monitor checks and supervises the extracorporeal blood circuit. The fluid monitor prepares the dialysis fluid to a desired temperature, conductivity and pressure within given specifications and supervises the dialysis fluid circuit.

In all experiments bicarbonate was used as dialysate fluid. Bicarbonate forms an insoluble salt, calcium carbonate, with calcium, so in order to avoid precipitation two sources of dialysate concentrate must be used, one with bicarbonate and one with the remaining dialysis components. To avoid that CO₂ diffuses from the bicarbonate container, which has a high CO₂-concentration, out into the air, which has a lower CO₂-concentration, a tight gasket was made around the pick-up tube. We also tried to use BiCart, pulverised bicarbonate where the dialysis fluid is prepared just before use to avoid CO₂ diffusion, but this resulted in inexplicable complications e.g. stoppage in the dialysis filter, Gambro, Plate Dialyzer 3N. The filter is freely permeable to water, small molecules and electrolytes but is impermeable to cells and proteins. The dialysis fluid was tempered to 37±0.5 °C. The bicarbonate concentration (HCO₃⁻) was set to 34 mmol/l and the sodium concentration (Na⁺) to 140 mmol/l.

Priming was performed on the AK 100 in order to remove air bubbles from the air detecting system. The priming was performed with 0.9 % sodium chloride with addition of Heparin, 5000 IU/ml, to avoid blood coagulation in the filter and in the blood tubes.

The ultrafiltration rate, that is ultrafiltration volume divided with treatment time, was kept almost at 0.6 l/h during all the experiments, otherwise a too high TMP would be achieved.

3.1.2 Measuring Equipment
The dialysis simulating experiments differs from normal dialysis treatment at several points, see Figure 3. A beaker partially filled with blood simulated a dialysis patient. This beaker was placed on a hot-plate to keep a normal body temperature at 37°C and was covered with plastic to prevent diffusion of blood gases out into the air. On the arterial side of the AK 100 the patients blood vessels were replaced with a tube, which was submerged into the blood beaker. Four opto holders with cuvettes and different light sources, were placed after the drip chamber on the venous side of the AK 100. Here is where the transmission measurements will take place. The loop is completed by letting the blood tube return to the beaker of blood. In order to simulate the human venous and arterial blood pressure, the beaker is located three decimetres above both
the opto holders and the roller pump. This arrangement was necessary for the AK 100 to work properly.

3.1.2.1 Cuvettes

A cuvette to insert in the blood line during dialysis, have not only optical demands but also biological and clinical ones.

One of the most important properties is that the cuvette is made of a rigid material with a constant cross section. In this case there will be no geometrical changes due to the pulsating blood pressure. With a rectangular cross section the optical path length is more accurately determined, but as blood is a highly scattering medium the importance to have a well defined optical path length is not so critical.

In order to avoid complicated flow and pressure changes which appear after the roller pump, the opto holders were placed after the dialysis filter. The dialysis filter and the air volume in the drip chamber makes the blood flow to change slower.
In order to avoid a blood flow dependence a turbulent flow, that causes a random orientation of the red blood cells, is desirable. Unfortunately, this might result in an increased degree of haemolysis and a laminar flow is therefore preferable. All sharp edges must be avoided to minimise the haemolysis.

Another aspect is the biocompatibility. The best optical material for biological purpose is Pyrex glass and PVC-plastic material [32]. From a clinical point of view, glass is unsuitable because of the infection risk at injuries. Also from an environmental point of view PVC is a better choice. According to [35] Pyrex glass had been tested with positive results and was there for used in the experiments. Plastic cuvettes were expensive and difficult to manufacture.

3.1.2.2 Opto Holders

A light emitting diode, LED, which emits green light at a wavelength of 555 nm with a full width at half maximum, FWHM, of 20 nm was used. As a triple isobestic point for HbR, HbO2 and HbCO is located at 548 nm, according to Chapter 2.2.2, this wavelength would have been desirable. Unfortunately, a LED that emits at 548 nm are not available. The cuvette used for measurements at this wavelength shown in Figure 4 is made of Plexi glass and has a rectangular cross-section with a short light path length. It was specially built for these experiments by Gambro Lundia AB. The sample thickness is only 0.5 mm because of the high absorption at this wavelength. Efforts were made to keep the degree of haemolysis to a minimum by creating smooth transitions from the circular blood tubes to the thin rectangular cuvette. To detect the transmitted light, a broad band photo detector with a built-in amplifier was used. The maximum sensitivity is at 745 nm. A second detector, equal to the first, was also attached to measure the incident light. To achieve a better signal to noise ratio the opto holder was screened from the surrounding light.

![Figure 4. Measurement geometry for green light transmitted through a thin cuvette of blood.](image)
Cuvettes with circular cross sections, see Figure 5, were used when performing experiments to evaluate transmission measurements in the near infrared part of the spectrum. One of the opto holders, including the cuvette, was designed by Hospal. The cuvette is made of PVC and the holder of aluminium. On the holder, the surface facing the cuvette is polished. The reason for this is that with a highly reflecting surrounding surface, the light scattered out from the cuvette will be reflected back into the blood again. This will decrease the effects of scattering [33]. The light source was a LED with a peak wavelength at 800 nm and a FWHM of 20 nm. The detector was a photo diode with a peak detection at 850 nm. Both detector and transmitter were thermally isolated from the aluminium holder.

![Figure 5. An opto holder for measurements in the near infrared part of the spectrum, using a cuvette with a circular cross section.](image)

The two remaining opto holders were almost identical with the one produced by Hospal and were also been built for these experiments by Gambro Lundia AB. The cuvettes are made of glass and the opto holders are made of aluminium. The two holders have polished interiors for the same reasons as the Hospal. One opto holder was equipped with a LED at 800 nm, identical with the one on the Hospal opto holder. The other holder had a laser diode with a narrow emission at 807 nm. Both holders had the same type of detector as the green opto holder. The LED at 800 nm had an identical detector for incident light. The laser diode, on the other hand, had an internally built-in power detector for controlling the incident intensity. None of the holders had thermal isolation for neither the detectors nor the transmitters. Glass tubes are better heat conductors than plastic tubes. Therefore, before starting the data acquisition, blood was pumped through the opto holders to reach thermal equilibrium. In Table 2 below, the four different light sources, their wavelength, materials and FWHM are enumerated.
A few experiments were also performed at 801 nm. The LED emitting at this wavelength was glued onto a patented measurement cell, Crit-Line. The purpose was to investigate whether a thin cuvette with a rectangular cross-section, or a thicker cuvette with a circular cross-section was to prefer. This measurement set-up is unfortunately not very rigid.

<table>
<thead>
<tr>
<th>Denomination</th>
<th>Wavelength (nm)</th>
<th>Material cuvette</th>
<th>Material opto holder</th>
<th>FWHM (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LED 555 nm</td>
<td>555</td>
<td>Plexi glass</td>
<td>Plexi glass</td>
<td>20</td>
</tr>
<tr>
<td>Hospal 800 nm</td>
<td>800</td>
<td>PVC</td>
<td>Aluminium</td>
<td>20</td>
</tr>
<tr>
<td>LED 800 nm</td>
<td>800</td>
<td>Glass</td>
<td>Aluminium</td>
<td>20</td>
</tr>
<tr>
<td>Laser 807 nm</td>
<td>807</td>
<td>Glass</td>
<td>Aluminium</td>
<td>Monochromatic</td>
</tr>
</tbody>
</table>

*Table 2. The four light source.*

### 3.1.3 The Data Acquisition

The way the information is acquired from the detectors on the opto holders and converted to data was the most important part of the measurement. To describe this complex system we begin with an overview. As shown in Figure 6 the light sources are pulsed with a square wave generated from a signal generator. The detectors were controlled by a Personal Computer using LabVIEW\(^9\). The computer activated the detectors and took one sample when the light was on, and one sample when the light was off. Signal processing was executed before storing the data in a file. On one of the holders a thermo element was also included to measure the temperature of the holder for reference purposes. All the opto holders had the same acquisition arrangement, except for Hospal, which will be discussed later.

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\(^9\) LabVIEW is a graphical data program for data acquisition, signal processing, analyses and presentation.
3.1.3.1 Light Modulation
When the light sources are pulsed, it becomes possible to perform a differential measurement and circumvent the influence from the background radiation. Another very important argument for using pulsed light sources is that a higher light intensity can be achieved. A non symmetrical square wave with a duty cycle of 40 % and a frequency of 4 kHz was used.

3.1.3.2 Detection
The data acquisition system was of course discrete. During one pulse period, the output signal from a detector was sampled eight times, five samples were taken on the low part of the period and three samples on the high part, according Figure 7.
One of the five values on the low part was chosen to represent the background radiation. Likewise, one of the three samples on the high part of the period was picked out to represent the transmitted light. The background radiation was assumed not to change during the 0.2 ms interval between a high and a low value. Therefore, the transmitted light could be calculated as the high value minus the low value. This procedure was done five times running and an average value was determined. The detector was activated according to Figure 8.
When all the detectors had been activated the computer waited a default time before restarting the iteration, alternatively the program terminated and data was stored in a file. The whole sequence, activating and averaging for all detectors, needed less than one second computer time.

3.1.3.3 Hospal’s Measuring Equipment

Hospal used a measuring method that is principally the same as for the other three cells. On the transmitter side the light source was pulsed with 250 Hz and on the detector side the output signal was first measured by a digital volt meter which performed the D/A conversion. In Figure 6, the Hospal arrangement is viewed. The signal was then directly sent on the external data bus, GPIB, to the computer. Both the transmitter and the detector driving circuits were manufactured by Hospal.

3.1.4 Blood Preparations

Due to risk of infection when experimenting on human blood, bovine blood was used during our experiments. Bovine blood was for us a satisfactory alternative to human blood.

The blood was delivered from the slaughterhouse where 10 ml Heparin, 5000 IU/ml, was added to 10 l bovine blood. It was kept cold in a refrigerator for not more than
three days. When an experiment was to be performed, blood was transferred to a
beaker which contained approximately 2 l blood but before that it was also strained of
larger particles which may have entered the blood at the time of slaughtering. To
avoid stoppage in the filter or in the drip chamber still more heparin was added to the
blood, approximately 2 ml for 2 l blood.

In certain experiments, e.g. when simulating dialysis treatments, it is desirable to have
a high oxygen saturation degree in the blood and also to keep it at normal body
temperature, 37°C. To oxygenate the blood a thin plastic tube with a diameter of about
1 mm was brought into the blood in the beaker. The tube was connected to a gas tube,
see Figure 3, containing 95% oxygen and 5% carbon dioxide[17], [34]. The gas
pressure was kept very low to avoid foam in the blood. Warming the blood was
performed with a heater Figure 3. A magnetic stirrer was dropped into the blood
beaker and the rotation velocity could be adjusted on the heater. Due to the stirring a
homogenous temperature was obtained in the blood and the blood did not sediment.
The magnetic stirrer was allowed rotating during the experiments.

3.1.5 Reference Instruments
During the experiments three reference instruments were used for measuring
important parameters on the blood.

3.1.5.1 OSM2, Radiometer, Copenhagen, Denmark
The OSM2 Hemoximeter is designed for photometric determination of the total
haemoglobin concentration (ctHb) and the oxygen saturation ($O_2$ sat) in whole blood
or blood concentrate.
The oxygen saturation $O_2$ sat is defined as:

$$O_2\text{ sat} = \frac{c[HbO_2]}{c[HbR] + c[HbO_2]}$$ (15)

where $c$ is the concentration, $HbO_2$ indicates oxyhaemoglobin and $HbR$ indicates
deoxygenated (reduced) haemoglobin. After the introduction of a blood sample by
either injection or aspiration the blood is haemolysed by ultrasound. The analyses
carried out by means of the Hemoximeter are based on absorbance measurements at
506.5 and 600.0 nm.

3.1.5.2 HemoCue, HemoCue AB, Ängelholm, Sweden
The HemoCue measures haemoglobin concentration in whole blood. In a microcyvette
the erythrocytes are haemolysed and transferred to MetHb. Absorbance measurement
at two wavelengths, 570 and 880 nm, is carried out to compensate for possible
turbidity.

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3.1.5.3 Stat Profile 5, NOVA Biomedical Corporation, Massachusetts, USA

The Stat Profile 5 Analyzer performs tests of serum, plasma, whole blood and expired gas for in vitro diagnostic use. Samples are analysed for:

- pH
- PCO₂
- PO₂
- Sodium (Na)
- Potassium (K)
- Chloride (Cl)
- Ionised Calcium (Ca)
- Haematocrite (Hct)

From directly measured results, calculated results can be derived at:
- Oxygen Saturation level (O₂SAT)
- Bicarbonate level (HCO₃⁻)
- Haemoglobin (Hbc)

The electrolyte concentrations are measured by using electrodes with ion-selective membranes.
- pH is measured using a hydrogen ion selective glass membrane.
- pCO₂ is measured with a modified pH electrode.
- pO₂ is measured amperometrically by the creation of a current at the electrode surface.
- Haematocrit is measured by using the known electrical resistance of red blood cells in blood samples.
3.2 Performances, Results & Discussions

3.2.1 Ultrafiltration Experiments

3.2.1.1 Performance

The ultrafiltration experiments were developed to simulate a real haemodialysis treatment and to relate the transmitted light to the haemoglobin concentration.

During a haemodialysis treatment fluid is removed from the blood. This results in a decreasing blood volume and thus an increasing haemoglobin concentration.

A beaker of blood simulated a patient and the oxygen saturation in the blood was kept between 95-100% during the experiments. The blood had to be diluted with saline solution\(^{10}\) in order to simulate the blood of an overhydrated patient. Thus, a low haemoglobin concentration down to 80 g/l was achieved.

The AK-100 was working in the volume control mode in order to use a treatment with controlled ultrafiltration. It was tuned to a UF-rate of 0.6 l/h and the blood flow was 250 ml/min. An experiment lasted about two hours. The experimental arrangement was shown in Figure 3.

The light transmission through the cuvettes was measured continuously by the detectors on the opto holders. To refer the transmitted light to the haemoglobin concentration and to measure the oxygen saturation, blood samples were taken every three minutes from the blood beaker, using a 1 ml plastic syringe, and analysed by the OSM2 and the HemoCue. The blood gases and the electrolyte concentrations were analysed approximately every ten minutes. These analyses were performed to discover failures in the experimental set-up or if any other parameter except for the haemoglobin influences the light transmission.

3.2.1.2 Results

In Table 3, typical variations of blood parameters during an experiment are given. Most of the experiments had a haemoglobin variation within the interval 80-180 g/l and an oxygen saturation of 100%.

<table>
<thead>
<tr>
<th></th>
<th>Hb (g/l)</th>
<th>O(_{2})sat (%)</th>
<th>pH</th>
<th>pCO(_2) (mm Hg)</th>
<th>Na (mmol/l)</th>
<th>K (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min</td>
<td>80</td>
<td>96</td>
<td>7,21</td>
<td>56</td>
<td>141</td>
<td>3,0</td>
</tr>
<tr>
<td>Max.</td>
<td>180</td>
<td>100</td>
<td>7,33</td>
<td>73</td>
<td>148</td>
<td>2,25</td>
</tr>
</tbody>
</table>

Table 3. Changes of relevant blood parameters in the experiments.

When the blood reached a haemoglobin concentration of about 180 g/l, the blood was diluted in order to start all over again or the experiment was ended.

\(^{10}\) With a saline solution means physiological 0.9% NaCl-solution.
In Diagram 3 to 6, the transmitted light from the different light sources is plotted against the haemoglobin concentration. In all four figures, data is taken from several haemolysis experiments.

**Diagram 3.** The light transmission through a glass cuvette with an interior diameter of 4 mm.

**Diagram 4.** The light transmission through a plastic cuvette with an interior diameter of 4 mm.
3.2.1.3 Discussion

In Diagram 3 and 6 above, data from different experiments can easily be distinguished. This is not the fact for the Hospal 800 nm and Laser 807 nm, in Diagram 4 and 5, where data from the different experiments are very similar. However, the curvature from the various tests in Diagram 3 and 6 looks very much the same, except for a vertical displacement. The most likely reason for this is that there have been inexplicable changes in the experimental arrangement.

From the theories discussed in Chapter 2.2.4, the transmission is not a linear function of the haemoglobin concentration. The equation suggested here is:

\[ T(c) = e^{-(\alpha c^2 + \beta c + \gamma)} \]  \hspace{1cm} (16)
were $T(c)$ is the transmitted light, $\alpha$ and $\beta$ are coefficients depending on the geometry and the wavelength of the light, $\gamma$ is a factor proportional to the emitted light and $c$ is the haemoglobin concentration. The coefficients $\alpha$ and $\gamma$ are adjusted to fit the Equation 15 for the curves in Diagram 3 to 6. In Table 4, the coefficients corresponding to the different opto holders are tabled.

<table>
<thead>
<tr>
<th>LED 800 nm</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$5.721 \times 10^{-5}$</td>
<td>$2.4116 \times 10^{2}$</td>
<td>$2.8573$</td>
</tr>
<tr>
<td>Laser 807 nm</td>
<td>$1.3239 \times 10^{4}$</td>
<td>$4.8052 \times 10^{2}$</td>
<td>$4.1023$</td>
</tr>
<tr>
<td>Hospal 800 nm</td>
<td>$4.9190 \times 10^{3}$</td>
<td>$2.0118 \times 10^{2}$</td>
<td>$1.6305$</td>
</tr>
<tr>
<td>LED 555 nm</td>
<td>$4.663 \times 10^{5}$</td>
<td>$1.5350 \times 10^{2}$</td>
<td>$2.0984$</td>
</tr>
</tbody>
</table>

*Table 4. Function coefficients for trendlines.*

In Diagram 7 to 10, the Equation 15, with coefficients for the different light sources are plotted against the haemoglobin concentration. Due to the fact that the experimental arrangement have been changed and developed, the number of experiments the diagrams below are based on varies for the different light sources.

*Diagram 7. The transmitted light as a function of the haemoglobin concentration.*
Diagram 8. The transmitted light as a function of the haemoglobin concentration.

Diagram 9. The transmitted light as a function of the haemoglobin concentration.
Diagram 10. The transmitted light as a function of the haemoglobin concentration.

The first intention was to measure relative haemoglobin concentration changes, but as the relation between haemoglobin concentration and transmitted light is non-linear, the measurement had to be absolute. The diagrams above can be used as calibration diagrams to determine the uncertainty of the haemoglobin concentration in a measurement.

In Diagram 10, the light transmission increases for a haemoglobin concentration of over 160 g/l and the explanation for this is that the oxygen saturation increased from 95-100% in the end of the experiments that this function is based on. The LED 555 nm is very sensitive of oxygen changes, see Chapter 2.2.2.

Under normal circumstances a dialysis patient has a haemoglobin concentration in the interval of 80-180 g/l [35] and a haemoglobin concentration of over normal values, see Chapter 2.1.8, increases the risk of hypovolemia. Therefore a good resolution is necessary in this interval to determine the haemoglobin concentration with accuracy.

The variations of different blood parameters except the haemoglobin concentration were of course affecting the light transmission. These effects will be discussed later.
3.2.2 Haemolysis Experiments

3.2.2.1 Performance

Before, during and after seven different experiments the degree of haemolysis was measured.

We wanted to find out if the roller pump, the cuvettes and the connections from the tubes to the four cuvettes caused any haemolysis. To avoid sharp edges both ends on the glass cuvettes were smoothen by heating. We also wanted to know if a high blood flow and the utilisation of a dialysis filter increased the degree of haemolysis. And of course we were interested in the time dependence of the haemolysis.

Two of the seven experiments had the purpose to see if we could maintain the haemoglobin concentration when the blood was circulated in the tubes. This was done to insure that we could rely on later experiments. Three experiments were simulations of dialysis treatments where fluid was removed from the blood when flowing through a filter, Gambro Plate Dialyzer 3N. In the remaining two experiments the blood flow dependence on the transmitted light was investigated.

When performing a haemolysis test, approximately 20 cl of blood was taken from the blood beaker on the dialyzer. We brought it to Development Lab, Disposable Division, Gambro Lundia AB, where the required equipment was available e.g. a spectrophotometer and a centrifuge. Below is a description of how the test was performed.

### Haemolysis test

Dilute 4 ml of blood with 5 ml 0.9% NaCl.
Prepare two test tubes, one with 10 ml 0.9% NaCl (Test 1) and one with 10 ml destilled water (Test 2).
Add 0.2 ml diluted blood to each of the test tubes.
Mix and centrifuge at 400 rpm for 15 minutes.
Carefully transfer the solutions to 10 mm cuvettes.
Measure the absorbances at 545 nm in a spectrophotometer.
Use destilled water as reference solution.
The degree of haemolysis = (absorbance Test 1/absorbance Test 2) *100

The destilled water in Test 2 haemolyses every single erythrocyte. The negligible concentration of electrolytes in destilled water makes the red blood cells swell until they burst. Because of the appropriate NaCl concentration in the other test tube there will not be further haemolysis.

3.2.2.2 Results

In Table 5 below the seven experiments, in which haemolysis tests were performed, are presented together with some important parameters.
Table 5. The seven experiments with some important parameters.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant test 1</td>
<td>0.35-1.23</td>
<td>3</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>No</td>
<td>148</td>
<td>100</td>
<td>7.0</td>
<td>148</td>
</tr>
<tr>
<td>Constant test 2</td>
<td>0.35-1.50</td>
<td>4</td>
<td>6</td>
<td>30</td>
<td>500</td>
<td>No</td>
<td>150</td>
<td>100</td>
<td>7.2</td>
<td>148</td>
</tr>
<tr>
<td>Dialysis</td>
<td>0.36-0.36</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>250</td>
<td>Yes</td>
<td>148-205</td>
<td>36-68</td>
<td>7.2</td>
<td>144-149</td>
</tr>
<tr>
<td>Dialysis</td>
<td>0.35-1.72</td>
<td>1</td>
<td>3</td>
<td>37</td>
<td>250</td>
<td>Yes</td>
<td>135-195</td>
<td>7.2-7.3</td>
<td>0</td>
<td>144-149</td>
</tr>
<tr>
<td>Dialysis, diluting the blood</td>
<td>0.37-0.93</td>
<td>2</td>
<td>3</td>
<td>37</td>
<td>250</td>
<td>Yes</td>
<td>80-180</td>
<td>100</td>
<td>7.2</td>
<td>144-148</td>
</tr>
<tr>
<td>Blood flow</td>
<td>0.56-1.90</td>
<td>1.3</td>
<td>2</td>
<td>37</td>
<td>42-505</td>
<td>No</td>
<td>142</td>
<td>100</td>
<td>7.2</td>
<td>0-7.3</td>
</tr>
<tr>
<td>Blood flow</td>
<td>0.60-0.60</td>
<td>1.3</td>
<td>2</td>
<td>35</td>
<td>30-450</td>
<td>No</td>
<td>141</td>
<td>100</td>
<td>7.2</td>
<td>0</td>
</tr>
</tbody>
</table>

In two of the experiments haemolysis tests were not only executed before and after the experiment but also during them. In Diagram 11 and 12 the degree of haemolysis is plotted versus time.

Diagram 11. The degree of haemolysis plotted versus time.
Diagram 12. The degree of haemolysis plotted versus time.

3.2.2.3 Discussion

In the seven experiments either a small increase in haemolysis or no haemolysis at all was achieved. As can be seen in Diagram 11, Diagram 12 and Table 5, no conclusions can be drawn concerning the time dependence, except that in 5 of the 7 experiments there is a small increase in haemolysis. In the remaining two no change occurred. We did not notice any difference in haemolysis between experiments with and without filter.

The 0.9 % NaCl solution, that is used for diluting the blood in order to decrease the haemoglobin concentration, seems to be suitable for the blood plasma since no increase in haemolysis originates from that specific experiment. When experiments to investigate the flow dependence were made, the haemolysis degree increased slightly in one experiment and was constant in the other. In short intervals the blood flow in these experiments was raised to 450-500 ml/min.

No conclusions could therefore be drawn about which parameters that might influence the degree of haemolysis and to what extent. An important conclusion is though that we always achieved a low degree of haemolysis, less than 2%. The influence of the degree of haemolysis to the transmitted light has not been investigated.
3.2.3 Oxygen Experiments

3.2.3.1 Performance

Four different oxygen experiments were performed to examine if the light sources emit at the isobestic points.

Measurements were made at 555 nm, 800 nm, 802 nm and 807 nm. According to [19], 800 nm is an isobestic point. Thus, if it is assumed that no other haemoglobin derivatives than HbR and HbO₂ are present in the blood, a change in oxygen saturation will not influence the intensity of transmitted light. If the transmitted light at 800 nm would prove to be dependent on the oxygen saturation, a LED at 802 nm would be tested for the purpose to investigate the oxygen dependence at a slightly longer wavelength. Because of problems in locating components which emits at isobestic points, other wavelengths have been examined. For example, there does not exist a commercial laser diode emitting at 800 nm and no company does to our knowledge manufacture a LED at 548 nm, the isobestic point in the green part of the spectrum. Therefore, it is of interest to find out how much the transmitted light will vary at wavelengths other than the isobestic points, when changes in the oxygen saturation occur.

When circulated in the tubes, the blood was slowly oxygenated by a method described in Chapter 3.1.4. During the oxygenation, the oxygen saturation and the transmitted light was measured. The OSM2 was used as a reference instrument for measuring the oxygen saturation. The Stat Profile 5 also presents an oxygen saturation value. However, this value is calculated, not measured.

In the experiments the oxygen saturation was varied between 70-100%. The saturation limits during most dialysis treatments are of 75-100% according to [35].

3.2.3.2 Results

In Table 6, the results from the four oxygen experiments are shown and for each light source there are values presented for the percentage change in transmitted light during the experiment.

<table>
<thead>
<tr>
<th>Hb (g/l)</th>
<th>LED 800 nm (%)</th>
<th>LED 802 nm (%)</th>
<th>Hospal 800 nm (%)</th>
<th>Laser 807 nm (%)</th>
<th>LED 555 nm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>131</td>
<td>3.5</td>
<td>-</td>
<td>5.2</td>
<td>7.0</td>
<td>50</td>
</tr>
<tr>
<td>124</td>
<td>5.0</td>
<td>-</td>
<td>3.5</td>
<td>4.0</td>
<td>30</td>
</tr>
<tr>
<td>140</td>
<td>-</td>
<td>3.0</td>
<td>5.0</td>
<td>3.0</td>
<td>-</td>
</tr>
<tr>
<td>141</td>
<td>-</td>
<td>3.5</td>
<td>3.0</td>
<td>7.0</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 6. Oxygen saturation variation between 70-100% with percentage changes in transmitted light for the different light sources.
In the Diagram 13 to 16 the oxygen saturation is plotted against the transmitted light for some typical experiments. The diagrams are standardised to easily evaluate the percentage change in transmitted light.

Diagram 13. Transmitted light plotted versus oxygen saturation at a haemoglobin concentration of 141 g/l.

Diagram 14. Transmitted light plotted versus oxygen saturation at a haemoglobin concentration of 141 g/l.

Diagram 15. Transmitted light plotted versus oxygen saturation at a haemoglobin concentration of 131 g/l.
3.2.3.3 Discussion

The expectation was that the transmitted light would not change at 800 nm when the oxygen saturation was varied. Unfortunately, a change in transmitted light was achieved for all light sources. This occurrence might be due to that;

- the isobestic point is not located at exactly 800 nm as we assumed.

- the assumption that the only existing haemoglobin derivatives present in the blood are HbR and HbO₂ is wrong. In human blood there are two other non negligible haemoglobin derivatives present, HbCO and MetHb, see Chapter 2.1.8. Hopefully, the bovines are non-smokers, so the amount of HbCO is practically zero. Normally the amount of MetHb present in the blood is below 1%, a figure which also depends on smoking. MetHb should therefore not considerably influence our measurements.

- the isobestic point is located at 800 nm when using a monochromatic light source, but not when using a broad spectrum LED. This is due to that HbO₂ and HbR are not symmetrical around the vertical axis at the isobestic points, see Diagram 2. Since broad spectrum light sources are used to examine the wavelengths 800 nm and 802 nm, there might have occurred a displacement of the isobestic point toward longer wavelengths [36].

The transmission curves when using LED 800 nm, LED 802 nm and Hospal 800 nm have positive slopes, according to Diagrams 13, 14 and 16. When the oxygen saturation increases an augmentation in transmitted light is achieved at these wavelengths. According to Diagram 2 in Chapter 2.2.1, an increase in transmission, when increasing the oxygen saturation, the wavelength has to be located at shorter wavelengths compared to the isobestic point.
For the LED 800 nm, LED 802 nm and Hospal 800 nm, changes of approximately 3-5% in the transmitted light occurred. When using a LED 802 nm, instead of a LED 800 nm, a slight decrease in the percentage change in the transmitted light could be noticed. This might be due to that 802 nm is closer to the apparent isobestic point than 800 nm. For Hospal this 3-5 percentage change gives rise to an uncertainty in the haemoglobin concentration of 4-8 g/l, when comparing with the Diagram 8 in Chapter 3.2.1.1. The uncertainty is not so large, up to 4-5 g/l, when using the LED 800 nm. This is due to that the calibration diagram for the LED 800 is steeper.

Using the same argument as for the three light sources just mentioned, it was realised that 807 nm, which is the wavelength emitted by the laser diode, is located at a longer wavelength compared to the isobestic point, as the curve in Diagram 15 has a negative slope. When comparing with the Diagram 9 for the laser, the percentage changes in light transmission as the oxygen saturation varies might result in an uncertainty in haemoglobin concentration of up to 10 g/l. This is not acceptable for the measurements and a laser diode closer to the isobestic point is desirable.

For the LED at 555 nm an oxygen saturation curve is achieved which has a steep positive slope. According to Diagram 1 in Chapter 2.1.1, 555 nm has to be located at a longer wavelength than the isobestic point. This supports the theory that an isobestic point is located at around 548 nm. The reason why the slope is so steep, when comparing to the slopes in the near infrared, is that the curves for HbR and Hb O_2 intersects at very large angles here. A small change in oxygen saturation has a large influence on the absorption coefficients. When the oxygen saturation is varied between 70-100%, the percentage change in transmitted light at 555 nm is therefore as large as 30-50%. For a change in oxygen saturation from 95 to 100%, an uncertainty of 30 g/l in haemoglobin concentration is achieved. It is therefore very important that when using a light source in this part of the spectrum it has to emit exactly at an isobestic point.
3.2.4 Sodium Experiments

3.2.4.1 Performance
The purpose with the sodium experiments was to understand to what extent large variations in the sodium concentration during a dialysis session will influence a light transmission measurement. By large variations means here ± 5 mmol/l [35].

To change the sodium concentration in the blood, three different experiments were performed

Experiment 1
The AK 100 was not equipped with a dialyzer in this experiment. A controlled amount of sodium chloride, NaCl, was poured into the blood in order to, in four steps, give a total change of 10 per cent of the sodium concentration, see Diagram 17. The salt was poured into the blood beaker during about half a minute to homogeneously distribute the salt. A blood sample was taken when the step response had reached its stationary value. The Stat profile 5 reference instrument, see Chapter 3.1.5, measured the sodium concentration.

Experiment 2
A set-up including a dialyzer was utilised which allowed us to create a sodium concentration gradient over the filter by changing the adjustment on the AK 100. Thereby it was possible to control the sodium concentration in the blood. This experiment best simulated the reality of the three experiments, since the sodium was already dissolved when diluted in the blood. However, it was very important to keep the blood volume constant and therefore the ultrafiltration rate was the lowest possible. The sodium concentration for the dialysis fluid was changed from 140 to 130 mmol/l on the AK 100. Three blood samples were taken in intervals of approximately 15 minutes. The osmosis process thus had enough time to equalise the sodium concentration on both sides of the dialyzer membrane.

Experiment 3
The dialyzer was used and the sodium concentration was varied between 130 mmol/l and 150 mmol/l in the dialysate but the method of pouring salt into the blood beaker was also employed, see Diagram 18. This experiment was performed as fast as possible in order to keep the remaining blood parameters as constant as possible.

In the Experiment 1 and 2, the measurements were carried out using the Laser 807 nm, the Hospal 800 nm and two other LEDs. The first had a peak wavelength at 802 nm and was placed into an opto holder, see Figure 5 Chapter 3.1.2.2, the other emitted at 801 nm and was glued onto a patented measurement cell, Crit-Line. In the third experiment the Crit-Line measurement cell was replaced by the LED 555 nm set-up, see Figure 4 in Chapter 3.1.2.2.

3.2.4.2 Results
Unfortunately Experiment 2 failed due to that the ultrafiltrational effect had a greater influence on the transmitted light than the osmotic process. The haemoglobin
concentration began at 144 g/l and ended up at a haemoglobin concentration of 152 g/l, referring to the OSM2. Therefore this experiment will not be exhibited here but is although mentioned to give an idea of how an experiment similar to a dialysis treatment might appear. Furthermore, it will later on be discussed how this experiment should be improved to achieve accurate results. To get a comprehension of what the light transmission looks like as a function of time, the first and the third experiment will be displayed below and where the sampling time for Experiment 1 is 10 s and for Experiment 2, 20 s:

Diagram 17. Experiment 1. Light transmission as a function of time. Salt was poured into the blood at sample number 15, 90, 140 and 190.

Diagram 18. Experiment 3. Light transmission as a function of time. Salt was poured into the blood at sample 84 and 158. At sample 210 the sodium concentration for the dialysate was changed from 140 to 150 mmol/l and at sample 254 from 150 to 130 mmol/l.

The diagrams below shows how well the various optical set-ups degraded the effects of the light transmission caused by the sodium changes. The plotting was done with values from the first experiment. Regarding the Laser 807 nm, it showed a much better result in the mixed experiment.
Trendlines were created for the diagrams above in order to find the slopes. It was assumed that the trendlines would be linear. The regression coefficient and the percentage changes of the light transmission due to a change in sodium concentration of 5 mmol/l are scheduled in Table 7.
### Table 7. The percentage change of the light transmission due to a change in sodium concentration of 5 mmol/l.

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1 per cent change</th>
<th>Regression coefficient 1</th>
<th>Experiment 3 per cent change</th>
<th>Regression coefficient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LED 802 nm</td>
<td>2.9 %</td>
<td>0.958</td>
<td>5.1 %</td>
<td>0.8878</td>
</tr>
<tr>
<td>Hospal 800 nm</td>
<td>1.5 %</td>
<td>0.9443</td>
<td>0.9 %</td>
<td>0.9696</td>
</tr>
<tr>
<td>Laser 807 nm</td>
<td>6.4 %</td>
<td>0.975</td>
<td>4.4 %</td>
<td>0.9319</td>
</tr>
<tr>
<td>Crit-Line 801 nm</td>
<td>(not linear)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LED 555 nm</td>
<td>-</td>
<td></td>
<td>9.5 %</td>
<td>0.9249</td>
</tr>
</tbody>
</table>

#### 3.2.4.3 Discussion

Despite all doubts regarding how dissolvable the salt was in the blood, Experiment 1 and 3 seemed to be the most successful, although unexpected effects appeared.

Before starting the experiments, it was not quite clear how changes of the mean cellular volume, MCV, would affect the scattering. It was however known that an increase of sodium in the blood would lead to a MCV decrease due to osmosis, see Chapter 2.3.1. The theory was that a decrease in MCV would imply a reduction of the scattering and thus an augmentation of the transmitted light. Still no references about how the scattering effect would change due to MCV changes have been found. However, this did not come true. In fact the opposite reaction occurred; a reduction of the transmitted light was displayed, see Diagrams 19 to 21.

One theory with enough scientific support was the “mace-effect” [37]. This means that when the blood cells are sensing a very high osmolality outside the cell membrane they will pour out all the contents inside the cells, including the haemoglobin, in order to reach osmolality equilibrium. After that the membrane will break down looking like a club with nails on the surface, a mace, see Figure 9 below. This rough surface will probably increase the scattering. The cell membranes will also stiffen on the surface, which will reduce the transmission of light through the cell walls.

![Figure 9. A red blood cell before and after the mace effect.](image)

The only problem with this theory is that the experiment would probably succeed better if changing the osmolality with the dialysate. However, for the third experiment the same kind of changes occurred in the light transmission when the osmolality was altered in this way.

Two theories can be presented in order to explain this phenomenon [34].

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When fluid is extracted from the blood, the intracellular space will get more dense and the refraction index will increase. This means that energy will be emitted to atoms and molecules when a photon passes the interface between two different media into a more dense medium. Hereby, the increased oscillating of charges will cause new scattering effects added to the other, thus decreasing the transmitted light.

A reduction in the MCV will decrease the Hct value without diminishing the haemoglobin concentration. This results in a reduction in transmission, see Diagram x. Hence, for a constant flow with decreasing Hct value for a constant haemoglobin concentration the transmission is decreased for slow blood flows (<70 ml/min), see Diagram 23, [29].

As further statements a few issues can be drawn

- The Hospal set-up was least sensitive to the variations of the scattering effects due to changes in the sodium concentration.
- For Hospal's equipment, a change of 5 mmol/l resulted in an error of 1.5 % in Experiment 1 and only 0.9 % in Experiment 3. When comparing with Diagram 8 from the ultrafiltration experiments a change of 1.5 % for the light transmission voltage gave an approximate uncertainty in haemoglobin concentration of 3 g/l for a haemoglobin concentration 140 g/l.
- The Laser 807 nm did not have any certain advantageous qualities for reducing errors originating from osmotic changes compared to broad spectrum LEDs.
- The reliability for these experiments is, as seen in the schedule x above, not very good since the variations between Experiment 1 and 3 for each diode are 1-2%.
- If further experiments would be performed in this area, it would be of interest to do the second experiment without the ultrafiltration effect. This would probably be
the case if a large amount of blood was used, e.g. 2.5 litres, and the AK-100 was equipped with a smaller dialyzer. The total effect of this would decrease the ultrafiltration effect 25 times. Moreover, the sodium concentration could be set to 130 mmol/l for the fluid and step-wise be augmented with 5 mmol/l until reaching 150 mmol/l.
3.2.5 Blood Flow Experiments

3.2.5.1 Performance
The purpose of these experiments was to examine how the blood flow influences the light transmission due to the behaviour of the red blood cells.

The blood was oxygenated, warmed up and then circulated in the tubes. In some of the experiments the dialysis filter was replaced with a glass tube to ensure no fluid withdrawal.

The blood flow dependence was examined stepwise from 0 ml/min to 400 ml/min. In some experiments many different blood flows were examined and the experiments lasted almost one hour. In others, the blood flow dependence on the light transmission was only examined for five or six different flows. These experiments did not last more than ten minutes.

To control that no relevant blood parameters had changed during the experiments two different supervisions were performed. First, blood samples were taken from the blood beaker and were analysed in the beginning and in the end of each experiment. Second, the blood flow was, after an increase from 0 to 400 ml/min, decreased in a few steps. Thus, if no blood parameters were changed, the same levels of light transmission would be achieved.

A short and simple test was performed also for turbulent blood flow in the cuvette trying to measure if the light transmission changes when varying the blood flow. The idea was to compress the blood tube to half it’s diameter just before the cuvettes, trying to create turbulence in the blood. This was only performed with the Hospal set-up.

3.2.5.2 Results
In Diagram 24 to 29 below, the relative light transmission due to changes in flow in a few typical experiments is shown. The haemoglobin concentration was 140 g/l.
Diagram 24. For a cuvette with an interior diameter of 4 mm a 28% change in the haemoglobin concentration gives rise to a 30% change in light transmission according to Diagram 7.

Diagram 25. For a cuvette with an interior diameter of 4 mm a 14% change in the haemoglobin concentration gives rise to a 12% change in light transmission according to Diagram 9.

Diagram 26. For the Hospal set-up with a cuvette with an interior diameter of 4 mm a 24% change in the haemoglobin concentration gives rise to a 30% change in light transmission according to Diagram 8.

Diagram 27. Light transmission through the Crit-Line cuvette using an LED at 801 nm.

Diagram 28. For a cuvette with a cross-section of 0.5 mm a 21% change in the haemoglobin concentration gives rise to a 5% change in light transmission, see Diagram 10.

Diagram 29. Two experiments, where in the one represented with circles a compressed blood tube was used to create a turbulent blood flow.
3.2.5.3 Discussion

The results in Diagrams 24 to 29 show how the light transmission is affected by blood flow variations.

At slow, laminar blood flows of 10-30 ml/min, the blood cells have a large total absorption area which results in low light transmission. The increase in light transmission at higher blood flows is probably due to that the red blood cells gather in the middle of the cuvette, thus leaving plasma gaps in the outer edges. The red blood cells are deformed into long ellipsoids, which might also explain the higher light transmission.

In blood flows higher than 150 ml/min, the light transmission is decreased. Because of a beginning turbulence, the red blood cells are more randomised orientated. Thus, the red blood cells have again a larger absorption area.

The degree of turbulence can be calculated by Reynolds number;

\[
Re = \frac{\rho v d}{\eta}
\]

where \(d\) the diameter of the cuvette, \(\rho\) is the density, \(\eta\) the viscosity, and \(v\) the velocity of the blood. A turbulent flow has a Reynolds number of 2000. When using a blood flow of 250 ml/min, as is the case in the experiments, Reynolds number is 500 [38]. The calculations were performed with a blood viscosity of \(\eta=0.0028 \text{ Ns/m}^2\) [39]. This suggests that full turbulence in the blood tubes is not developed.

For the slowest blood flows (<10 ml/min) no increase in light transmission could be noticed due to aggregation of the blood cells which leaves plasma gaps.

Another amazing fact is that the Hospal set-up shows less dependence on the blood flow compared to the laser diode and the LED at 800 nm, which all have similar opto holders. The difference might be due to that the cuvettes used by Hospal is made of PVC instead of glass.

The short wavelength (555 nm) propagating through the cuvette with a rectangular cross section showed changes that are difficult to explain. According to [29] the light transmission in the green part of the spectrum would show a less dependence of the blood flow compared to wavelengths in the infrared. This is due to that the scattering coefficient for green light is small compared to the absorption coefficient and flow changes affect the scattering most. When the flow was decreased after reaching 400 ml/min, it was difficult to achieve the same light transmissions for equal flows. This might be due to the very thin cuvette used. The blood thickness is just 0.5 mm. Disturbances in the blood flow through the cuvette in form of coagulation could be noticed.

Unpredictable results were also noticed for the Crit-Line cuvette. The cuvette was tested in horizontal as well as in a vertical position.
The last experiment performed, see Diagram 29, was an experiment where the blood tube before the Hospal set-up was compressed to create a turbulent flow. This action seems to result in a slightly lower flow dependence.
4. Scattering Simulations

Scattering simulations were made to compare to the experimental results from Chapter 3.2. The Monte Carlo simulation method was chosen to simulate the multiple scattering that occurs in blood.

4.1 Materials and Methods

The fundamental assumption of the Monte Carlo simulation is that the photon is treated as a neutral particle, not as a wave phenomenon. Events that the model simulates are refraction, absorption and scattering.

When starting a simulation, a packet of photons with a certain weight is sent into the sample. The Monte Carlo simulation program allows different layers to be defined with different refractive indices, and in each layer a grid is defined. The sample is divided by the grid into small boxes, and during the simulation the photons are registered in the grid elements corresponding to where in the sample the absorption took place. A principal picture of the scattering is shown in Figure 10.

![Diagram of photon propagation through a scattering media.](image)

**Figure 10.** Photon propagation through a scattering media.

4.1.1 Photon Propagation

When a photon package is sent into the blood sample a part of it is refracted due to different refracting indices according to Figure 11. $n_0$ and $n_1$ are the refracting indices...
The refraction follows the relation:

$$T_r = \frac{(n_0 - n_1)^2}{(n_0 + n_1)^2}$$ \hspace{1cm} (18)

where $n_0$ and $n_1$ are the refracting indices in each layer and $T_r$ is the back scattered fraction.

Figure 11. The refraction of light in a surface.

Inside the sample, the distance the photons travel before any events occur is dependent on the mean free path, $s$, between two particles. Through a random parameter, $\xi$, the step $s_1$ to $s_n$ is calculated and emulates the path length between scattering events. The steps have the mean value $s$.

4.1.2 Photon Absorption

Once the photon package has progressed the step $s_1$, the photon weight must be decreased due to absorption. The fraction is $\xi/(\sigma_s + \sigma_a)$, and is stored in the local grid element. The rest of the package will suffer scattering.

4.1.3 Photon Scattering

In the scattering phase the direction of the photon package is changed and subsequently the deflection angle, $\theta$, and the azimuth angle, $\psi$, must be generated. The probability distribution equation:
well approximate photon scattering in tissue and in this equation $g$ is the anisotropy factor. Rewriting the expression, with the same random parameter, $ξ$, as was used to calculate the step, it is possible to determine the new direction of the photon package. Subsequently a new step can be generated and the iteration is fulfilled.

### 4.1.4 Boundary and Photon Termination

When the photon package reaches the boundary of the sample, it can undergo complete internal reflection or partial reflection and partial transmission. The angles between incident, $θ_i$, transmitted, $θ_t$, and reflected light are given by Snell’s formula

$$n_i \sin θ_i = n_r \sin θ_r$$

(20)

where $n_i$ and $n_r$ are the refracting indices for the sample and the ambient medium. Using the formula

$$R(θ_i) = \frac{1}{2} \left[ \frac{\sin^2(θ_i - θ_r)}{\sin^2(θ_i + θ_r)} + \frac{\tan^2(θ_i - θ_r)}{\tan^2(θ_i + θ_r)} \right]$$

(21)

an average of the reflectans of the two orthogonal directions is determined.\[40\] The random parameter, $ξ$, is finally used to decide whether the photon package is reflected or transmitted.

### 4.1.5 Termination

Termination of the iteration can occur in two ways. Firstly, if the photons are transmitted out of the sample, or secondly, the photon package is insufficiently decreased so that it falls under a threshold value. Whether there is transmission or the weight of the photon package falls under the threshold, all the remaining weight is put in the last cell.
4.2 Performance

4.2.1 Input Parameters
To make a reliable simulation a number of input parameters have to be given for the optical properties and geometrical extensions of the sample. Only simple geometries with large prolongation in two directions compared with the third are easily emulated in the Monte Carlo simulation code used. A geometry like the cuvette used for 555 nm was here simulated with three layers. Two surrounding layers with characteristics of Plexi glass and one in the middle with optical properties similar to blood. In Table 8 below, the characteristics of each layer are represented.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Refracting indices</th>
<th>Thickness/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.65</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1.495</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>1.65</td>
<td>3</td>
</tr>
</tbody>
</table>

*Table 8. Characteristics of the different layers.*

The wave lengths at 550 nm and 800 nm are of interest and their corresponding absorption and scattering coefficients have to be given as input parameters to the program. Also the g-factor has to be given. The specific values for blood with a haemoglobin concentration of 140 g/l, are given in Table 9 below [34].

<table>
<thead>
<tr>
<th>λ/nm</th>
<th>μ/μm/cm</th>
<th>μs/μm/cm</th>
<th>g-factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>550</td>
<td>125</td>
<td>4,8</td>
<td>0,985</td>
</tr>
<tr>
<td>800</td>
<td>1,99</td>
<td>4,8</td>
<td>0,98</td>
</tr>
</tbody>
</table>

*Table 9. The optical absorption and scattering coefficients.*

4.2.2 Simulations
The number of photons simulated is a decisive parameter when discussing the accuracy of the simulation. About 200 000 photons is a normal value but to be confident, simulations with different amounts of photons preferable. In Figure 12 the simulated volume is showed and as the problem is cylindrical symmetrical the shadowed area is of interest. It is in the program represented as a matrix.
4.2.3 Results

The results of the simulations of the two wavelengths can be studied in Figure 13 to Figure 16. These are three-dimensional pictures over the absorption in the blood layer. The horizontal plane is the simulated area with the light source in the left far corner and the detector in the right far corner. In the vertical plane the logarithm of the light intensity is viewed. The depth of the scattering layer was for light at 550 nm 0.5 millimetre, and the simulated radius was 1 millimetre. For 800 nm the corresponding values were for both 5 millimetre.

Figure 13. Light at 800 nm and a haemoglobin concentration of 80 g/l.
Simulations have also been made for various haemoglobin concentrations from 80 to 180 g/l. In Diagram 30 and Diagram 31, transmitted light in the straight forward direction is plotted against the haemoglobin concentration. Both simulated and
experimental data from Chapter 3.2.1, are here plotted in the same diagram. Two exponential functions with second ordered polynoms have been fitted to the data and their coefficients can be studied in Table 10.

![Diagram 30. Simulated and experimental data for 550 nm.](image1)

![Diagram 31. Simulated and experimental data for 800 nm.](image2)

<table>
<thead>
<tr>
<th>Wave length/nm</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>550</td>
<td>1.56\times10^{-5}</td>
<td>8.294\times10^{-1}</td>
<td>1.5202</td>
</tr>
<tr>
<td>800</td>
<td>1.50\times10^{-8}</td>
<td>4.442\times10^{2}</td>
<td>1.5137</td>
</tr>
</tbody>
</table>

*Table 10. Polynomial coefficients for the exponential function fitted to the Monte Carlo simulated data.*

### 4.3 Discussions

Though the sources of errors are not few, the Monte Carlo simulation program is robust and some conclusions from the results can be drawn.

The values of the absorption and scattering coefficients are very uncertain and they are determined for human blood while bovine blood was used in the experiments. In addition incident light of 550 nm was simulated, while in the experiments a LED emitting at 555 nm was used. Yet, when looking at the three dimensional figures in Figure 13 to 16, a simulation of a high haemoglobin concentration absorbs and
scatters more compared to a simulation of low concentration in accordance to the results from experiments. The fact that light at 550 nm seems to be scattered more than light at 800 nm is misleading and is due to different scales in the horizontal plane.

In Diagram 30 and Diagram 31, the curves from the simulations for both wavelengths are more linear than the experimental. When looking at the polynomial coefficients the non-linear $\alpha$-term is one to forty compared to the experimental and the linear $\beta$-term is three times larger than the correspondent experimental coefficients. The bad correspondence between experimental and simulated data, might be due to the uncertain absorption and scattering coefficients. It should also be noticed that the transmitted intensity is much higher for 800 nm than for 550 nm even though the blood layer for 800 nm is ten times thicker.
5. Summary & Conclusions

During a dialysis treatment, solutes are removed from the blood. Due to ultrafiltration, which is performed in order to remove accumulated water in the body, the patient's blood volume will decrease. It is therefore desirable to continuously measure these volume changes. Because of a reciprocal relationship between blood volume and haemoglobin concentration, an optical transmission method is used to measure how dense the blood is. This was performed by shining through four different cuvettes attached to the blood tube during simulated dialysis sessions with different light sources and wavelengths.

Different parameters have been found during the preparative research to influence the light transmission through the blood; the blood flow, the osmolality, the oxygen saturation and the degree of haemolysis. These parameters have been investigated in several experiments, which are described in Chapter 3. Some conclusions from this chapter will be drawn below together with some mechanical aspects.

5.1 Ultrafiltration Experiments

The ultrafiltration experiments imitated a dialysis session and was made to determine how the light transmission changes with the haemoglobin concentration changes. Several experiments were performed to see if there was a good repeatability. Data from different experiments for Hospal 800 nm and Laser 807 nm were very similar, probably because these two had the most rigid set-ups. For LED 800 nm and LED 555 nm data from different experiments could easily be distinguished, see Diagram 3 and 6.

Calibration curves have been fitted to all four opto holders and the chosen functions are exponential expressions with a second ordered polynomial in the exponent. In Chapter 2.2.4 different scattering theories are discussed and all of them suggest exponential expressions but with different exponents. However, both Janssen’s and Andersson and Sekelj’s theories can with truncated Taylor approximation, be expressed with a second ordered polynomial. Even the Monte Carlo simulations showed curvatures similar to the experimental. The fact that the curves are non-linear implicates the need of absolute measurement instead of relative. To compare the different opto holders and decide which one that gives the best resolution, data from a specific test has to be plotted in the corresponding calibration diagram and the maximum deviation from the curves is an indication of the resolution.

5.2 Changes in the Degree of Haemolysis

In several experiments haemolysis tests have been performed in order to examine if the cuvettes, the connections from the tubes to the cuvettes, the roller pump, the utilisation of a dialyzer and a high blood flow increased the degree of haemolysis. Either a small increase in haemolysis or no haemolysis at all was achieved. No conclusions could be drawn about which parameters that might influence the degree
of haemolysis and to what extent. An important conclusion is that a low degree of haemolysis, <2%, was always achieved. No experiment was performed to investigate how the degree of haemolysis influences the transmitted light.

5.3 Changes in Oxygen Saturation

The purpose with the oxygen experiments was to investigate the dependence between light transmission and oxygen saturation. The oxygen saturation was varied between 70-100%. The saturation limits during most dialysis treatments are 75-100%. Blood is, as discussed in Chapter 2.1.8, composed of many different haemoglobin derivatives and the most important are \( \text{HbO}_2 \) and \( \text{HbR} \). For certain wavelengths, two or three of the derivatives have the same absorptivity and these wavelengths are called isobestic points. The two isobestic points investigated here are 548 nm and 800 nm.

The reason for examining 548 nm is that it is the triple isobestic point for \( \text{HbCO} \), \( \text{HbO}_2 \) and \( \text{HbR} \). No company, to our knowledge, manufactures a light source emitting at 548 nm, so a LED at 555 nm was used instead. This was of course too far away from the isobestic point to get accurate results in any of the experiments. The strong oxygen dependence displayed in the experiments is a result of the steep intersection of the two absorption curves in Diagram 1. When using a light source in this part of the spectrum, experiments showed the need to find a light source emitting at the exact location of the isobestic point.

The other isobestic point mentioned above is located at 800 nm. This might be true for a monochromatic light source but for LEDs with bandwidths of 20 nm, as used in the experiments, the oxygen experiments showed that the isobestic point is located above 802 nm. For the LED 800 nm, LED 802 nm and Hospal 800 nm, changes of approximately 3-5% in the transmitted light occurred. For the LED 800 nm, that suppressed changes in oxygen saturation the best, this resulted in an uncertainty in haemoglobin concentration of 4-5 g/l. The LED 802 nm was not examined thoroughly enough to draw any conclusions about the uncertainty in haemoglobin concentration.

A monochromatic light source was also tested. Because there does not exist a commercial laser diode emitting at 800 nm, 807 nm was used. The negative slope in Diagram 15 indicated that the isobestic point is located towards shorter wavelengths. The oxygen saturation changes here resulted in an uncertainty in haemoglobin concentration of up to 10 g/l. This is because 807 nm is located too far away from the isobestic point.

5.4 Changes in Sodium Concentration

According to [27] and [35] during dialysis the sodium gradient between plasma water and dialysate fluid may change up to ±5 mmol/l. Changes in the sodium concentration of the blood was achieved both by dissolving sodium chloride directly by pouring it into the blood beaker and by changing the sodium concentration in the dialysate fluids. Because of the fluctuating reliability of reference instruments, accurate conclusions of how the MCV changes influences the transmitted light can not be drawn.
Since the multiple scattering can be described with a g-factor, an absorption and a scattering coefficient, see Chapter 2.2.4, and the amount of blood cells remains constant, it is believed that the absorption coefficient does not change and the change in transmitted light is due to scattering changes.

An increase in sodium concentration in the blood would lead to a decrease in MCV and the experiments showed that this led to a decrease in the transmitted intensity. This might be due to that the light transmission is decreased for a constant haemoglobin concentration and blood flow and a decreasing Hct value. Another theory is that when fluid is extracted from the cells, the interior of the cell will get more dense and the refraction index will increase. This will increase the scattering to the cell membranes and the transmitted light is decreased.

The idea that a highly reflecting layer on the inside of the aluminium holder to minimise the scattering effects seems to be unnecessary according to the Monte Carlo simulations. In the three-dimensional Figures 13 to 16 in Chapter 4.2.3, the fraction of the light scattered to a radius of 2 mm of the simulated radius of 5 mm is very small. Another point is that the effect, the reflecting layer has, only compensates for scattering in two dimensions, while light scattered in the third dimension, the flow direction, not will be compensated for.

The Hospal opto holder was least sensitive to the variations of the scattering effects due to changes in the sodium concentration. A change of 5 mmol/l resulted in an uncertainty in haemoglobin concentration of 3 g/l for a haemoglobin concentration of 140 g/l. During a dialysis session the sodium changes are small and will cause only small errors which confirms the theory in Chapter 2.3.1.

5.5 Changes in Blood Flow

To determine the transmitted light dependence on the blood flow using the different opto holders, a series of flow experiments were performed where the blood flow was changed between 0-400 ml/min. The red blood cell distribution changes for various flow rates results in changes in the transmitted intensity. During the experiments, the light transmission increased as long as the blood flow was slower than 100-150 ml/min and after that it decreased. This could be an indication of a change in the cellular distribution and a transition from laminar to turbulent flow.

At wavelengths in the green part of the spectrum, the light transmission would, according to Chapter 2.2.2, show a less dependence on the blood flow compared to wavelengths in the infrared. This, however was not the case and unpredictable changes in the light transmission occurred. This might depend on that a very thin cuvette was used.

In one experiment the blood tube was compressed right before one of the cuvettes in order to create a turbulent flow. This resulted in a slightly decreased flow dependence.
The transmitted light through the Hospal cuvette changes the least due to blood flow changes. This might be due to that the cuvettes used by Hospal is made of PVC. A total change of 12% in light transmission gives rise to a 14% change in haemoglobin concentration. To reduce these changes in transmitted light the blood flow has to be measured and compensated for.

5.6 Mechanical Aspects

The opto holders have to be designed and produce to meet the requirements of a precise fit to the cuvette. An accurate fit is important, because a small displacement of the cuvette will cause a significant error in the transmitted light.

The choice of material for the cuvette is also of interest. The benefit with PVC is that it has a refracting index close to the blood, which will minimise the reflection to the surface, and it is good thermal isolator. Glass on the other hand, has better optical properties.
6. Improvements

To guide future researches to make improvements of the results achieved in this thesis, the authors will recommend some interesting work.

- Oxygen experiments with a laser and a LED, both emitting at the isobestic point would give interesting information about if a narrow banded light source is preferred due to oxygen variations.

- Investigations if a highly reflecting film or an absorbing film on the inside of the infrared opto holders would be preferable. This could be done with two identical opto holders, one with reflecting and one with absorbing surface facing the cuvettes. The test should be performed simultaneously during an experiment with scattering changes, e.g. a sodium experiment or change the scattering properties in some other way.

- If further experiments with green light will be performed, it is necessary to find a narrow banded light sources that emits at a wavelength very close to the isobestic point at 548 nm. A more powerful light source is also desirable for detecting more of the transmitted light and for increasing the signal to noise ratio. A higher intensity will result in the possibility to use a thicker cuvette which might decrease the uncertainty of the measurement.

- To investigate any advantages with collimated as compared to diffuse light in the green and infrared spectrum. A diaphragm could be located in front of the detector in order to filter out the direct transmitted light. This might decrease the influence from scattering changes.

- To get rid of, or at least minimise, the influence of the rapid flow changes caused by the roller pump on the AK 100, the sampling of the detectors could be synchronised with the pump. An alternative is to sample very fast and to obtain an average value of the flow. This is of interest only if the haemoglobin detector is located close to or directly after the roller pump.

- Detection of scattered light and light in the straight forward direction simultaneously to see if there is any correlation between them. One could try to detect the scattered light in various angels to the incident beam. By using this light an algorithm to minimise the influence of the scattering changes in the blood could be found.

- Due to the demand of high accuracy on the measurements, the emitted light from the light sources have to be constant or continuously measured. Even if the electrical power to the light sources is kept constant by output power regulation the light conversion coefficient of the diodes might vary. An experiment to investigate if there is any temperature dependence of the diodes could easily be done. An alternative is to use detection of the incident light.

- More accurate scattering and absorption coefficients have to be determined for human as well as for bovine blood to be able to improve the Monte Carlo simulations.

- To perform experiments, comparable to the sodium experiment, for investigation of the scattering, intralipids could be dissolved in the blood to increase the scattering coefficient of the blood.
• It would be desirable to perform the experiments using human blood instead of bovine blood. Many parameters are the same for the two blood types but several parameters e.g. the volume of the red blood cells differs.
7. References


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