The Effects of Acid-Base Parameters, Oxygen and Heparin on the Ability to
Detect Changes in the Blood Status of End-Stage Renal Disease Patients Undergoing
Hemodialysis Using Whole Blood-Based Optical Spectroscopy

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ABSTRACT

Relative changes are detectable in the blood of end-stage renal disease (ESRD) patients during hemodialysis (HD) treatment using optical spectroscopy. However, the potential impacts of several confounding factors that could affect the detection of these changes have not been evaluated. The objectives of this thesis were to: 1) investigate how the variations and/or changes in acid-base and oxygen parameters during HD treatment can affect the optical signature of whole blood of ESRD patients, 2) to investigate the effect of heparin on the optical properties of whole blood and its impact on our method.

Blood samples were drawn from 23 ESRD patients at 5 time points during a 4 hour HD treatment and sent for blood gas and blood spectroscopy analyses. No significant correlations were found between the changes in the blood transmittance spectra and acid-base and oxygen parameters. This indicates that the perturbations in these parameters due to HD procedures do not confound the detection of changes in the blood transmittance spectra of ESRD patients during HD treatment. Additionally, the effect of heparin in modifying the optical properties of whole blood does not confound the detection of changes in the blood of ESRD patients due to HD treatment using whole blood-based optical spectroscopy.

ANOVA revealed significant ($P<0.05$) measurable changes in the blood transmittance spectra of ESRD patients during HD treatment. Significant spectral differences ($P<0.05$) were found between ESRD patients. The lack of uniform spectral characteristics across patients is useful for developing treatment strategies that can potentially meet the need of individual patients.

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LIST OF ABBREVIATIONS

AT   Antithrombin

CKD   Chronic Kidney Disease

CRF   Chronic Renal Failure

COP   Cyclo-olefin polymer

Da   Dalton

EDTA   Ethylenediaminetetraacetic acid

ESRD   End-stage renal disease

GFR   Glomerular filtration rate

HCO_3- Bicarbonate

HD   Hemodialysis

HHb   Deoxy-hemoglobin

HV   High Voltage

IR   Infrared

KDOQI   Kidney disease outcome quality initiative

KRC   Kidney Research Centre

MDRD   Modification of Diet in Renal Disease

MW   Molecular weight

NIR   Near-infrared

NKF   National Kidney Foundation

O_2 Oxygen

OHREB   Ottawa Hospital Research Ethics Board

OLIS   On-Line Instrument Systems

O$_2$Hb   Oxyhemoglobin

PbS   Lead sulphite

PCT   Proximal tubule

PMT   Photomultiplier tube

pO$_2$ Partial pressure of oxygen

pCO$_2$ Partial pressure of carbon dioxide

RBCs   Red blood cells

RRT   Renal replacement therapy

Scr   Serum creatinine

SO$_2$ Oxygen saturation

UFH   Unfractionated heparin

URR   Urea reduction ratio

USRDS   United States renal disease system

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first of its kind in our family! I did not do this; I was simply a tool you utilized and thank you for making me that tool!

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Foremost, this journey has enhanced my view on the value of life and it has awarded me with the tool to humbly face life's challenges with dignity!

1. INTRODUCTION

Chronic Kidney Disease (CKD) is a global health and economic crisis with a prevalence of 7.2% and 30% in people over 30 and 64 years of age, respectively (Zhang, 2008). It was projected that by the end of 2010, 2 million people will develop stage 5 CKD also known as end-stage renal disease (ESRD) worldwide, a condition requiring dialysis or kidney transplantation to sustain patients' lives. Moreover, the average annual cost for CKD therapy (excluding kidney transplantation) was between US $70 and 75 billion worldwide in 2001. The rise in the number of patients with ESRD and the increasing cost of dialysis treatment is a serious global concern that requires immediate attention (Lysaght, 2002).

The majority of ESRD patients rely on hemodialysis (HD) treatment to remove accumulated toxins from the blood stream in order to prolong their long-term survival. However, HD treatment selectively removes some toxins from the blood while others are retained (Vanholder et al., 2003). The lack of the removal of potentially toxic solutes from the blood leaves patients at risk for cardiovascular disease (Parfrey and Foley, 1999; Mann et al., 2000; Haroun et al., 2003), contributing to a mortality rate greater than 20% per year (USRD, 2004). Patient outcome can be improved with adequate HD treatment. The efficacy of dialysis treatment is based on urea clearance, although urea is a surrogate marker and not a blood toxin (Gotch et al., 1974). However, urea clearance during HD treatment has been shown to be an ineffective prognosticator of uremic toxicity and long-term patient outcome. Although there is a clear need, no proven and clinically accepted

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methods currently exist to assess toxins that are more indicative of dialysis adequacy or long-term patient outcome (Depner, 2001).

Accordingly, previous work in the Visual Optics laboratory of The Ottawa Hospital Research Institute has focused on using a novel whole blood-based optical spectroscopy method to avoid the shortcomings of marker detection by correlating the complete spectral properties of whole blood (the optical fingerprint) to disease status. The general concept is that whole blood contains most markers resulting from renal insufficiency, some of which may represent toxic effects and disease progression.   This technique potentially allows the simultaneous measurement of all possible uremic solutes; both toxic and non toxic (even those unknown to us) thus avoiding the difficulties and lack of specificity associated with the detection of a few specific markers. We have previously shown that relative changes can be detected in the state of whole blood of ESRD patients during HD treatment using optical spectroscopy with greater than 95% specificity (Lagali et al., 2006; Lagali et al., 2007). The long-term goal of this is to eventually use this method *in vivo*, as an online system to monitor the progression and the efficacy of HD treatment. However, the potential impact of several confounding factors that could affect the detection of changes in the blood status of ESRD patients during HD treatment has not been evaluated.

This thesis investigates the effect of the perturbations of the acid-base and oxygen parameters due to HD procedures on our ability to monitor changes in ESRD patients during HD treatment via whole blood-based optical spectroscopy. The possible impact of

acid-base (pCO_2, HCO_3^- and pH), and oxygen parameters (SO_2 and pO_2) were quantified as potential confounding factors. Furthermore, the effect of heparin in modifying the optical properties of whole blood as a potential confounding factor was also investigated.

1.1. The Kidney

The kidney is a critical component of the body and it is involved in the regulation of vital homeostatic functions that can broadly be described as excretory, regulatory and endocrine. The nephron, the functional unit of the kidney is comprised of segments that are responsible for controlling the excretory, regulatory and endocrine functions within the kidney. The regulatory functions of the kidney include the regulation of extracellular fluid (ECF) osmolality and volume, electrolyte and acid-base balance (Koeppen et al., 2001). The excretory functions comprise the excretion of metabolic by-products (i.e. urea) and foreign substances (i.e. drugs) from the blood into the urine. The endocrine functions include the production and secretion of important hormones; renin, calcitrol (1, 25-dihydroxyvitamin D3) and erythropoietin. These hormones play an essential role in maintaining blood pressure control, calcium reabsorption by the gastrointestinal tract and deposition in bones, and red blood cell production, respectively (Koeppen et al., 2001). Dysfunctions within the nephron segments can cause severe impairment of kidney function and can lead to kidney failure (Koeppen et al., 2001).

1.1.1. Assessment of Kidney Function

The level of kidney function is conventionally assessed using the glomerular filtration rate (GFR) and it is estimated from serum creatinine concentration (Koeppen et al., 2001). Creatinine clearance is considered to be a good indicator of the level of kidney function (Smith, 1951) because creatinine is not reabsorbed, secreted, or metabolized by the cells of the nephron (Gaspari et al., 1997). Therefore, the amount that appears in the urine equals the amount filtered across the glomerular filtration barrier and it is reported as ml/min/1.73 m^2 (Koeppen et al., 2001). Accordingly, in patients suspected of having CKD, GFR is estimated from serum creatinine concentration based on predetermined equations (Levey et al., 1999).   Currently, the most widely used equations to estimate GFR are the Modification of Diet in Renal Disease (MDRD) study equation and the Cockcroft and Gault equation (Verhave et al., 2003; Cockcroft and Gault, 1976; Kuan et al., 2005). The formula to estimate GFR from the MDRD equation is defined below and contains 4 variables, all of which affect GFR: serum creatinine (Scr), age, gender, and ethnicity (Bailie et al., 2005).

$$\text{GFR (ml/min/1.73 m}^2) = 186 \times (\text{Scr})^{1.154} \times (\text{age in yrs})^{-0.203} \times (0.742 \text{ if female}) \times (1.21 \text{ if African-American.})$$

A typical GFR for a healthy young human adult Caucasian male is 120 ml/min per 1.73 m^2 (normal range being 90–160 ml/min/1.73 m^2) and declines with age (Johnson et al., 2004). An abnormal decrease in GFR is indicative of the onset or progression of kidney disease (Koeppen et al., 2001).   The National Kidney Foundation (NKF) Kidney Disease Outcome Quality Initiative (KDOQI) practice guideline recommends that in

order to reduce the prevalence of CKD through early detection, serum creatinine alone is not sufficient to assess the level of kidney function (GFR) as it is insensitive in detecting the early to mild stages of CKD (NKF KDOQI guideline, 2002).

1.2. Chronic Kidney Disease

CKD is defined as kidney damage for $\geq$ 3 months, characterized by structural or functional abnormalities, with or without decreased GFR (Levey et al., 2003; Daugirdas et al., 2006). CKD is diagnosed by either pathologic abnormalities or markers of kidney damage, such as proteinuria (which indicates the presence of protein in the urine) (Daugirdas et al., 2006). CKD is not often detected until it has progressed too far and it is unrecognized in 80-90% of cases because of the asymptomatic nature of this disease (John et al., 2004). The underlying causes of CKD are multifaceted with evidence suggesting that diabetes is the leading cause worldwide followed by hypertension (Haroun et al., 2003; Perneger et al., 1994).

The characterization of CKD is based on the estimated GFR normalized to body surface area as it is the best measure of the overall kidney function (Smith, 1951; Daugirdas et al., 2006). The NKF-KDOQI guideline suggests classifying CKD from stage 1 (the mildest) to stage 5 (the most severe), where stages 1 to 4 represent the progression of CKD while stage 5 is marked with kidney failure with a GFR below 15 ml/min/1.73m^2 and is also referred to as ESRD (Table I). Patients diagnosed with ESRD have irreversible loss of kidney function (Daugirdas et al., 2006), whereby the kidney can no longer detoxify or eliminate unwanted compounds from the blood stream (Vanholder

and De Smet, 1999; Vanholder et al., 2001). Consequently, both non toxic (uremic retention solutes) and toxic (uremic toxins; capable of affecting body functions) compounds accumulate in the blood stream (Vanholder et al., 2008). Hence, ESRD patients suffer from uremic syndrome; the accumulation of toxic solutes, which has harmful effects on various biological functions within the body including cardio-vascular damage leading to increased morbidity and mortality in CKD patients (Parfrey and Foley.1999; Vanholder et al., 2005; Van Biesen et al., 2007).

In order to reduce the harmful effects caused by the accumulations of uremic toxins, ESRD patients require renal replacement therapy (RRT), kidney transplantation or dialysis (hemo and peritoneal) to sustain their lives (Daugirdas et al., 2006). Kidney transplantation is the ultimate therapy with a markedly lower mortality rate among transplant recipients than dialysis patients (McDonald and Russ, 2002). However, the lack of kidney donors contributes to the limitations of kidney transplantation (Knoll, 2008) and as a result; the majority of ESRD patients rely on hemodialysis treatment to remove accumulated uremic toxins from the blood stream in order to prolong their long-term survival.

Table I: The characterization of chronic kidney disease based on the estimated glomerular filtration rate (GFR) as recommended by the National Kidney Foundation Kidney Disease Outcome Quality Initiative guideline.

Stage	Description	GFR (ml/min/1.73m^2)
1	Kidney damage with normal GFR	$\geq$ 90
2	Kidney damage with mild decline in GFR	60-89
3	Moderate decline in GFR	30-59
4	Severe decrease in GFR	15-29
5	Kidney failure; ESRD	<15

1.3. Hemodialysis Treatment

Hemodialysis (HD) treatment is a blood-based renal replacement therapy that provides fluid management (Convenor et al., 2005), maintains electrolyte and acid-base balance (Power et al., 2009), and removes uremic retention solutes from the blood stream of patients with kidney failure (Daugirdas et al., 2006).

However, there are several dialysis approaches designed for the removal of uremic retention solutes based on molecular weights and/or protein-binding. These solutes are classified into three categories based on the physico-chemical characteristics that affect their removal during HD treatment (Vanholder et al., 2003). The first category of uremic retention solutes is small water-soluble molecules (MW<500 Da) such as urea and creatinine, and these are removable by any of the currently available dialysis approaches. These compounds are selectively removed from the blood into the dialysate via diffusion through a semi-permeable membrane (Daugirdas et al., 2006) as illustrated in figure 1. Although these compounds can easily be removed, they are considered not necessarily toxic (Vanholder et al., 2008; Lesaffer et al., 2000).

The second category of uremic retention solutes is classified as the middle molecular weight molecules (MW >500 Da), which include leptin and β_2-micro-globulin. These molecules can only be removed by high-flux HD strategies employing convection; ultrafiltration mechanisms whereby solutes are dragged out of the plasma along with water molecules (Ledebo, 1998).

The third category of uremic retention solutes is the protein-bound molecules (i.e. phenols and indoles) which can be any molecular weight and are difficult to remove by any of the currently available dialysis strategies (Lesaffer et al., 2000). Many of the compounds in both category 2 and 3 of uremic retention solutes are toxic and these solutes have a vast range of biological impacts (Vanholder et al., 2008; Lesaffe et al., 2000), including cardiovascular damage (Parfrey and Foley.1999; Vanholder et al., 2005; Van Biesen et al., 2007). In order to assess whether these solutes are being removed during hemodialysis, the patient's blood is tested once a month to measure the adequacy of dialysis treatment.

Figure 1. The mechanism of hemodialysis therapy. In hemodialysis, the patient's blood is pumped through the inflow blood line to the dialyzer compartment. In the dialyzer, blood is exposed to a semi-permeable membrane whereby water molecules and low MW solutes are removed from the blood. The dialyzed blood is returned back to the patient's body through the outflow blood line. There are various components that are attached to the inflow and outflow blood lines, and these are used to infuse heparin and saline, monitor blood and dialysate pressures, and detect and trap air.   There is also the dialysate circuit system which makes dialysis solution online from purified water and pumps the solution via a separate compartment of the dialyzer (Daugirdas, et. al., 2006). The image is taken from: *http://cassandracaminow.com/linked%20web%20pages/Dialysis.htm*
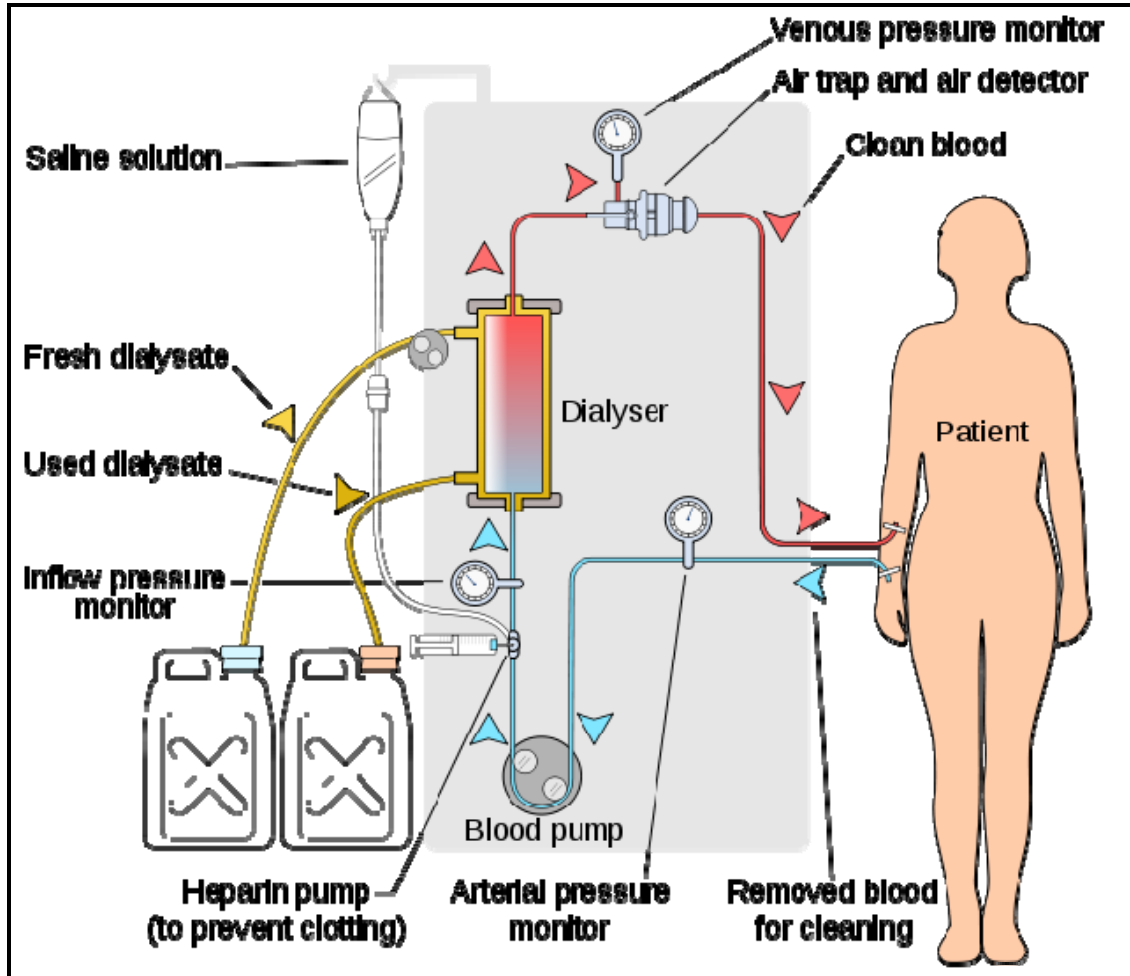
1.3.1. Assessment of the Hemodialysis Treatment Adequacy

Urea was the first marker successfully used to quantify dialysis adequacy (Gotch et al., 1974). Given that toxins responsible for uremic syndrome are not well known, urea clearance is typically used to assess the adequacy of the delivered dose of HD treatment. Urea was chosen as an index of dialysis adequacy since its concentration rises the most in the blood of uremic patients; it has a low molecular weight (60 Da) which facilitates its rapid diffusion between compartments and easy removal during HD treatment and it is easy to measure in the blood and dialysate (Gotch et al., 1974). Therefore, urea clearance is used as a surrogate measure of the removal of other potentially toxic solutes that have accumulated in the blood stream of patients with renal insufficiency (Vanholder et al, 2003). In clinical practice, there are two ways of assessing the adequacy of dialysis as outlined below (Gotch and Sargent, 1985).

1. Fractional clearance of urea, conventionally expressed as Kt/V, measures urea clearance during a single HD treatment normalized to the amount of fluid in a patient's body. $Kt/V = (K_{urea} \times T_d)/V_{urea}$, where K_{urea} is the effective dialyzer urea clearance in milliliters per minute. T_d is the time in minutes for the duration of the treatment, and V_{urea} is the patient's volume of distribution of urea expressed in milliliters (Daugirdas et al., 2006).

2. Urea reduction ratio (URR), evaluates the reduction in urea concentration during HD. URR is calculated from pre- and post-dialysis blood urea nitrogen (BUN) (Daugirdas et al., 2006) as follows: URR percent = preBUN-postBUN/ preBUN x 100.

For ESRD patients, the NKF-KDOQI guideline recommends a minimum of 3 hours per dialysis treatment, 3 times per week and a minimum Kt/V of 1.2 or URR of 65% with thrice weekly treatments. Studies have shown that below these values, there is increased mortality rate. However increasing the Kt/V or URR above these values confers no significant improvement on mortality outcomes (Lowrie et al., 1998; Gotch and Sargent, 1985). Although the HD treatment method prolongs the long-term survival of ESRD patients, it also has many limitations that contribute to health complications independent of kidney disease (Parfrey and Foley.1999; Vanholder et al., 2005).

1.3.2. Limitations of the Hemodialysis Treatment

HD treatment only sustains the lives of patients afflicted with ESRD and it does not restore kidney function. Consequently, there are many disadvantages with the current HD treatment regime. The two major limitations include: 1) the lack of an adequate marker to assess dialysis adequacy or long-term patient outcome, 2) the lack of suitable techniques to identify all uremic retention solutes in order to understand their physico-chemical behaviours to enhance their removal during HD treatment.

Firstly, urea clearance is used as an index of HD treatment adequacy however, it is a marker solute not a uremic toxin and it is only a representative of small water-soluble molecules. However, the ratios of the middle and large molecules as well as protein-bound molecules vary across patients (Vanholder et al., 2008). Therefore, small molecular weight solutes (i.e. urea and creatinine) are poor prognosticators of uremic toxicity and long-term patient outcome on HD treatment. For instance, the effects of

increased urea clearance on mortality or morbidity outcomes in ESRD patients on HD treatment were assessed in a large randomized clinical trial in the HEMO study (Eknoyan et al., 2002). This study concluded that increased urea clearance had no significant influence on either mortality or morbidity outcomes. Still, clinical prognosticators of long-term outcome such as cytokines and albumin (Daugirdas et al., 2006) have not been combined to form a good mortality index and only represent a partial picture of the complex uremic state in CKD patients.

Secondly, most of the small-water soluble solutes that are not necessarily toxic (i.e. urea) are removable by any of the available dialysis approaches (Vanholder et al., 2008). Meanwhile it is difficult to remove potentially toxic solutes; middle, large and protein-bound molecules (Ledebo, I. 1998; Lesaffer et al., 2000) that contribute to the vast range of biological impacts such as cardiovascular disease, the leading cause of death in CKD patients (Parfrey and Foley.1999; Vanholder et al., 2008; Van Biesen et al., 2007). In order to address these issues, the European Uremic Toxin Work Group was established in 2000 to classify individual toxins based on clinical importance. Unfortunately, there were no techniques that could identify all retained uremic toxins and no methods were readily available to remove uremic toxins that had already been identified. Additionally, no specific uremic toxin that could accurately describe disease state and HD treatment efficacy was found amongst the known uremic retention solutes because of the complex uremic state in CKD patients (Vanholder et al., 2003).

Numerous potentially toxic blood solutes (known as uremic toxins) are retained during the ESRD state. However, these solutes are just starting to be identified (116 uremic solutes have been identified) and their roles are not well understood (Vanholder et al., 2003). Therefore, the detection of a specific uremic marker or toxin is limited by the specificity between these solutes and disease status. Furthermore, only solutes for which a prior knowledge exists can be monitored, resulting in an incomplete picture of disease status. Moreover, no clinical method exists that can simultaneously assess all uremic retention solutes in order to identify the ones that are more indicative of HD treatment adequacy and long-term patient outcome.

1.4. A Novel Tool for Assessing Hemodialysis Treatment Adequacy and Long-Term Patient Outcome

As discussed above, no single component of whole blood provides a good prediction of HD treatment adequacy or long-term patient outcome. The ability to monitor multiple or all uremic retention solutes could significantly improve our ability to assess HD treatment adequacy and disease status.

Accordingly, whole blood-based optical spectroscopy has been proposed as a non-invasive health-monitoring tool in ESRD patients on HD treatment. Whole blood-based optical spectroscopy is an *in vitro*, non-invasive optical probing method (details in next section) that potentially allows the measurement of total changes in whole blood by means of the interaction of all uremic retention solutes (both toxic and non toxic) and red blood cells (RBCs) with light. This allows us to obtain an optical signature of a patient's

whole blood without attempting to isolate individual molecular components. The optical signature (the optical fingerprint) can then be correlated to disease status or HD treatment efficacy as required.

This approach differs significantly from earlier studies which successfully used optical spectroscopy for the detection of specific analytes in spent dialysate rather than uremic retention solutes in the blood of patients with renal insufficiency (Jensen et al., 2004; Olesberg et al., 2004). Until the work of Lagali and colleagues, none have attempted to monitor whole blood fingerprinting to encompass all markers of renal insufficiency simultaneously (Lagali et al., 2006; Lagali et al., 2007).

1.4.1. The Basic Principle of Optical Spectroscopy

Spectroscopy is a technique that utilizes the light energy (electromagnetic radiation usually quantified by its wavelength) dependent interaction with a sample to probe matter. The output is a wavelength dependent signal displayed as a spectrum (Jones, 2000).

Biological substances, as all matter, can interact with light energy and produce a variety of optical responses; reflection, remission, refraction and absorption (Jones, 2000). Each of these responses is wavelength dependent and one could chose to monitor any one. In biological tissue, the two most effective tools are usually the absorption and remission (usually referred to as scatter) of light which can probe molecular bonds and cellular structures (as is broadly used in chemistry). Our discussion will be limited to

absorption as it is the tool used for the purpose of this thesis. Based on these complex interactions between the different components of whole blood and light, and the changes in the blood composition of ESRD patients under dialysis treatment, it should be possible to monitor and quantify HD treatment progression using spectroscopy of whole blood.

The basic principle of optical spectroscopy as used in our experiments is illustrated in figure 2. Broadband radiant energy (white light) is first split into separate wavelengths of light with a monochromator. Then using a slit, light of a specific wavelength is isolated and then directed onto the sample (in this case whole blood) which is usually contained inside a cuvette of path length (l). The light then interacts with the sample and optical radiation detectors (and necessary optics) are positioned to capture light after it interacts with the sample (Jones, 2000). After obtaining the response for a given wavelength, another wavelength is automatically selected and the measurement is repeated. This procedure is repeated until a sample response is obtained for each of the wavelengths to be investigated, thus producing a wavelength dependent response, the spectrum of the interaction with the sample. Figure 2 shows two optical paths in the spectrometer, a reference path and a path for interaction with the sample. The reference path is used to measure the amount of light that is actually incident on the sample in order to be able to quantify the strength of the interaction with the sample. This is required since all optical sources fluctuate in time (1-10%) and measuring their intensity at the time of the experiments is required for high accuracy, and because the performance of optical sources changes over long periods of time due to filament evaporation, coating of the glass enclosure and other mechanisms beyond the scope of this thesis.

Figure 2. The basic principle of optical spectroscopy. Spectroscopy requires an energy source (white light in this case), a monochromator (capable of scanning the range emitted by the source) and a detector. The arrows represent the light path as it travels from the source and it is split into separate wavelengths by a monocromator. It then interacts with the sample and signals are transmitted to the detector then displayed as a spectrum. *Modified from Jones, 2000*

Energy source (White light)　Monochromator　Incident light (Io)　Sample　Transmitted light (I_1)　Detector　Spectrum

Reference path

www.manaraa.com
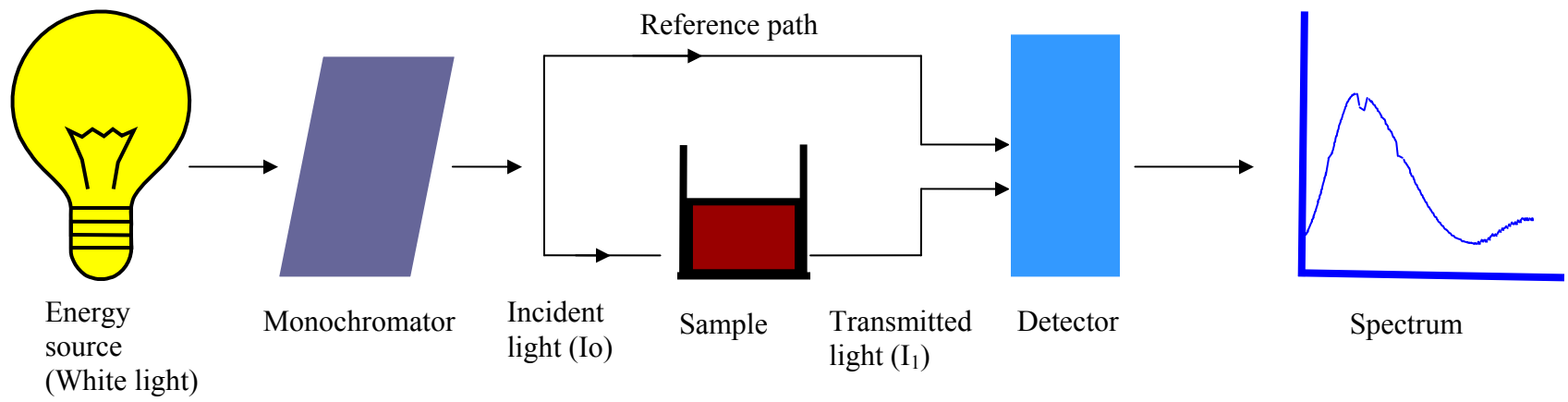
1.5. Previous Application of Whole Blood-Based Optical Spectroscopy in Investigating the Blood Status of ESRD Patients during Hemodialysis Treatment

The feasibility of the whole blood-based optical spectroscopy method has been successfully demonstrated in detecting relative changes in whole blood of ESRD patients during HD treatment in two pilot studies (Lagali et al., 2006; Lagali et al., 2007).

The first pilot study set out to investigate whether changes in the state of whole blood as a result of HD treatment were detectable using optical spectroscopy. Transmission through and backscatter from the blood samples (pre- and post-dialysis) of 8 ESRD patients on HD treatment was measured for wavelengths 500 nm to 1700 nm (<500nm, no transparency and >1700nm, spectrum is dominated by water). The result showed that HD treatment induced significant, measurable changes in whole blood spectra observed in a diverse cross-section of patients, and that spectral change had a characteristic shape with variation in amplitude across patients. This result is critical since it outlined the potential for quantifying patient-specific HD-induced spectral information that can be used to design treatment strategies to meet the need of individual patients.

In the second pilot study, the feasibility of optical spectroscopy was further investigated by comparing the optical spectra of whole blood of ERSD patients during HD treatment and healthy subjects. The goal of the study was to determine if it was possible based on the optical fingerprint alone to differentiate between the

blood of an ESRD patient before and after HD treatment and that of a healthy

subject. A total of 20 subjects (10 ESRD and 10 healthy) were recruited for this

study and measurements were carried out in the same way as the first pilot study.

Using a linear discriminant analysis of the blood spectra, the authors were able to

classify the 3 groups (pre-dialysis blood, post-dialysis blood and blood samples

from healthy subjects) with a specificity of 95% accuracy, as illustrated in figure 3.

Figure 3. Mean absorption spectra of whole blood samples from ESRD patients on HD treatment and healthy subjects. Mean spectra are shown for healthy subjects (green line), ESRD pre-dialysis (black line) and ESRD post-dialysis (red line). Arrows indicate the eight wavelength regions with significant absorption differences between pre- and post-dialysis and between ESRD patients and healthy subjects. *Modified from Lagali et al., 2007*
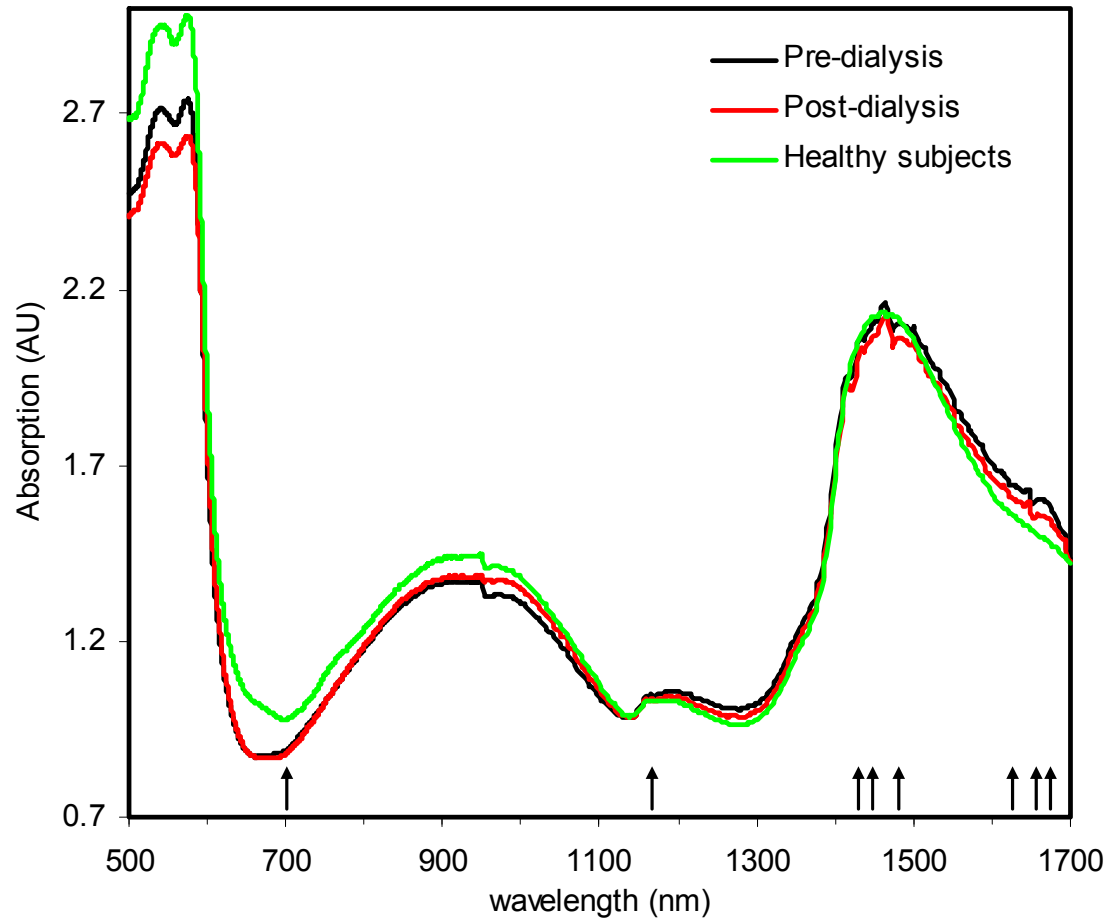
1.6. Validation of the Whole Blood-Based Optical Spectroscopy by Investigating the Effects of Potential Confounding Factors

Although Lagali and colleagues have successfully demonstrated that whole blood-based optical spectroscopy can detect measurable differences in the blood of ESRD patients during HD treatment, it is still unclear what is being measured. Therefore, we need to better understand whether we are reproducing standard clinical measures or providing novel clinical information indicative of HD treatment adequacy and long-term patient outcome.   Consequently, the possible impact of potential confounding factors such as medications (i.e. heparin), medical conditions (i.e. diabetes), and blood chemistry changes (i.e. acid-base and oxygen parameters) during HD treatment needs to be addressed.

The purposes of this thesis are: A) to investigate how the variations in acid-base and oxygen parameters during HD treatment can potentially affect the optical signature of whole blood of ESRD patients, and how this might impact our ability to monitor the health status of ESRD patients using this novel technique; and B) to investigate the effect of heparin in modifying the optical properties of whole blood of ESRD patients on HD treatment. The next few sections explain the basis of the changes in the acid-base and oxygen parameters and the role of heparin during HD treatment.

1.6.1. The Effect of Hemodialysis Procedures on Acid-Base and Oxygen Parameters

1.6.1.1. Acid-Base Parameters; HCO$_3$-, pCO$_2$ and pH as Potential Confounding Factors

In ESRD patients, acid-base balance is regulated by the HD procedures whereby metabolic acidosis due to an excess acidity in the blood is corrected. Metabolic acidosis is an acid-base imbalance characterized by a decrease in serum bicarbonate (HCO$_3$-) level. It is predominant in the majority of ESRD patients undergoing HD treatment; even though some patients are in acid balance (Gennari, 1985). More than one third of these patients have pre-dialysis serum HCO$_3$- levels below 22 mEq/L (Uribarri et al., 1999). In both ESRD patients and healthy individuals, systemic pH is dependent on the ratio of pCO$_2$ to HCO$_3$-. Nevertheless, the maintenance of acid-base balance between these two groups differs. In healthy individuals, there is a homeostatic feedback loop system that allows the kidney to maintain acid-base balance by excreting acids and regenerating HCO$_3$-, which predominantly occurs in the proximal tubule of the nephron. However, in ESRD patients, acid-base balance is regulated by the HD procedures whereby endogenous acid is buffered by HCO$_3$- in the dialysate during HD treatment (Gennari, 2000). As HCO$_3$- continuously diffuses into the blood stream, it buffers endogenous acids and this causes an abrupt increase in pH. Acid-base balance is achieved when the rate of HCO$_3$- addition equals the rate of endogenous acid production; equilibrium is established between these two rates by the end of a single HD treatment (Gennari, 1985). Normally, this physiological process takes days to reach completion within the kidney (Koeppen et al., 2001), however it takes a short time (on average 4

hours) to occur in ESRD patients during HD treatment, and this exerts tremendous fluctuations in the blood chemistry. Consequently, the perturbations in the blood chemistry cause variations in Acid-base parameters: pCO_2, HCO_3-, pH and oxygen parameters: pO_2 (details in section 1.6.1.2) and SO_2 (details in section 1.6.1.3) during HD treatment.

Understanding that acid-base events during HD treatment cause fluctuations in blood chemistry and thus acid-base and oxygen parameters, our goal is to ensure that we are not merely measuring these variations and are providing novel clinical information on HD efficacy and long-term patient outcome.

1.6.1.2. The Effect of the Hemodialysis Procedures on the Partial Pressure of Oxygen as a Potential Confounding Factor

As mentioned in the previous section, there are variations in the partial pressure of oxygen (pO_2) during HD treatment that can potentially affect the optical signature of whole blood being measured using optical spectroscopy. Hypoxemia, defined as a decrease in pO_2 level in the blood below 60 mmHg, affects the affinity of hemoglobin for oxygen and contributes to changes in blood chemistry during HD treatment.   The occurrence of hypoxemia during HD treatment was first reported in the early 1970's; whereby a decrease in pO_2 and an increase in pH were reported during HD treatment (Johnson et al., 1970). Studies have since reported a decrease in pO_2 with both acetate and bicarbonate-HD (Feriani, 1998; Nielsen et al., 1995). However, studies showed that during HD, pO_2 decreases by 10-20% with acetate

dialysate. This decline does not affect oxygen saturation (SO_2) of hemoglobin (see next section) due to Bohr's effect (Ahmad et al., 1982). This occurs via an increase in pH which shifts the oxyhemoglobin dissociation curve to the left rendering hemoglobin with increased affinity and oxygen is less available to tissues as illustrated in figure 4.

Although the occurrence of hypoxemia has been reported, the causes are not well known with certainty but have been postulated. The proposed mechanisms causing hypoxemia during HD treatment include a rapid correction of chronic metabolic acidosis which subsequently disrupts tissue oxygenation by counteracting Bohr's effect, resulting in a decreased pO_2 level (Barnert et al., 1988).

Irrespective of the cause, fluctuations in pO_2 during HD treatment contribute to changes in the blood chemistry and the oxygen saturation of hemoglobin that could potentially affect blood spectral signals detected using whole blood-based optical spectroscopy. In order to improve this novel method, the effect of the fluctuations in pO_2 during HD treatment on the optical signature of whole blood was investigated.

Figure 4. The oxyhemoglobin dissociation curve describes the blood oxygen carrying capacity which is dependent on the relationship between oxygen saturation (SO_2) of hemoglobin molecules and partial pressure of oxygen (pO_2). It also shows the various factors that affect this relationship such as 2, 3-diphosphoglycerate (2, 3 DPG), carbon monoxide (CO), temperature, and pH. The image was obtained from: *(http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=cm&part=A1479)*
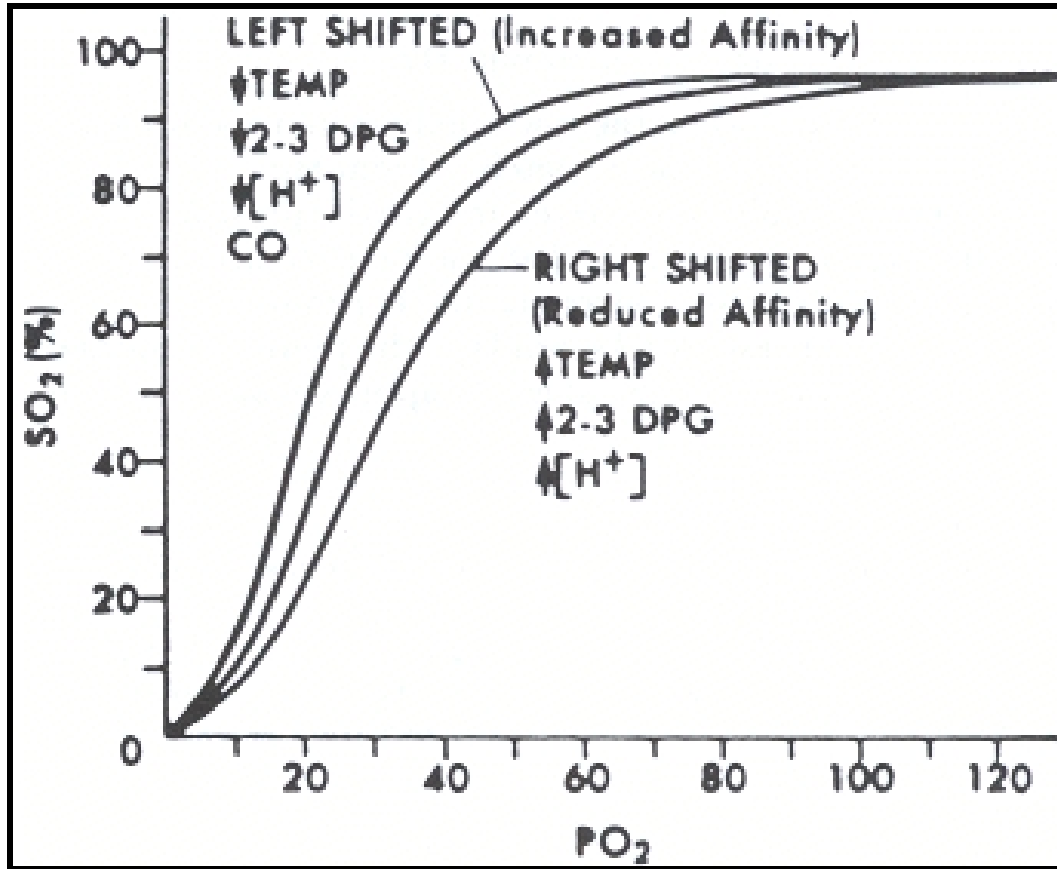
1.6.1.3. The Variations in Oxygen Saturation as Potential Confounding Factors

Oxygen saturation (SO_2) is another factor being investigated as a potential confounder on whole blood-based optical spectroscopy method for two reasons. Firstly, the process of correcting metabolic acidosis during HD treatment affects the ability and capacity of blood to carry oxygen to tissues, which is primarily dependent on the relationship between SO_2 and pO_2 in the blood (Randall et al., 1997). Hemoglobin affinity for oxygen is also dependent on other factors such as pH, temperature, carbon dioxide, and 2, 3-diphosphoglycerate (Egger et al., 1973), figure 4. During HD treatment, there is an increased affinity of hemoglobin for oxygen. This increased affinity is greatly enhanced in the post-dialysis period due to an extensive rise in pH (Egger et al., 1973); causing variations in SO_2.

Secondly, hemoglobin is a strong light absorbing protein found inside red blood cells (Frieble and Meinke, 2005; MacRae et al., 1961). The absorption spectra of human whole blood in the visible (VIS) and near-infrared (NIR) region below 1000 nm (Meinke et al., 2007) are predominantly due to light absorption by hemoglobin molecules. The two derivatives of hemoglobin; oxyhemoglobin and deoxyhemoglobin interact with light in very discrete manners and thus produce unique optical absorption spectra (Prahl, 1999), figure 5. However, the absorption of light by hemoglobin molecules changes with the number of oxygen molecules that occupy its binding sites in a hemoglobin mixture.

Therefore, any small change in the number of oxygen molecules can result in significant spectral change. Therefore, the spectral response of whole blood to light in the visible and NIR region of the spectrum is dominated by SO_2 and the detection of other analytes in the blood is confounded by it (Meinke et al, 2007). Hence, it is important to ensure that our method is providing novel information on HD status independent of variations and dominance of SO_2. In order to achieve this goal, analysis outcomes of SO_2 measurements obtained from blood gas results and whole blood oximetry are correlated to blood transmittance spectra obtained at 5 testing time points during HD treatment.

Figure 5. The extinction coefficients of deoxyhemoglobin (HHb) and oxyhemoglobin (O_2Hb) of human whole blood. Hemoglobin derivatives dominate the absorption spectra of whole blood in the visible (VIS) and near-infrared (NIR) wavelengths region below 1000 nm (Prahl, 1999).
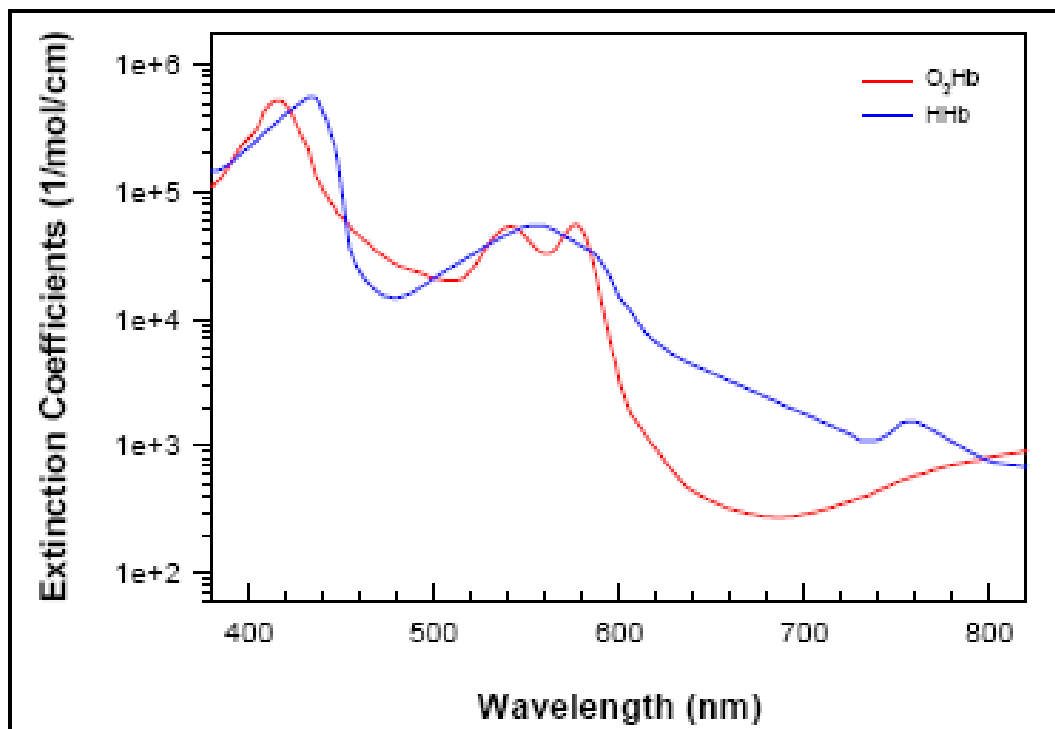
1.6.1.3.1. Oxygen Saturation Measurement using Whole Blood Oximetry

Oxygen saturation (SO_2) is the parameter that is used clinically as a health indicator and it is the value usually referred to regarding oxygen measurements (oximetry), in a clinical setting (Moyle, 1998). Oxygen (O_2) is transported through the blood stream to living tissues by hemoglobin molecules contained inside red blood cells (Randall et al., 1997). A hemoglobin molecule has four O_2 binding sites and the total fraction of binding sites occupied by O_2 molecules in a hemoglobin mixture is referred to as the oxygen saturation which is usually calculated as:

$$SO_2 = \frac{O_2Hb}{O_2Hb + HHb}$$

Oximetry is a method used to determine the oxygen content of blood and it is a standard clinical tool used to monitor the health status of patients (Moyle, 1998).   The working principle of oximetry is dependent on the different extinction coefficients of de-oxyhemoglobin (HHb) and oxyhemoglobin (O_2Hb) molecules (figure 5), and the Beer-Lambert law. The Beer-Lambert law states that the absorbance is directly proportional to the concentration of the absorber in solution and assumes that the intensity of light transmitted through the sample depends only on light absorption and no scattering. It is usually expressed in short form as:

$$A(\lambda) = \varepsilon(\lambda) \cdot l \cdot c$$

Where $\varepsilon(\lambda)$ *is* the molar extinction coefficient, l is the path length of light through the sample (optical path) and c is the concentration of the substance.

In the clinical laboratory, oxygen saturation is measured using the CO-oximeter (considered a gold standard), whereby a blood sample is haemolysed using ultrasound to form a hemoglobin solution and the Beer-Lambert law is applied to it. The absorbance of hemoglobin solution is measured at multiple wavelengths and the measured absorbance spectrum is expressed as a linear combination of the extinction coefficients of HHb and O_2Hb to determine SO_2 as well as acid-base parameters such as pCO_2 (Moyle, 1998).

The same working principle of oximetry is applied to determine oxygen content in whole blood. The advantage of whole blood oximetry is that results are readily available at the point of care and it provides continuous monitoring in urgent care situations such as surgery. However, in whole blood oximetry, there are a number of challenges that need to be addressed since whole blood is not a homogenous mixture. Firstly, in whole blood, hemoglobin molecules are located inside red blood cells (RBCs) that are known to scatter light flatten the absorption peaks and change the absorbance (Caicai et al., 1998), causing deviations from the Beer-Lambert law. Secondly, RBCs are not the only light absorbing chromophores present in whole blood (i.e. platelets); however, the absorbance due to other substances in whole blood is negligible (Meinke et al., 2007). Furthermore, it has been shown that the optical density (O.D.) of whole blood is not proportional to the blood layer thickness or the concentration of hemoglobin (MacRae et al., 1961) and that light scattering increases with increased whole blood layer thickness (Drabkin, 1946; Fine, 1993). Nevertheless, previous work in our laboratory demonstrated the

feasibility of whole blood oximetry whereby it was determined that the shapes of the absorbance spectra were dependent on optical path thicknesses (unpublished data). Furthermore, whole blood oximetry was obtained with an accuracy of 3% using a multiple linear regression approach between 515 and 595 nm with measurements performed using optical path thickness of 100 μm or less. Thus oximetry is used to calculate SO_2 from whole blood samples of ESRD patients at 5 testing points during HD treatment. This extra step is necessary since blood samples used for spectroscopy measurements are manipulated differently (exposure to air causes the sample to be highly saturated with oxygen) from samples used for blood gas analysis. Therefore, comparing SO_2 values from a fully saturated blood sample to variance in a fully saturated blood spectroscopy measures is essential to eliminate bias.

1.6.2. The Effect of Heparin in Modifying the Optical Properties of Whole Blood as a Potential Confounding Factor

Heparin is the final potential confounding factor being addressed in this thesis. During HD treatment, the patient's blood is exposed to several artificial surfaces and air, and this may initiate blood clotting in the extracorporeal circuit and cause catastrophic episodes leading to death in some cases (Hirsh et al., 2001). The solution is to provide most patients with intravenous anticoagulant during HD treatment. Heparin is the most commonly used anticoagulant during intermittent HD treatment to prevent thrombogenesis in the extracorporeal circuit (Daugirdas et al., 2006). However the inhibition of coagulation might have an impact on the

optical properties of blood and thus impact the ability of whole blood-based optical spectroscopy to monitor HD efficacy and long-term patient outcome.

Unfractionated heparin (UFH) is the anticoagulant of choice in most HD centers with a molecular weight that ranges between 3000 to 30000 Da (Hirsh et al., 2001). Heparin is a sulfated polysaccharide, consisting of d-glucosamine and d-glucuronic acid (Park and Chakrabarti, 1977), as shown in figure 6 (Quader et al, 1999). The sulfate groups on glucosamines are central for the anticoagulant activity of heparin (Desai, 2000).

The carboxyl group on the d-glucuronic acid group of heparin is the light absorbing component of the molecule and contributes to its optical properties. However, the optical properties of heparin vary with pH due to the acid-base status of the carboxyl group (Park and Chakrabarti, 1977). Studies pertaining to the optical properties of heparin have been extensively performed in the ultra violet (UV) and mid infrared (IR) wavelength regions (Park and Chakrabarti, 1977; Sushko et al., 1993; Grant et al., 1991); however these regions are outside the VIS and NIR regions (400-1760nm) used to perform measurements in this thesis.

For our measurements, the blood samples collected at pre-dialysis contain no heparin. However, samples obtained after patients are already on HD treatment have heparin. Therefore, it is important to ensure that differences in blood spectra

are not due to the effect of heparin in modifying the optical properties of blood and

we are reporting real change as a result of HD treatment.

Figure 6. The structure of heparin with repeated units of sulfated d-glucosamine and d-glucuronic acid. The carboxyl group on the d-glucuronic acid group in heparin is the light absorbing component of the molecule and contributes to its optical properties (Quader et al, 1999).

Repeat unit of heparin
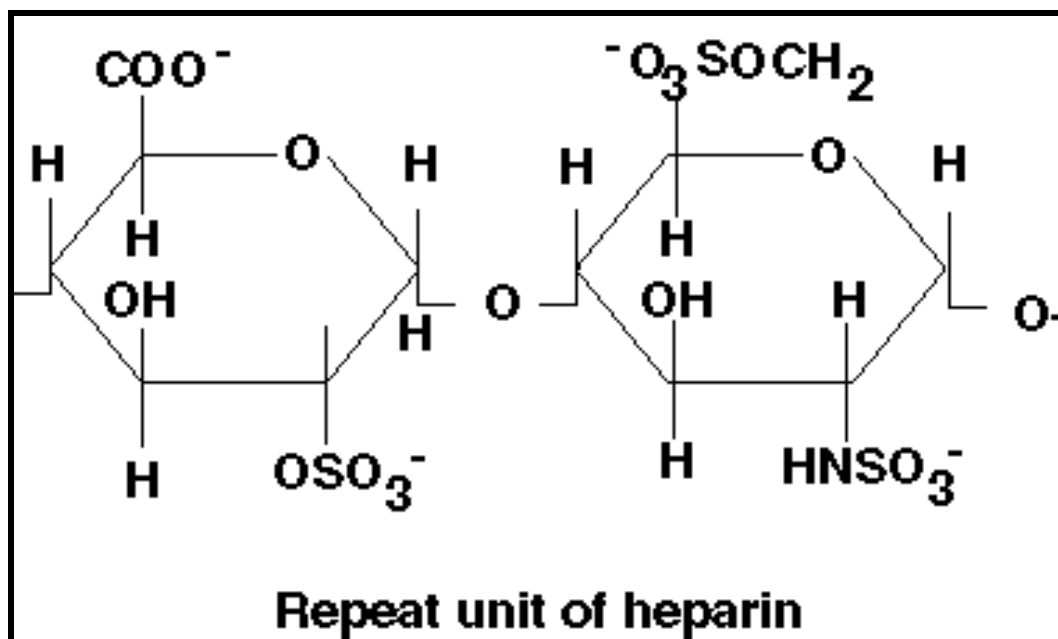
1.7. Project Rationale and Overview

ESRD is a state whereby there is irreversible loss of kidney function resulting in accumulations of toxic solutes, causing substantial morbidity and mortality (Parfrey and Foley.1999; Vanholder et al., 2005; Van Biesen et al., 2007). ESRD patients require HD treatment to remove accumulated toxins from the blood stream in order to prolong their long-term survival. However, HD treatment selectively removes some toxins from the blood while others are retained (Vanholder et al., 2003). Conventionally, urea is used as an index of dialysis treatment adequacy but unfortunately, urea is only a surrogate marker (Gotch et al., 1974). Additionally, urea removal during HD treatment only represents the removal of small MW substances and not large MW or protein-bound compounds (Vanholder et al., 2003). The lack of the removal of potentially toxic solutes from the blood and inadequate assessment of HD treatment efficacy leave patients at risk for cardiovascular disease, contributing to a mortality rate greater than 20% annually (USRD, 2004). Moreover, no adequate clinical methods exist to measure toxins more indicative of HD treatment adequacy or long-term patient outcome (Depner, 2001).

Lagali and colleagues have previously proposed to use whole blood-based optical spectroscopy to avoid the shortcomings of marker detection by correlating the complete spectral properties of whole blood (the optical fingerprint) to disease status. It has been shown that optical spectroscopy method can detect significant measurable changes in the blood of ESRD patients during HD treatment (Lagali et

al., 2006; Lagali et al., 2007). However, it is unclear what is being detected in the blood status of ESRD patients during HD treatment and therefore it is important to ensure that the optical spectroscopy method is providing novel clinical information on HD status independent of confounding factors. Therefore, understanding how perturbations in the acid-base and oxygen parameters during HD treatment can potentially affect optical signature of whole blood is warranted. Furthermore, it is important to investigate the effect of heparin in modifying the optical properties of whole blood and its impact on the optical signature of blood.

1.8. Objectives

1. The main objective of this thesis was to investigate the effect of perturbation of acid-base and oxygen parameters due to HD procedures on the ability to monitor changes in ESRD patients during HD treatment via whole blood-based optical spectroscopy. The specific goals to meet this objective were:
   a) To quantify the possible impact of acid-base parameters; pCO_2, HCO_3^- and pH as potential confounding factors by correlating blood gas results obtained at each HD time point to blood spectra measured using 1000 μm and 100 μm optical path thicknesses.
   b) To quantify the possible impact of oxygen parameters; SO_2 and pO_2, as potential confounding factors by correlating SO_2 (blood gas lab) obtained at 5 HD time points to blood spectra measured using 1000 μm and 100μm optical path thicknesses.
   c) To calculate whole blood SO_2 values using the Beer-Lambert absorbance

model at each HD time point, and correlated to blood spectra measured in 1000 μm and 100 μm optical path thicknesses.

2. The second objective was to determine the effect of heparin in modifying the optical properties of whole blood as a potential confounding factor on the ability to monitor changes in ESRD patients during HD via whole blood-based optical spectroscopy.

1.9. Hypotheses

Experiments were designed to address 4 specific working hypotheses to meet the goals stated in the section above:

1) Acid-base parameters; pCO_2, HCO_3- and pH will not impact the ability to detect significant measurable changes in the blood of ESRD patients due to HD treatment via whole blood-based optical spectroscopy.

2) Oxygen parameters; SO_2 and pO_2 will not impact our ability to detect significant measurable changes in the blood of ESRD patients during HD treatment, via whole blood-based optical spectroscopy.

3) Whole blood SO_2 will not impact our ability to detect significant changes in the blood of ESRD patients during HD treatment via whole blood-based optical spectroscopy.

4) The effect of heparin in modifying the optical properties of whole blood will not impact and confound the ability to use whole blood-based optical spectroscopy to monitor the health status of ESRD patients during HD treatment.

2. MATERIALS AND METHODS

2.1. Patient Recruitment and Selection

The inclusion criteria for the protocols discussed in the two pilot studies in this thesis consisted of end-stage renal disease (ESRD) patients on hemodialysis (HD) treatment at the Ottawa Hospital, General Campus and dialyzed 3 times per week, 4 hours per treatment. The exclusion criteria consisted of patients on peritoneal dialysis, patients with acute renal failure, patients with active hepatitis B or C, or any other active infections and patients who were not able to voluntarily give an informed consent as listed in Table II. Patients were recruited into this study with assistance from the Kidney Research Centre (KRC) clinical trials research coordinators from a population of ESRD patients on HD (Hemodialysis Unit of the Ottawa Hospital, General Campus). All patients provided an informed written consent as per Ottawa Hospital Research Ethics Board (OHREB) requirements following an information session with each patient. A total of 23 patients were recruited for the two pilot studies discussed in this thesis: 18 patients were recruited for the investigation of the effect of perturbations of acid-base and oxygen parameters due to HD procedures on whole blood-based optical spectroscopy method. The other 5 ESRD patients were recruited for the investigation of the effect of heparin in modifying the optical properties of whole blood as a potential confounding factor.

Table II: End-stage renal disease (ESRD) patient selection criteria for the investigation of the effect of perturbations of acid-base and oxygen parameters due to hemodialysis procedures on whole blood-based optical spectroscopy in the first pilot study discussed in this thesis

Inclusion criteria	Rationale
ESRD patients on HD	The focus of our research
Patients treated at the General Campus	Logistics issues
Exclusion criteria	**Rationale**
Peritoneal dialysis patients	None HD patients
Acute renal failure patients	On HD for a short time
Hepatitis B or C	Risk of infecting staff
Other active infections	Risk of infecting staff
Patients who can not provide an informed consent	Against Research Ethics regulation

2.2. Blood Sample Collection and Preparation

The blood collection tubes and blood gas syringes were labelled by a research coordinator using randomly assigned codes and patient information was withheld from all laboratory staff. Labelled blood tubes and syringes were subsequently delivered to the hemodialysis unit prior to the day of the study.

Blood samples were drawn by a dialysis nurse from a pre-existing vascular access on the scheduled HD treatment day. Both patient populations whose vascular access were central venous catheters (CVC) and arteriovenous fistulae (AVF) were included. CVC is an access line created by inserting a catheter into a large vein whereas an AVF is constructed by surgically joining an artery to an adjacent vein, re-directing blood flow from artery to vein (Daugirdas et al., 2006). A CVC line provides venous blood with low oxygen saturation (below 70 %) while an AVF provides mixed-arterial blood with a level of oxygen saturation greater than 90 % (Daugirdas et al., 2006). Mixed-arterial blood samples were drawn via the AVF from 14 patients and venous blood samples were drawn via the CVC from 9 patients.

2.2.1. The Effect of Perturbations of Acid-Base and Oxygen Parameters due to Hemodialysis Procedures on Whole Blood-Based Optical Spectroscopy

For each patient, a total of 15 tubes of blood were drawn by a dialysis nurse from a pre-existing vascular access right before heparinization, 1 hour-intradialysis, 2 hour-intradialysis, 3 hour-intradialysis and immediately post-dialysis.

At each HD time point, approximately 3 ml of blood was drawn into a plasma separator tube (PST) (Becton, Dickinson and Company, New Jersey, USA). Samples were picked up at each HD time point from the hemodialysis unit by a member of the Visual Optics laboratory and delivered to the Biochemistry laboratory (Ottawa Hospital, General Campus) for regular assessment of blood urea nitrogen, as an index of dialysis treatment adequacy.

Approximately 1.5 ml of blood was drawn into a blood gas syringe containing 7.00 units of lithium heparin (anticoagulant) (Vital Signs Colorado Inc, Colorado, USA). Samples were put on ice slurry and delivered immediately to the blood gas laboratory (Ottawa Hospital, General Campus) for measuring acid-base (pCO_2, HCO_3-, and pH) and oxygen (SO_2 and pO_2) parameters.

Approximately 3 ml of blood was drawn into a tube containing ethylenediaminetetraacetic acid (5.4g of K_2-EDTA) (Becton, Dickinson and Company, New Jersey, USA), used as anticoagulant. Samples were put on ice slurry and taken directly to the Visual Optics laboratory at the University of Ottawa

Eye Institute (located within the Ottawa Hospital, General Campus) for whole blood optical spectroscopy and oximetry (a method for determining oxygen content in the blood) measurements.

Upon delivery to the Visual Optics laboratory, the blood sample in the EDTA tube was mixed continuously on a rotator for approximately 5 minutes to maintain homogenous mixture of red blood cells and serum. The sample was prepared for measurement in two optical cells (cuvettes) with 1000 μm and 100 μm optical path thicknesses. The cuvette with 100 μm optical path thickness was used for oximetry calculation since it has a higher light transmission that is compatible with the Beer-Lambert law. However, the cuvette with 1000 μm optical path thickness has a lower light transmission and its spectral output is a mixture of both forward scatter and transmittance; it is suitable for measuring relative changes. Additionally, the differences in light transmission between these two optical path thicknesses may reveal a different set of features in the blood spectra that could be useful in monitoring the health status of ESRD patients on HD.

Initially, sample preparation for measurement using the optical cell with 1000 μm optical path thickness was performed by removing the top of the tube to allow access to the blood sample. Subsequently, three draws of 100 μl of blood were taken from the tube, and transferred using gel loading pipette tips (AdvanTach, DiaMed Lab Supplies Inc., Mississauga, ON) to an optical cell (Schott

B270, Germany). The optical cell was closed with a Teflon lid and sealed with parafilm to secure it.

The optical cell with the 100 μm path thickness was used for blood spectroscopy and whole blood oximetry measurements.   Oxygen saturation (SO_2) was determined on whole blood because optical spectroscopy measurement was also performed on whole blood samples.   However, the SO_2 values obtained from CO-oximetry measurement from the blood gas laboratory were performed on hemoglobin solutions. Therefore the comparison of blood samples that were equally manipulated was essential to fully elucidate the potential effect of SO_2 as a confounding factor on whole blood-based optical spectroscopy method.

As illustrated in figure 7, a whole blood sample of 0.2 ml was transferred from the tube using a 1.00 ml syringe (Becton, Dickinson and Company, New Jersey, USA) with a needle attached.   Then the needle from the syringe was inserted through the filter (for aerosol inhibition) of a 20 μL pipette tip (AdvanTach, DiaMed Lab Supplies In., Mississauga, ON).   The pipette tip was filled with blood by pressing on the syringe and the sample at the tip was discarded first. Then the sample was transferred into an optical cell with 100 μm optical path thickness (made from UV transparent cyclo-olefin polymer (COP) films, SpecVette, Aline, Inc., Redondo Beach, CA) by pressing on the syringe. The pipette tip at the end of the needle facilitated coupling to the optical cell to facilitate blood filling. All blood samples were measured at room temperature within 1 hour of sample extraction from patients.

Figure 7. A schematic illustration of blood sample transfer to an optical cell with 100 μm optical path thickness. 1) A blood sample is transferred from the tube using a syringe with a needle attached. 2) Then the needle from the syringe is inserted through the filter (acts as an aerosol inhibitor) of the pipette tip. 3) The sample is transferred into the optical cell.
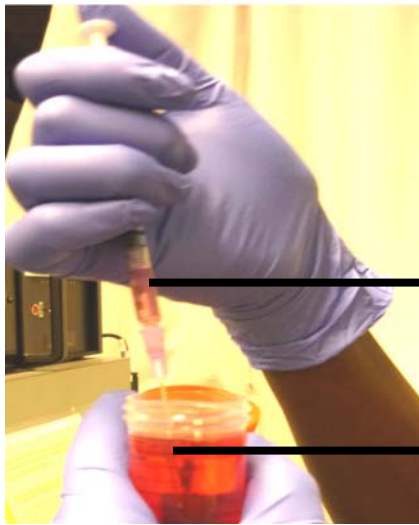
Syringe

Tube containing blood sample
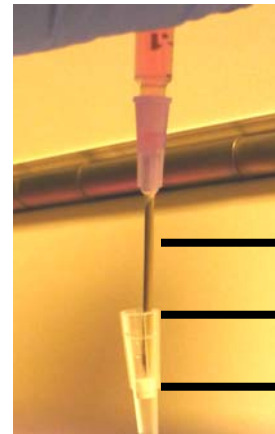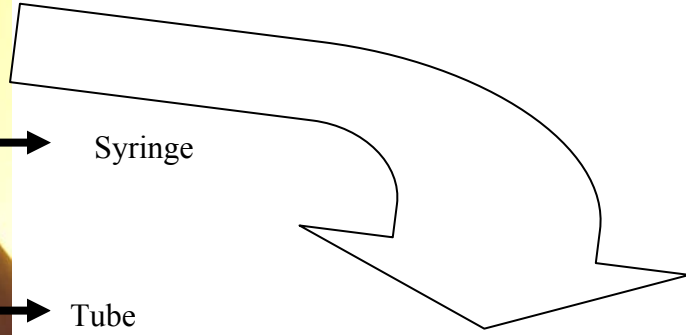
Needle

Pipette tip

Filter
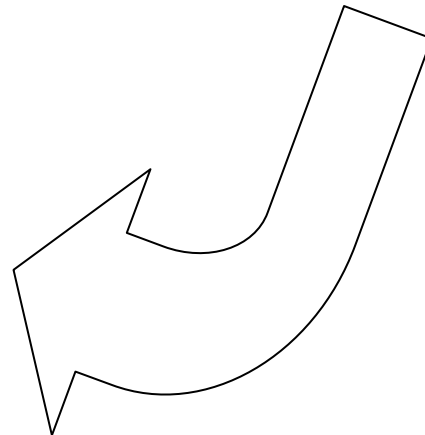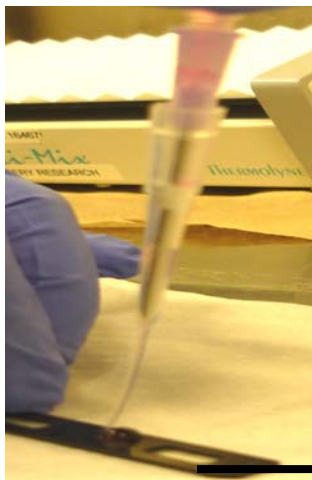
An optical cell with 100 μm optical path thickness

2.2.2. The Effect of Heparin on Whole Blood

A blood sample of approximately 3 ml was drawn by a dialysis nurse from a pre-existing vascular access right before heparinization into an EDTA tube from 5 ESRD patients. Blood samples were placed on ice slurry and immediately picked up from the hemodialysis unit and taken directly to the Visual Optics laboratory for whole blood optical spectroscopy measurements.

Upon delivery to the Visual Optics laboratory, the blood sample in the EDTA tube was mixed continuously on a rotator to maintain a homogeneous distribution of red blood cells (RBC) and serum before mixing with heparin and testing. Our aim was to investigate the effect of heparin in modifying the optical properties of whole blood as a potential confounding factor. We chose four different concentrations of heparin, starting with a known concentration of 5.3×10^{-3} units/µL of heparin; a standard dosage administered to patients during HD treatment (Brunet et al., 2008). For each patient, a blood sample of approximately 3 ml was obtained prior to HD treatment and divided into 4 sub-samples of 500 µL and each sample was mixed with 2.7 µL of the different concentration of sodium heparin (Pharmaceutical Partners of Canada Inc., Richmond Hill, ON Canada). The final concentrations of heparin in the four blood samples were 5.3×10^{-3} units/µL, 1.3×10^{-3} units/µL, 6.6×10^{-4} units/µL and 5.3×10^{-4} units/µL.   A range of concentrations was chosen to determine the concentration-dependent effect of heparin in modifying the optical properties of whole blood.

Subsequently, three draws of 100 μl of the blood sample with heparin were transferred to an optical cell of 1000 μm path length (Schott B270, Germany). The optical cell was closed with a Teflon lid and additionally sealed with parafilm to secure it. As a negative control, blood was diluted with 2.7 μL of deionized water in order to assess whether heparin affects the optical properties of blood or it simply dilutes the sample. A blood sample without heparin or water was also measured as a positive control. All blood samples were measured at room temperature within 3 hours of extraction from patients as previous works in our laboratory have demonstrated stable spectral measurements of up to 8 hours.

2.3. Biochemical and Blood Gas Measurements

Protocols were obtained from the Analytical Procedure Manual (Pathology and Laboratory Medicine, Division of Biochemistry, the Ottawa Hospital) and measurements were performed by the blood gas and biochemistry laboratory personnel.

2.3.1. Blood Urea Nitrogen (BUN)

Upon receipt by the biochemistry laboratory personnel, tubes containing blood samples were centrifuged at room temperature and the serum was used for serum urea nitrogen concentration determination. Sample urea measurements were determined using the bichromatic rate reaction on the Dimension Vista instrument (Siemens, Burlington, ON, Canada).

2.3.2. Acid-Base and Oxygen Parameters

Upon receipt by the blood gas laboratory personnel, samples were kept on ice slurry and analyzed immediately.   Syringes containing samples were mixed thoroughly and checked for clots and excess bubbles (air >10 %). Blood samples were analyzed by the blood gas analyzer (GEM Premier 4000 analyzer, Instrumentation Laboratory, Warrington, United Kingdom) and provided values for pH, pCO_2, pO_2, HCO_3^-, SO_2 and electrolytes.

2.4. Optical Spectroscopy

2.4.1. The OLIS Spectrometer

The OLIS spectrometer (On-Line Instrument Systems Inc, Bogart, Georgia, USA) as depicted in figure 8, has two chambers; sample and reference chamber. The Chopper, (a mirror) alternates and sends light into either the sample or reference chamber. The sample chamber contains an integrating sphere and two optical cell holders (entrance and exit port), where the optical cells can be placed for sampling. Only the exit port was used for our experiments. In the OLIS system, 3 signals are measured and referred to as chamber signal, chamber reference and sphere (measures backscatter; reflected signal from the sample).

2.4.1.1. Types of Signals in the OLIS Spectrometer

A) Chamber Signal (T)

This represents the amount of light transmitted through the sample chamber and it is a measure of the amount of light (I_1) that passed through the sample as depicted in figure 9.

B) Chamber Reference (T_{Ref})

This represents the amount of light going through the reference chamber and it is a measure of the amount incident light (Io) on the sample as depicted in figure 9. It is used as a reference for T and the light path is kept empty during experiments unless study design changes (see section 2.4.2.2).

C) Sphere (B)

This represents the backscatter signal (the amount of light reflected back from the sample) of the sample measured from the integrating sphere. These measurements were not used in the analysis presented in this thesis.

2.4.1.2. Types of Measurements in the OLIS Spectrometer

There are three different measurements acquired during an experiment that are necessary for spectral data processing as illustrated in figure 10.

A) *Empty Chamber (E)*

The empty chamber (E) measurement is used to compare T_E and T_{Ref} without anything placed in the sample chamber. This allows us to know the actual amount of incident light (Io) on the sample (illustrated in figure 9) from the measurement of the amount of light going through the reference chamber. If the signals are not equal (as is assumed in the transmission calculation) this will result in errors in transmission calculations. Figure 11 illustrates typical signals for the OLIS spectrometer and shows clearly that T_E and T_{Ref} are not equal and also their differences are wavelength dependent. The correction factor is the ratio of the two signals and will be called the beam intensity correction factor (CF).

B) Empty Cuvette (C)

An empty cuvette (optical cell) is placed into the holder of the sample chamber and it allows for the correction of the transmission properties of the optical cell during spectral data processing as illustrated in figure 10.

C) Sample (S)

This measures the signal due to the blood sample and the cuvette. The effect due to cuvette is compensated for during spectral data analysis.

2.4.1.3. The OLIS Detectors and Settings

As illustrated in figure 8, the OLIS spectrometer is setup to measure 2 wavelength ranges: Visible (VIS) and near infrared (NIR). VIS signals are detected with photomultiplier tubes (PMT) detectors; one detector is in the sample chamber whereas the other one is in the sphere chamber. The PMT detector in the sample chamber measures the chamber signal (T) and chamber reference (T_{Ref}) signal while a second detector in the sphere chamber measures the backscatter (BS) signal in VIS wavelength. The sensitivity of the PMT detector is controlled by adjusting applied high voltage (HV).

The NIR signals are detected with lead sulfide (PbS) detectors; one detector is in the sample chamber whereas the other one is in the sphere chamber. The PbS detector in the sample chamber measures the T and T_{Ref} signals in the NIR wavelength while a second PbS detector in the sphere chamber measures the backscatter (BS) signal in the NIR wavelength. There is no direct way of controlling the sensitivity of the PbS detectors. However; the slit width of the monochromator can be adjusted. The slit width of the monochromator controls the light intensity and bandwidth, which is the ultimate control over signal strength in the NIR wavelength.

Figure 8. The overview of the OLIS spectrometer with four components. 1) Prism and grating monochromator (splits white light into different wavelengths) and the slit selects the wavelength of light directed to the sample. 2) Chopper, a mirror that alternates sending light into either the sample or reference chamber. 3) Chambers; reference and sample chamber. The sample chamber contains an integrating sphere which was not relevant to our measurements and two cell holders (entrance and exit port), where the optical cells can be placed for sampling, only the exit port was used for our experiments. 4) Detectors; 2 Photomultiplier tubes (PMT), visible and UV detectors (250 to ~900nm) and 2 Lead sulphite (PbS), NIR detector (up to 2500nm). The PMT detector in the sample chamber measures the chamber signal (T) and chamber reference (T_{Ref}) signal while a second detector in the sphere chamber measures the backscatter (BS) signal in VIS wavelength. The PbS detector in the sample chamber measures the chamber signal (T) and chamber reference (T_{Ref}) signal in the NIR wavelength while a second PbS detector in the sphere chamber measures the backscatter (BS) signal in the NIR wavelength.

58
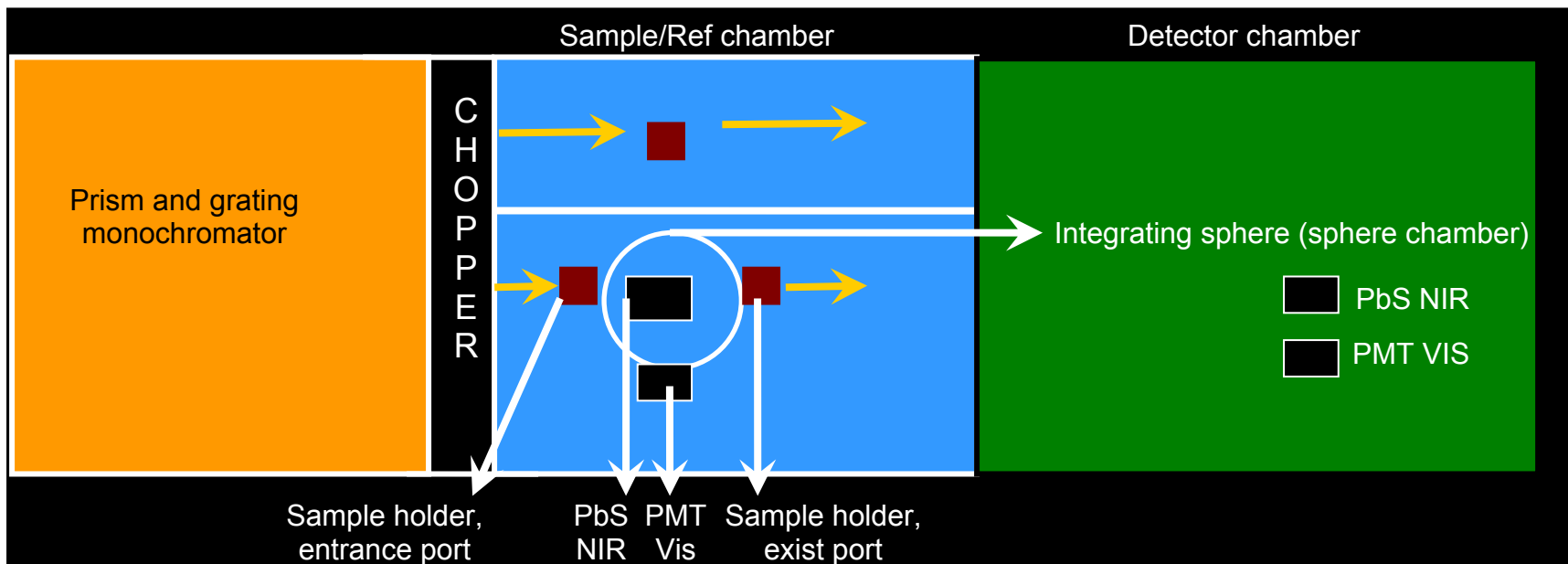
2.4.2. Whole Blood-Based Spectroscopy Measurements and Procedures

Two types of optical cells were used for measuring whole blood spectra: 1000 μm and 100 μm optical path thicknesses. The optical cell with 1000 μm optical path thickness was used for blood spectroscopy measurements whereas the 100 μm optical path thickness was used for both spectroscopy and whole blood oximetry measurements.

2.4.2.1. Whole Blood Spectroscopy Measurements with the Optical Cell of 1000μm Thickness

Empty chamber measurements were performed for at least 20 minutes to allow the lamp to warm up to obtain stable measurements (Appendix V illustrates intra-sample variability of 0.01%). Subsequently, three types of measurements (empty chamber, empty cuvette and blood sample) were performed using the same parameters in the VIS and NIR wavelength ranges. Spectral data were acquired separately using a computer controlled program in the VIS and NIR wavelength ranges because the visible detector (PMT) is more sensitive and requires little light with a slit width of only 0.5 mm. However, the NIR detector (PbS) requires more light with a slit width of 1.0 mm.

The optical cells containing blood samples were placed in the sample holder at exit port of the spectrometer and illuminated with light from a 150W Halogen lamp. Samples were properly mixed in between measurements in the VIS and NIR wavelength ranges. Optical cells were removed from the sample holder of the

spectrometer, blood samples were agitated, and then placed back into the sample holder, and approximately 45 seconds was given for the sample to re-equilibrate before spectral data acquisition commenced.

Blood spectral data were obtained for the 18 ESRD patients from 375 nm to 780 nm and from 780 nm to 1755 nm, in the VIS and NIR wavelength ranges respectively, using OLIS Optics spectrometer (On-Line Instrument Systems Inc, Bogart, Georgia, USA). Acquisition times ranged from 1 to 3 minutes in the VIS wavelength and 4 to 6 minutes in the NIR wavelength. After the completion of data acquisition, the instrument produced three different signals: chamber signal (T), chamber reference (T_{Ref}), and sphere (B; backscatter) for each type of measurement acquired. Each sample was automatically measured four times and the average spectrum was reported and used for spectral data processing described in figure 10. The transmittance due to blood was calculated by dividing the normalized transmitted signal of the blood sample by the normalized transmitted signal of an empty cuvette (optical cell). Normalization was performed by dividing spectral signals at each wavelength by the area under the entire curve.

2.4.2.2. Whole Blood Oximetry Measurements with the Optical Cell of 100μm Thickness

The optical cells containing blood samples from 8 ESRD patients were inserted into an adapter and then placed into the sample holder at the exit port of the spectrometer and illuminated with light from a 150W Halogen lamp. Concurrently, an optical cell filled with de-ionized water was inserted into an adapter and then

placed into the sample holder in the reference chamber. This was performed to optimize the match of the reflections from the optical cell surfaces between the sample and the reference, water having an index of refraction closer to blood than air.   Spectra were acquired using a computer controlled program while the sample remained undisturbed from 490 nm to 810 nm. A slit width of 0.5 mm was used and acquisition times ranged from 1 to 3 minutes. Each sample was automatically measured four times and the average spectrum was reported and used for spectral data processing (illustrated in figure 10). The transmittance due to blood was calculated by dividing the normalized transmitted signal (T_S) of the blood sample by the normalized transmitted reference signal (T_{Ref}) of an optical cell filled with de-ionized water.   The spectral data were compensated for beam intensity difference (correction factor) between reference and sample path as outlined in appendix VII. The final equation for calculating blood transmittance for oximetry measurement is:

$$\left[\frac{[I_S(\lambda) * T_{COP\ film} * T_S] + [I_S(\lambda)]}{[IR(\lambda) * T_{COP\ film} * T_{deioned\ water}]} \right] * CF \quad [1]$$

Where I_S = Intensity of incident light in the sample chamber

$T_{COP\ film}$ = Transmitted signal of COP film

T_S = Transmitted signal of measured sample

I_R = Intensity of incident light in the reference path

$T_{de\text{-}ionized\ water}$ = Transmitted signal of de-ionized water

CF = Beam intensity correction factor.

2.4.2.3. Whole Blood Spectroscopy Measurements of Pre-dialysis Blood Samples Spiked with Heparin using the Cuvette with 1000 μm Optical Path Thickness

Measurements were followed as in section 2.4.2.1 with a few exceptions explained below. Spectral data were obtained on the four final concentrations of heparin in the blood samples of 5.3×10^{-3} units/μL, 1.3×10^{-3} units/μL, 6.6×10^{-4} units/μL and 5.3×10^{-4} units/μL from 400 nm to 800 nm in the VIS and from 760 to 1760 nm in the NIR wavelength ranges. Spectroscopy measurements were also performed on blood samples (positive control) and blood samples diluted with water (negative control).

2.5. Data Analyses

2.5.1. The Effect of Hemodialysis Procedures on Acid-Base and Oxygen Parameters

2.5.1.1. Spectral Data Processing

The blood transmittance calculations were the same for spectral data obtained in both 1000 μm and 100 μm optical path thicknesses. Refer to figure 10 of the flow chart illustrating spectral data processing.

2.5.1.1.1. Transmittance Correction Factor

In the OLIS system, the reference chamber (T_{Ref}) was used to measure the intensity of incident light; Io (refer to figure 9) on the sample; however, T_{Ref} and T_E are not equal. Therefore the differences in the light intensities between T_E and

T_Ref were compensated for, by using a beam intensity correction factor (CF) measuring the ratio of light intensities between the two chambers as:

$CF = T_E/T_{Ref}$ [2]

Where CF = Correction factor

T_E = Transmitted signal of the empty chamber

T_{Ref} = Transmitted signal used as a reference for T_E

2.5.1.1.2. Transmittance Calculations

A) Empty Cuvette (Optical cell)

The transmittance was used to compensate for the transmission properties of the optical cell during spectral data processing as illustrated in figure 10.

The transmittance (T_C) of light through an empty cuvette was calculated as:

Cuvette Signal= $T_C = I_S (λ) * T_C$ [3]

Reference signal = $T_{Ref} = I_r (λ)$ [4]

Where I_S = Intensity of incident light in the sample chamber

I_r = Intensity of incident light in the reference chamber

T_C = Transmitted light through the cuvette

T_{Ref} = Transmitted signal used as a reference for T_C

To get the transmittance due to an empty cuvette, equation 3 is divided by equation 4 as follows:

Transmittance $_{Empty\ Cuvette}$ = $T_C = Is(λ) * Tc / Ir (λ)$ [5]

Transmittance $_{Empty\ Cuvette}$ = T_C [6], transmittance due to empty cuvette only.

B) Blood Sample

The transmittance (I_1); the fraction of incident light that passes through the blood sample taking into account the fraction of light that passes through the cuvette was calculated as:

Sample Signal = T_S = I_S (λ) *T_C * T_S [7]

Reference signal = T_{Ref} = I_r (λ) [8]

Where I = Intensity of incident light in the sample chamber.

I_r = Intensity of incident light in the reference chamber

T_C = Transmitted light through the cuvette

T_S = Transmitted light through the blood sample

T_{Ref} = Transmitted signal used as a reference for T_S

To get the transmittance due to the blood sample in the cuvette, equation 7 is divided by equation 8 as follows:

Transmittance = I_S(λ) * T_C * T_S $\Big/$ I_r (λ) = T_C *T_S [9]

To get the transmittance due to blood, equation 9 is divided by equation 6 as follows: *Transmittance $_{sample}$ = T_S = [T_C * T_S/T_C]* [10], the transmittance due to the blood sample only.

Figure 9. The light path as it travels through an optical cell (cuvette). The incident light on the sample (I_0) is measured in the OLIS system with an empty chamber (T_{Ref}). Transmitted light ($I_1 = T$) is the light intensity after interaction with the sample with concentration, c and an optical cell of path length l. The Beer-Lambert law states that the transmitted light through a substance, is logarithmically dependent on c, absorption coefficient; a and l. Image was taken from: (*http://en.wikipedia.org/wiki/File:Beer_lambert.png*).

c, α

I_0

I_1

l
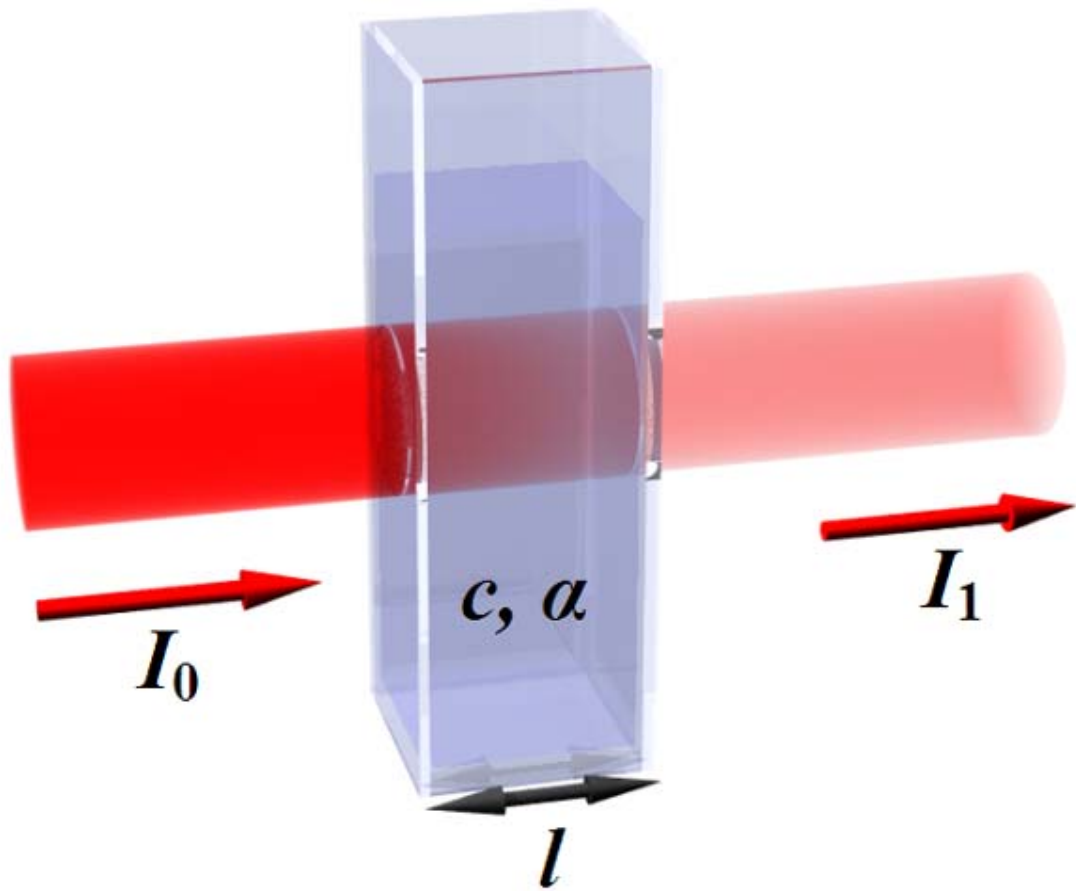
2.5.1.2. Whole Blood Oximetry using the Beer-Lambert Absorbance Model

From equation 10, the absorbance (A) of the blood sample was calculated as

follows: A = -log (T$_S$) = $A(\lambda) = \varepsilon(\lambda) \cdot l \cdot c$ [11]

Where T$_S$ is the transmittance of blood which is associated with the properties of

the blood through $\varepsilon(\lambda)$ its molar extinction coefficient, l the path length of light

through the sample (optical path) and c the concentration of the substance. This is

commonly referred to as the Beer-Lambert law (Moyle, 1998), for a single light

absorbing solution. When multiple light absorbing substances are present in a

mixture, the Beer-Lambert law is expressed as a sum of the contribution of n known

substances of concentrations c_n, $A(\lambda) = \sum_n \varepsilon_n(\lambda) \cdot c_n \cdot l$ [12]

For blood, the measured absorbance spectrum is expressed as a linear combination

of the measured extinction coefficients of HHb and O$_2$Hb (Prahl, 1999) at each

wavelength using a multiple linear regression approach over the wavelength range

between 515 nm and 595 nm. The optical properties of O$_2$Hb and HHb are distinct

in these regions and it had previously been applied to quantify oxygen saturation

(Diaconnu, 2009).

The result of the fit is: $A_{mes}(\lambda) = a \cdot \varepsilon_{O_2Hb}(\lambda) + b \cdot \varepsilon_{HHb}(\lambda) + K$ [13]

Where $A_{mes}(\lambda)$ is the measured absorbance of whole blood, $\varepsilon_{O_2Hb}(\lambda)$ are the O$_2$Hb

extinction coefficients, $\varepsilon_{HHb}(\lambda)$ are the HHb extinction coefficients, a and b are the

fitting parameters and K is a fitting constant. Oxygen saturation is calculated from

equation 13 as follows: $SO_2 = \dfrac{a}{a+b}$ [14]

Figure 10. A flow chart describing the spectral data acquisition and processing. In column 1 are three types of measurements performed: empty chamber, empty cuvette and blood sample. Within each column, three types of signals are obtained for each type of measurement and reported as: chamber signal (T), chamber reference (T_{Ref}) and sphere (B); backscatter signal. To get the transmittance in any type of measurement, normalized chamber signal (T) is divided by normalized chamber reference (T_{Ref}) as indicated in column 2. The third column represents the spectral response of the blood sample taking into account the effect of the cuvette. *1) T_E= Transmitted signal of an empty chamber, BS_E =backscatter signal of an empty chamber. *2) T_C = Transmitted signal of the empty cuvette, BS_C =backscatter signal of the empty cuvette. *3) T_S = Transmitted signal of cuvette with blood sample, BS_S =backscatter signal of cuvette with blood sample. Note that T_{Ref} in all three types of measurements simply represent the reference used for the transmitted signal. The backscatter, BS signal is ignored in our analysis as it is beyond the scope of this thesis.

*1) Empty chamber (E)

- T_E
- BS_E
- T_{ref}

Correction factor$=T_E/T_{Ref}$

*2) Empty Cuvette (C)

- T_C
- BS_C
- T_{ref}

Normalized Transmittance$=T_C/T_{Ref}$

Normalized Transmittance $= [T_S*T_C/Tc]$

*3) Blood sample (S)

- T_S
- BS_S
- T_{ref}

Normalized Transmittance$=T_S*T_C/T_{Ref}$
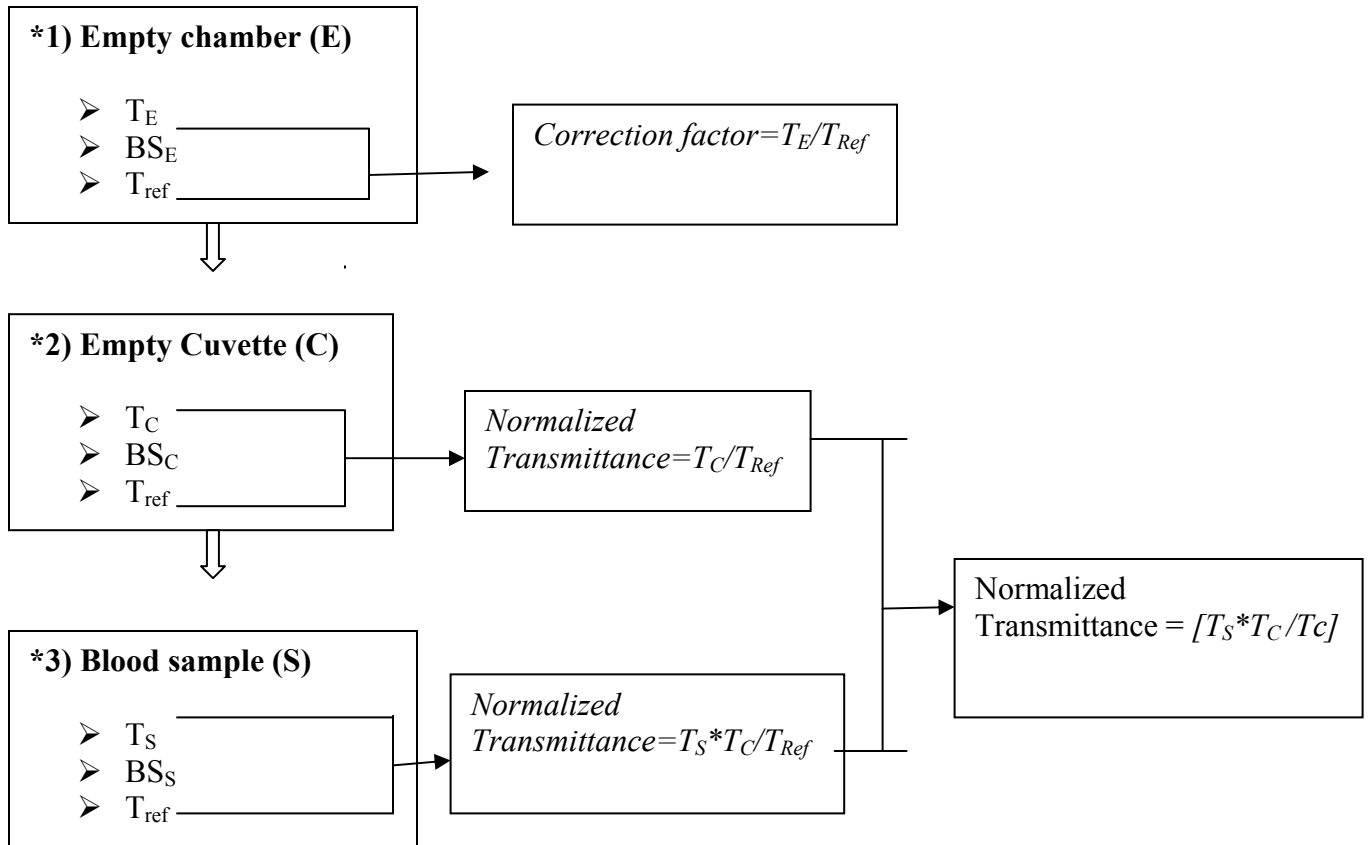
Figure 11. Empty chamber measurement comparing signals from the sample (T_E) and reference chamber (T_{Ref}). T_E and T_{Ref} are not equal and also their differences are wavelength dependent due to system over correction.
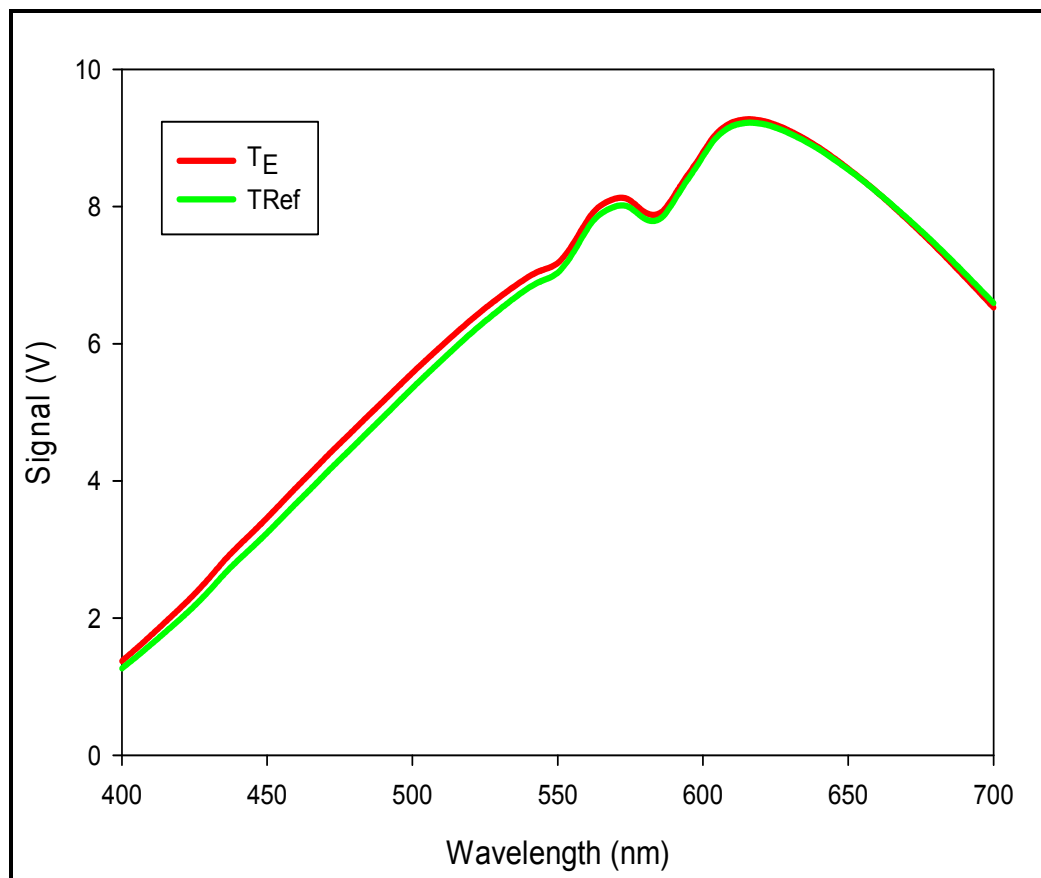
2.5.1.3. Whole Blood Spectroscopy Measurements of Pre-dialysis Blood Samples Spiked with Heparin using the Cuvette with 1000 μm Optical Path Thickness

The transmittance signals of the blood samples spiked with 4 concentrations of heparin were calculated as in section 2.5.1.1 but the data was not normalized.

2.6. Statistical Analyses

2.6.1. Multiple Linear Regression

Multiple linear regression analysis (Sigma Plot® 11.0, Systat Software, Inc.) was used to express the measured absorbance of whole blood as a linear combination of the theoretical extinction coefficients of HHb and O_2Hb. The fitting parameters a and b were derived from the fit between the measured and theoretical absorbance, and were used to calculate whole blood SO_2 values as illustrated in equation 14. The value of the coefficient of determination (R^2) close to 1 was chosen to indicate the reliability of the fit curve.

2.6.2. Analysis of Variance (ANOVA)

In order assess to whether there were statistically significant differences in the mean acid-base and oxygen parameter values among the different time points during HD treatment, a One Way analysis of variance (ANOVA) was performed. A P value <0.05 was chosen to indicate statistically significant changes in acid-base and oxygen parameters during HD treatment.

To determine whether there were statistically significant differences in the mean coefficient of determination (R^2) at each time point during HD treatment, ANOVA was performed using the Mann-Whitney Rank Sum Test. A P value <0.05 was chosen to indicate statistically significant differences in the mean R^2 values between each time point during HD treatment.

In order to assess whether changes in the blood transmittance spectra during HD treatment were significant, the Holm-Sidak multiple comparison tests on a Two-way ANOVA was performed. A P value <0.05 was chosen to indicate statistically significant spectral changes and differences between patients during HD treatment.

2.6.3. Pearson Product Moment Correlation

Pearson Product Moment Correlation (Sigma Plot® 11.0, Systat Software, Inc.) was used to correlate the analysis outcomes of variance in whole blood spectra to variations in acid-base and oxygen parameters to assess each parameter as a potential confounding factor. A Pearson Correlation Coefficient (r) of 0.8 was chosen to indicate a high correlation between the two variables. A P value <0.05 was chosen to indicate a significant relationship between the two variables.

3. RESULTS

3.1. Patient Demographics and Clinical Indicator of Hemodialysis Adequacy

To assess whether the patients selected for this study were representative of a normal ESRD population, patient demographics and clinical indicator of HD adequacy are presented. Patient demographics for the 18 ESRD patients enrolled in this study are summarized in Table III. Enrolment included 11 females and 7 males, age ranged from 23 to 88 years old, 77.7 % were hypertensive and 38.9 % were diabetics. Mixed-arterial blood was obtained from 11 patients (AVF) and venous blood samples were drawn from 7 patients (CVC). Table IV summarizes the urea reduction ratio (URR); an index of HD treatment adequacy for the 18 ESRD patients selected for this study. Blood urea nitrogen (BUN) was measured at every testing time point but only the pre- and post-dialysis values were used to calculate URR as per KDOQI guideline (K/DOQI Clinical Practice Guidelines, 2002). The average URR was 78.3% in all cases but one, and exceeded the minimum recommended value of 70% as per KDOQI guideline on HD treatment adequacy. All the patients in this study received adequate HD treatment except patient 15 with a URR of 63.5% as per KDOQI guideline.

Table III: Characteristics of the 18 patients with end-stage renal disease selected for the investigation of the effect of perturbations of acid-base and oxygen parameters due to hemodialysis procedures on whole blood-base optical spectroscopy in the first pilot study.

Patient	Age	Sex	Hypertensive	Diabetic	Vascular access
1	53	F	Yes	No	AVF
2	78	M	Yes	No	CVC
3	63	M	Yes	No	CVC
4	80	M	N/A	No	AVF
5	72	F	No	Yes	CVC
6	60	F	Yes	Yes	CVC
7	57	F	Yes	Yes	AVF
8	76	M	Yes	No	AVF
9	76	M	Yes	No	AVF
10	77	F	Yes	No	AVF
11	56	F	Yes	Yes	CVC
12	88	F	Yes	No	AVF
13	33	M	Yes	No	AVF
14	23	F	No	No	CVC
15	44	M	No	Yes	CVC
16	83	F	Yes	Yes	AVF
17	58	F	Yes	No	AVF
18	65	F	Yes	Yes	AVF
Average	63				

Table IV: Urea reduction ratio (URR) as a clinical indicator of hemodialysis treatment adequacy for the 18 patients with end-stage renal disease selected for the investigation of the effect of perturbations of acid-base and oxygen parameters due to hemodialysis procedures on whole blood-based optical spectroscopy in the first pilot study. All patients received adequate dialysis treatment as per KDOQI guideline.

Patient	Urea (mmol/L)		
	Pre-dialysis	Post-dialysis	URR (%)
1	9.5	0.9	90.5
2	11.5	2.2	80.9
3	20.5	5.1	75.1
4	16.8	3.0	82.1
5	9.7	2.0	79.4
6	26.3	7.4	71.9
7	26.8	8.0	70.2
8	13.2	3.1	96.5
9	17.6	5.0	71.6
10	36.0	5.6	84.4
11	27.5	5.6	79.6
12	16.5	4	75.7
13	9.8	2.1	78.6
14	28.3	8.1	71.8
15	11.8	4.3	63.5
16	7.3	1.5	79.4
17	21.8	4.4	79.8
18	19.1	3.2	83.2
Average			78.3
StDev			7.8

Figure 12. Flow diagram of the study design for the first pilot study; investigation of the effect of perturbations of acid-base and oxygen parameters due to HD procedures on whole blood-based optical spectroscopy. A total of 18 ESRD patients were recruited and blood samples were collected at 5 time points during HD treatment. Samples from each time point were sent for blood gas and blood spectroscopy analyses. 15 complete blood gas data were obtained. For 3 patients, blood gas data were not reported at every time point on HD either because of error by laboratory personnel (patients 14 and 22) or because of coagulation problems (patient 15). Blood spectroscopy measurements were performed using two cuvettes; 1000 μm cuvette was used for spectroscopy, 100 μm cuvette was used for both spectroscopy and oximetry measurements. Utilizable spectral data were obtained from 14 patients, for 4 patients measurements were performed when proper acquisition parameters were not established. 14 (1000 μm cuvette) and 8 (100 μm cuvette) complete data sets were used for correlation to blood gas parameters and analysis outcome of variance in blood transmittance spectra during HD treatment.
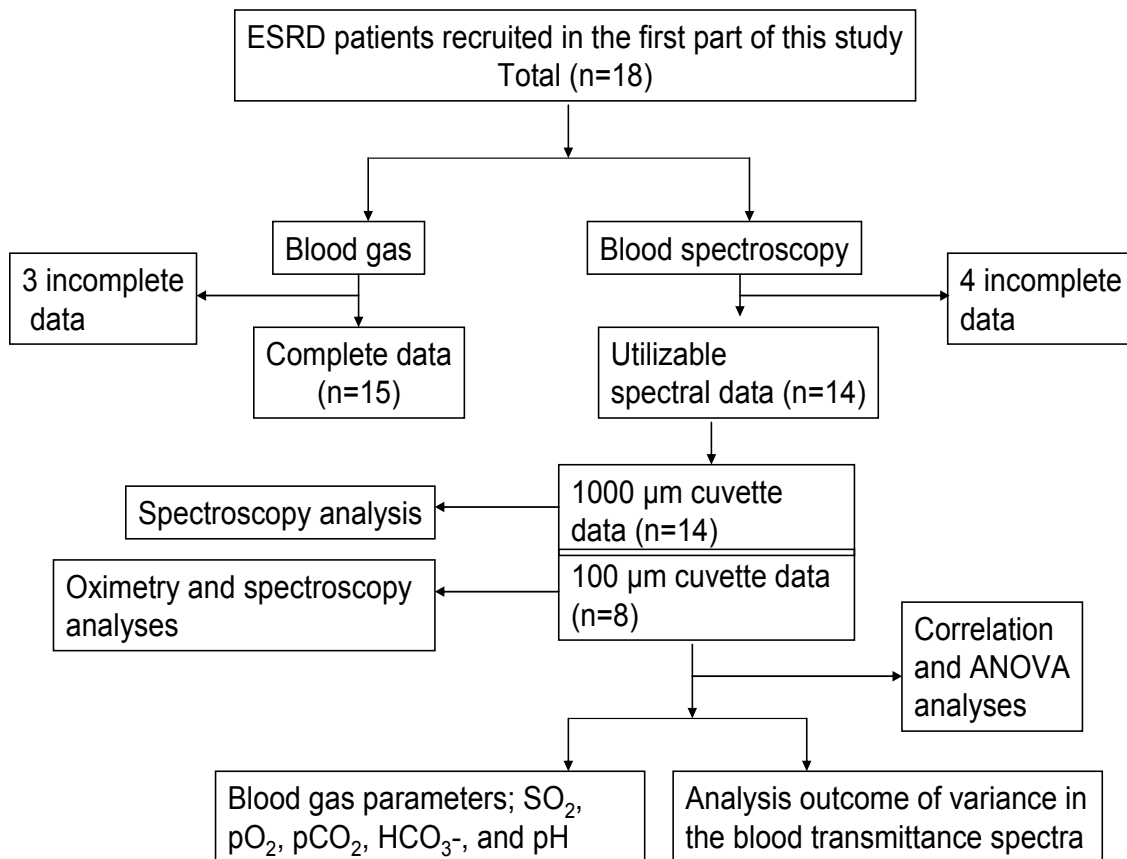
3.2. Changes in Whole Blood Transmittance Spectra during Hemodialysis Treatment

Since the set-up used in this study was different from that of Lagali et al., it was necessary to validate our results to ensure that our findings were in agreement. Having acquired data at 5 different time points during hemodialysis, one of question that is of interest is whether the changes detected during HD treatment are significant across time points.

In order to assess whether there were significant measurable changes in whole blood transmittance spectra during HD treatment, the blood transmittance signals were compared to the instrument repeatability level of 0.01%. We found that the changes in the blood transmittance spectra during HD treatment were above the instrument noise level indicating that we are measuring real changes as a result of HD treatment.

Further statistical evaluation of the wavelength dependence of changes detected in whole blood transmittance spectra during HD treatment was performed. ANOVA was performed on changes in the blood normalized transmittance spectra obtained using the 1000 μm cuvette (14 patients), in both the VIS and NIR wavelength regions. Statistically significant ($P<0.05$) changes in whole blood transmittance spectra were found across all 5 time points during HD treatment in isolated regions between 508 nm and 526 nm, 562 nm and 580 and at 688 nm. Significant measurable changes were also detected between pre- and post-dialysis in regions between 598 nm and 634 nm and between 724 nm and 778 nm (figure 13 A) in the VIS wavelength. In the NIR regions, statistically significant ($P<$

0.05) measurable changes were found only between pre- and post-dialysis (figure 13 B; 780 nm to 824 nm, 956 nm to 1044 nm).

In the visible wavelength, every patient had a characteristic spectrum with significant (P<0.05) differences in whole blood transmittance spectra found in the entire VIS region. In the NIR wavelength, there were no significant (P>0.05) differences between patients across the entire region.

Analyses were also performed on the changes in whole blood normalized transmittance spectra for the spectral data obtained using the 100 μm cuvette (8 patients), across the VIS wavelength. There were no significant (P>0.05) changes found in whole blood transmittance spectra across time points during HD in the entire VIS region; 496 nm to 800 nm. However, every patient had a characteristic spectrum. There is a lack of consistency between spectral data measured using 1000 μm and 100 μm cuvette for the same set of patients. This suggests that relative changes as the result of HD treatment can be detected with a thicker cuvette (1000 μm optical thickness), while the thinner cuvette (100 μm) is compatible with oximetry calculations only.

Overall, the result shows that relative changes are detected during HD treatment using optical spectroscopy and these are consistent with the work of Lagali et al. Now we can proceed and assess the impact of potential confounding factors on our results.

Figure 13. Representative blood transmittance spectra showing wavelength regions with significant transmittance differences during hemodialysis (HD) treatment. A) In the visible wavelength, red arrows indicate regions with significant changes across all 5 time points during HD, blue arrows indicate regions with significant differences only between pre- and post-dialysis. B) In the near-infrared wavelength, the blue arrow also indicates regions with significant differences between pre-and post-dialysis.

A
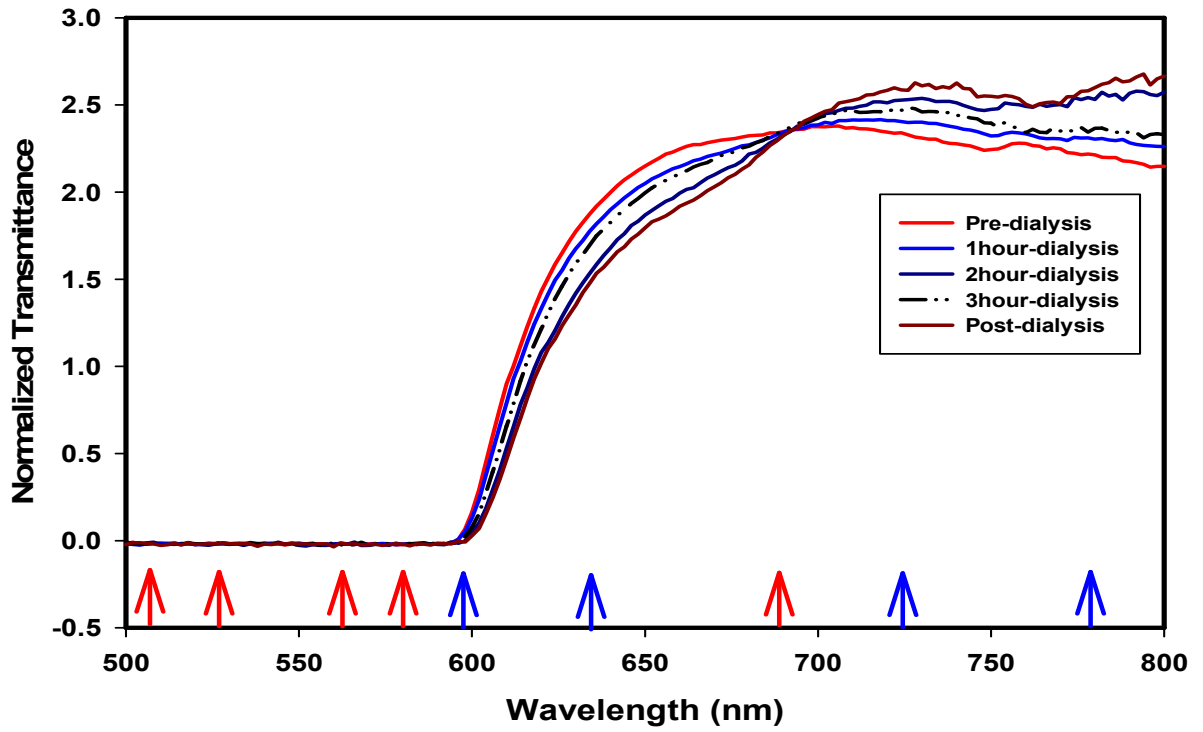
B
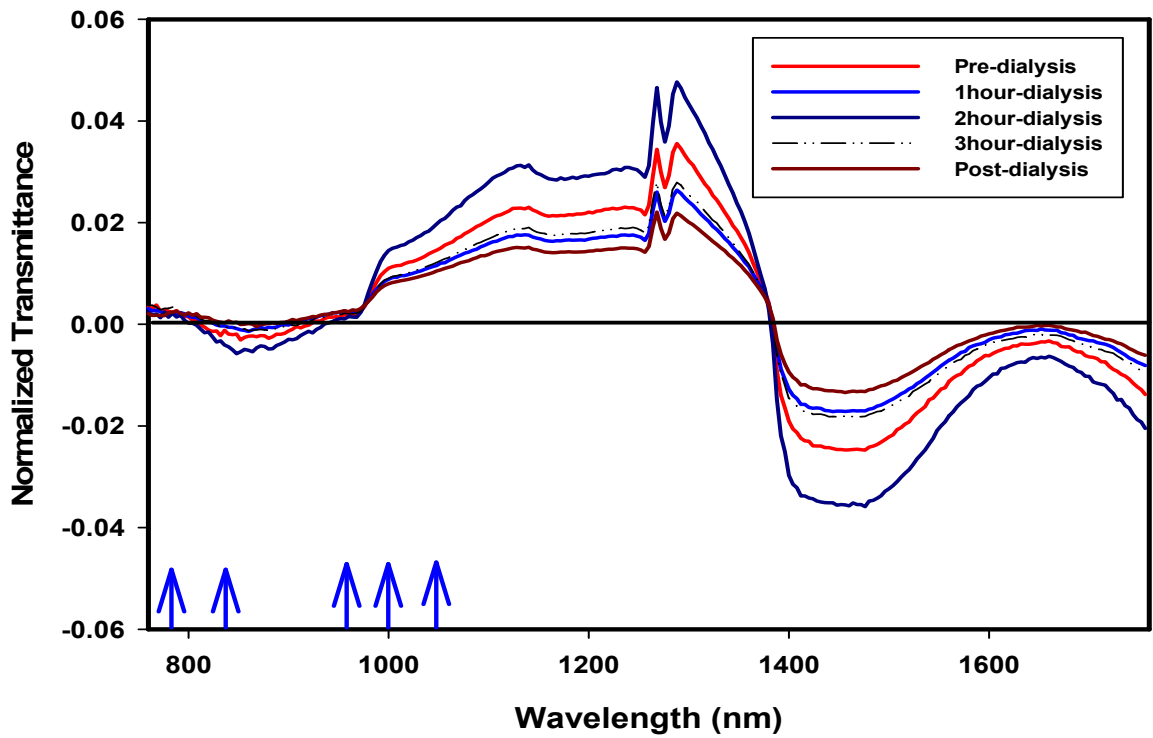
3.3. The Effect of Hemodialysis Procedures on Acid-Base and Oxygen Parameters

3.3.1. Variations and Changes in Acid-Base and Oxygen Parameters during Hemodialysis Treatment

Blood gas measurements were performed on blood samples drawn from 18 ESRD patients at 5 time points during HD treatment. However complete blood gas data were obtained from 15 ESRD patients (figure 12) and presented in this section.

Time dependence of acid-base parameters; pCO_2, HCO_3-, and pH during HD treatment are presented for individual patients (figures 14 A to 16 A). Because of the large differences in oxygen content between arterial ($pO_2 > 90$ mmHg) and venous blood ($pO_2 < 40$ mmHg), arterial and venous pO_2 were plotted separately and are presented in figures 17 A and 18 A, respectively. Mixed-arterial and venous SO_2 values were also plotted separately and are presented in figures 19 A and 20 A, respectively. Individually patients show significant changes in the acid-base; pCO_2, HCO_3- and pH, and oxygen parameters; SO_2 and pO_2 (figures 14 A to 20 A) during HD treatment when compared to laboratory test resolution for each parameter (Appendix VIII, page 201). This is critical so that it is possible to correlate changes in blood spectra in individual patients to changes in clinical parameters during HD treatment.

Additionally, the dependence of the acid-base; pCO_2, HCO_3- and pH, and oxygen parameters; SO_2 and pO_2 on each time point during HD is presented for the group (figures 14 B to 20 B) to validate that this group of patients was representative of the population. As a group, there is a large interpatient variability in acid-base and oxygen parameters at

all 5 time points during HD treatment, as demonstrated by the large standard deviation for the population.

For the acid-base parameters, there is no significant change in pCO_2 (P=0.312) between time points of the 4hour HD treatment. For statistical analysis of the changes in HCO_3- during HD treatment, the normality test failed and ANOVA on Ranks was performed. A significant increase in HCO_3- (P <0.001) is noted at all 4 HD time points compared to pre-dialysis values (Dunn's pairwise multiple comparison test). Similarly there was a significant change in pH (ANOVA) which revealed (using the Holm-Sidak's pairwise multiple comparison method) a significant increase in pH (P = 0.040) only between the post-dialysis and pre-dialysis values. The increase in both HCO_3- and pH are consistent with previous findings (Gennari, 1985; Gennari 2000; Uribarri et al., 1999).

In order to assess whether there were changes in the oxygen parameters during HD treatment, Kruskal-Wallis One-Way ANOVA on Ranks was performed for all the oxygen parameters since the normality test failed. There is no significant change in the laboratory provided oxygen parameters; pO_2 (P = 0.834) and SO_2 (P = 0.949) or the derived whole blood SO_2 (P = 0.798) during the 4 hour HD treatment.

Figure 14. A) Variations in partial pressures of carbon dioxide (pCO_2) during hemodialysis (HD) treatment for 15 individual patients with end-stage renal disease (ESRD). B) The average and standard deviations (error bars) of the group. There is no change in pCO_2 (P=0.312) during the 4 hour HD treatment.

A
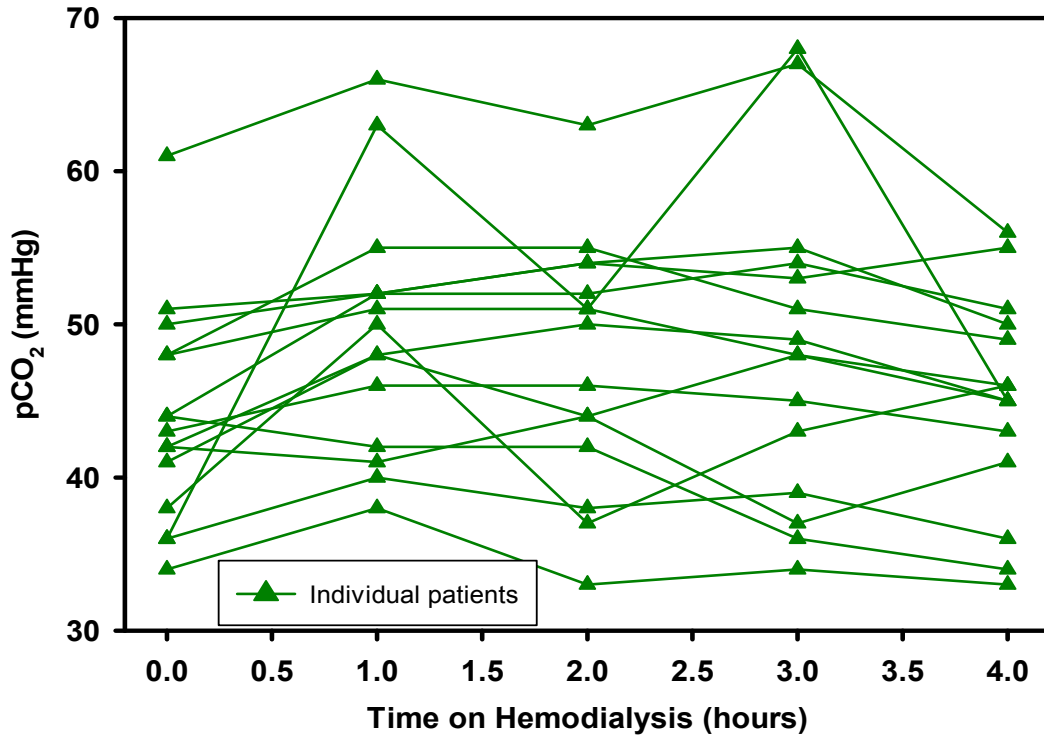
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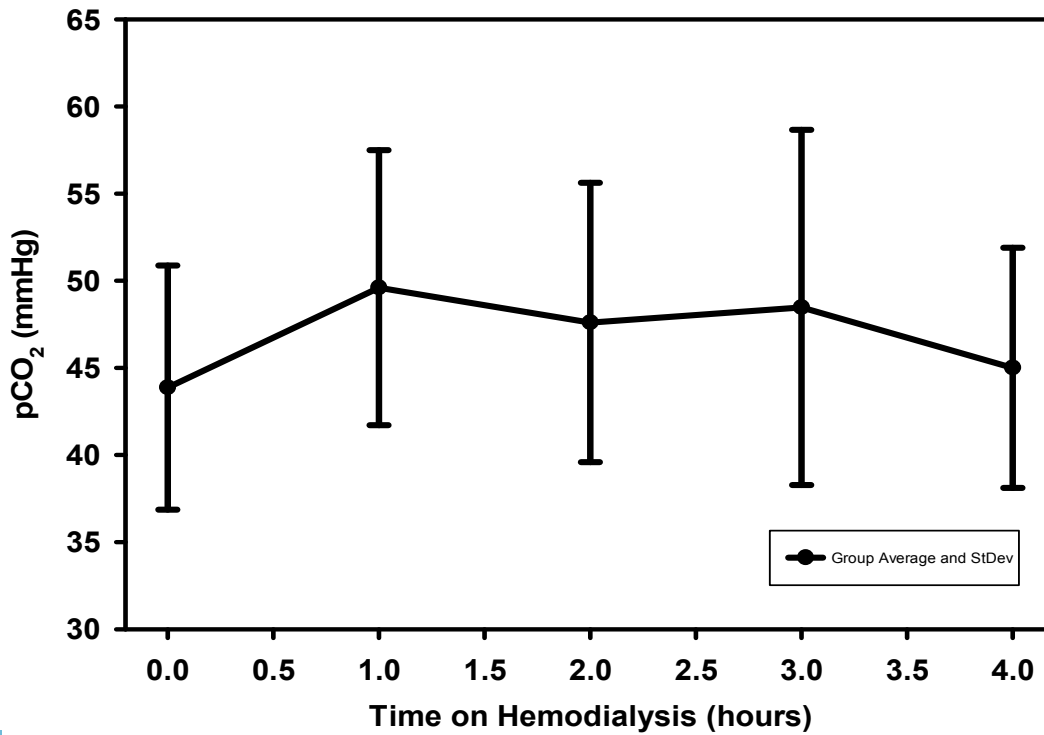
Figure 15. A) Variations in bicarbonate (HCO_3^-) during hemodialysis (HD) treatment for 15 individual patients with end-stage renal disease (ESRD). B) The average and standard deviations (error bars) of the group. A significant increase in HCO_3^- ($P < 0.001$) is found at all 4 HD time points compared to pre-dialysis values.

A
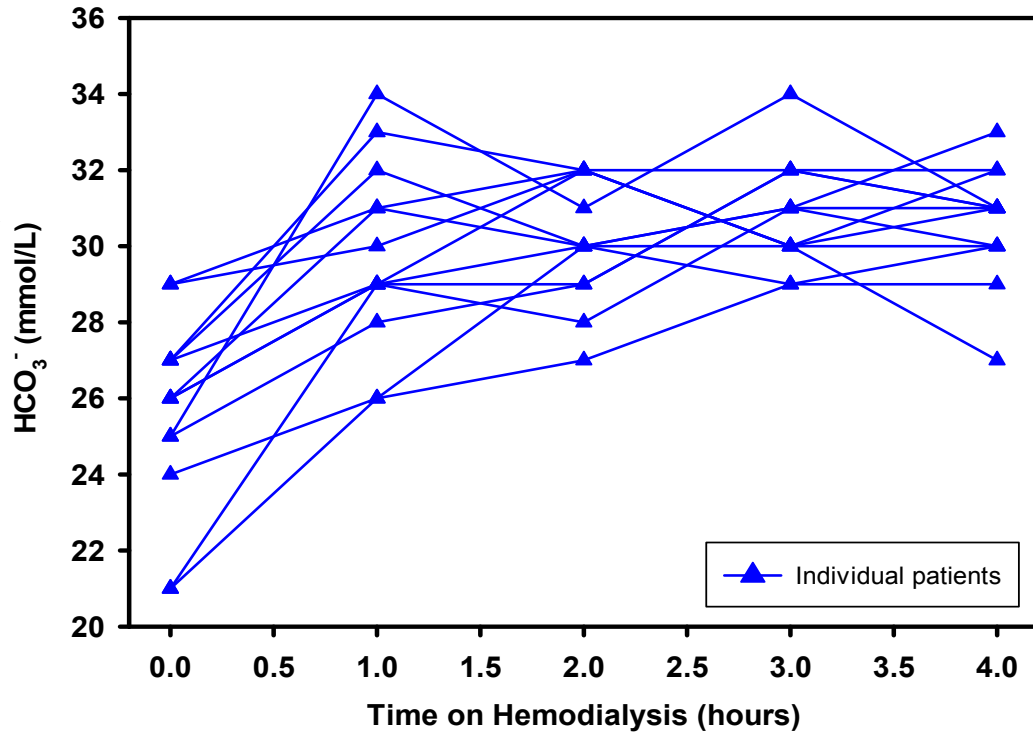
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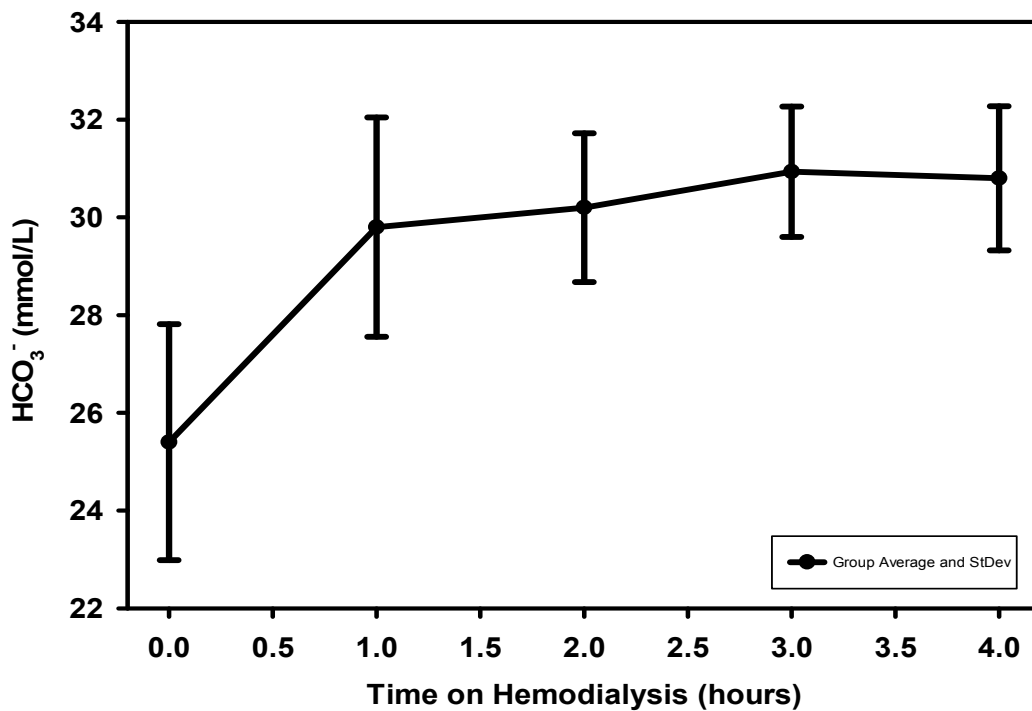
Figure 16. A) Variations in pH during hemodialysis (HD) treatment for 15 individual patients with end-stage renal disease (ESRD). B) The average and standard deviations (error bars) of the group. A significant increase in pH (P = 0.040) is observed post-dialysis compared to pre-dialysis values.

A
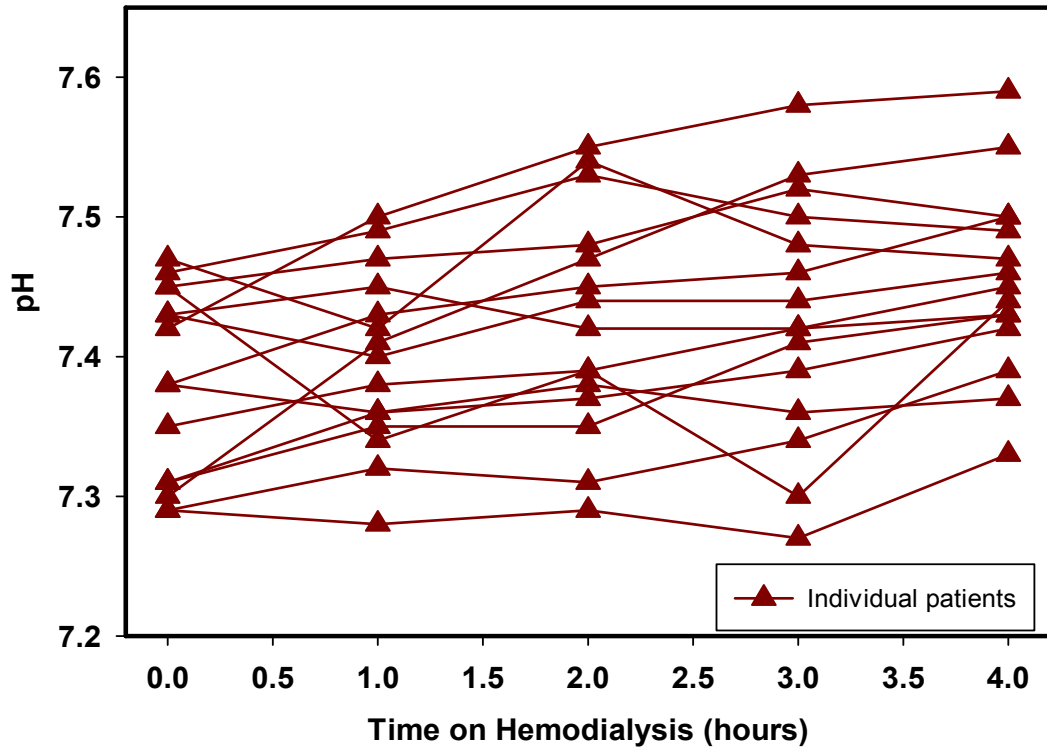
B
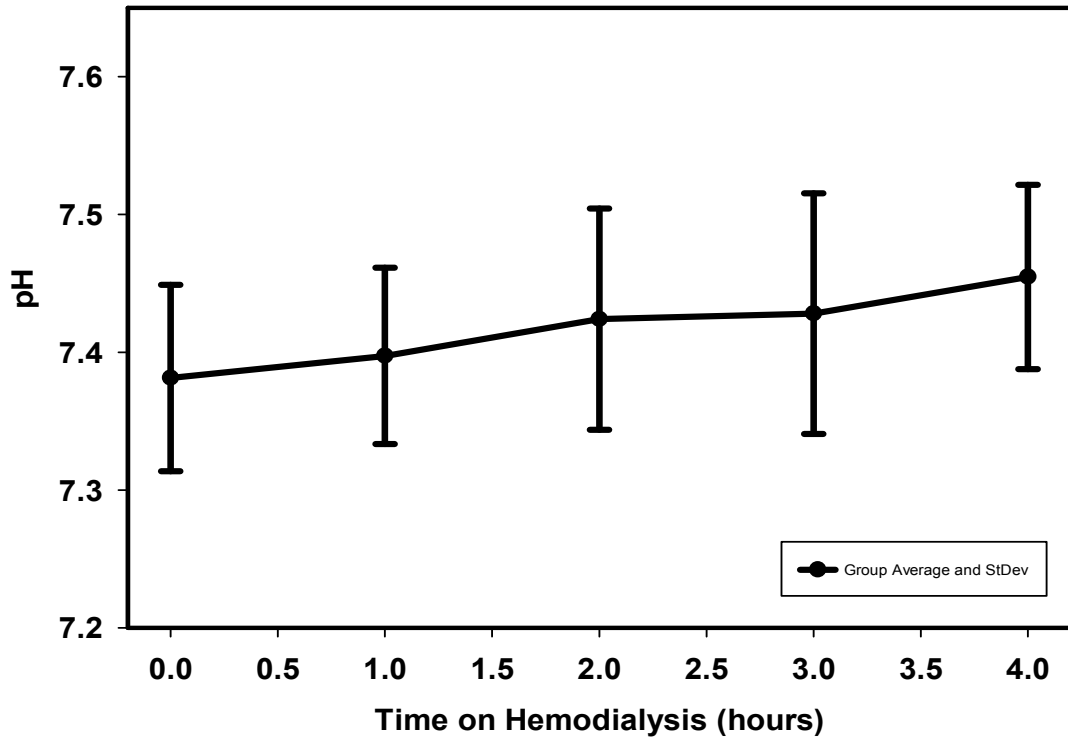
Figure 17. A) Variations in partial pressures of oxygen (pO_2) of mixed-arterial blood (arteriovenous fistulae; AVF) during hemodialysis (HD) treatment for 9 individual patients with end-stage renal disease. B) The average and standard deviations (error bars) of the group. There is no change in pO_2 ($P = 0.834$) during the 4 hour HD treatment.

A
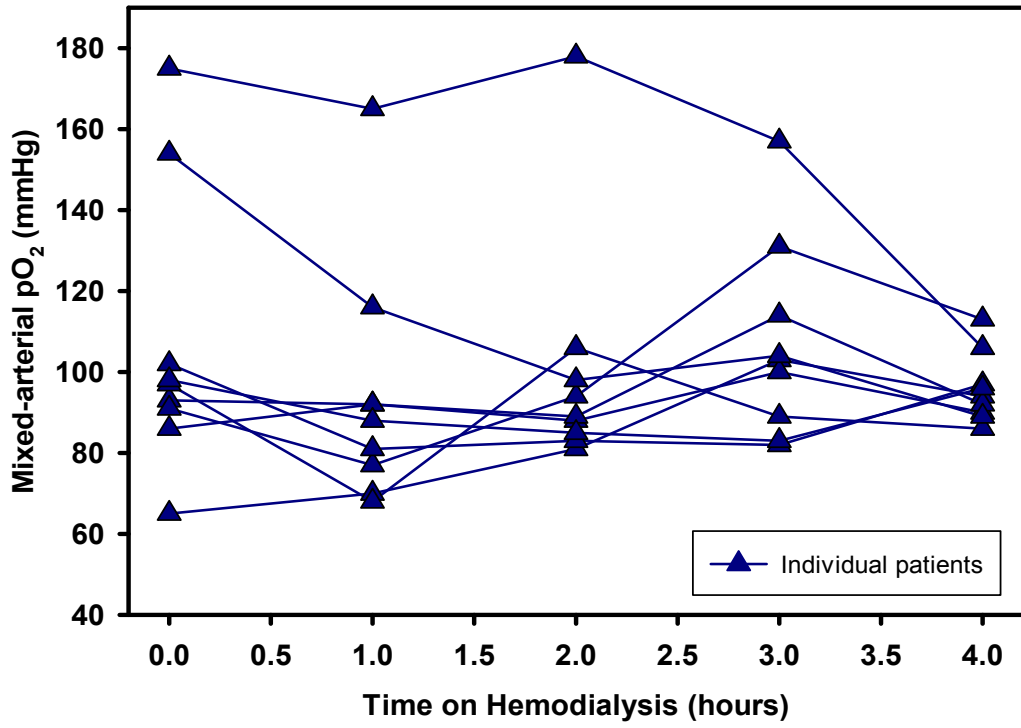
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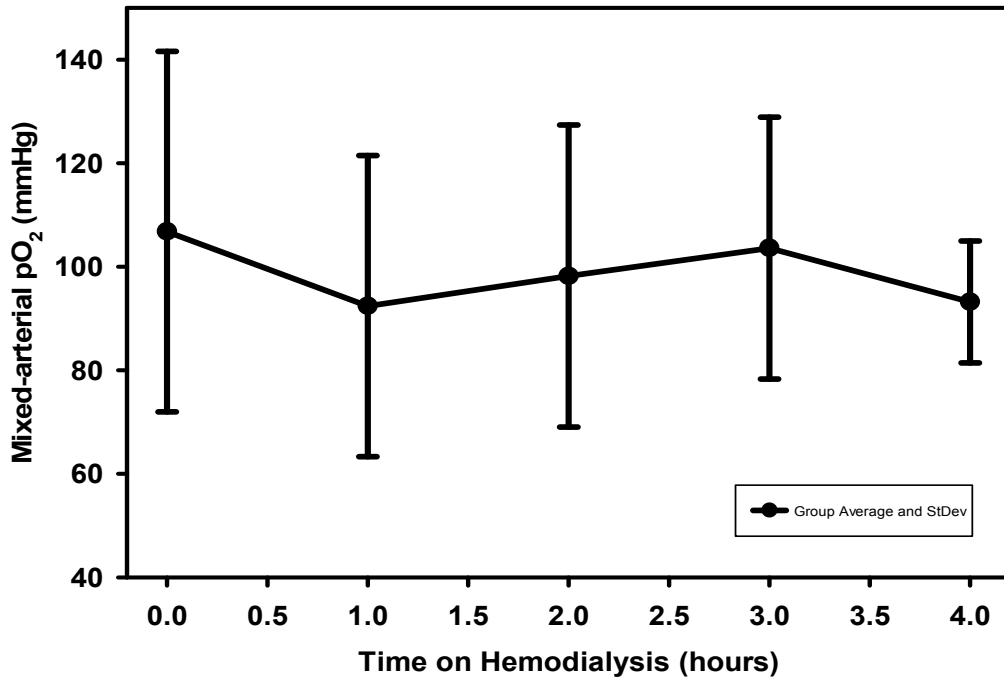
Figure 18. A) Variations in partial pressures of oxygen (pO_2) of venous blood (central venous catheter; CVC) during hemodialysis (HD) treatment for 6 individual patients with end-stage renal disease. B) The average and standard deviations (error bars) of the group. There is no change in pO_2 (P = 0.834) during the 4 hour HD treatment.

A
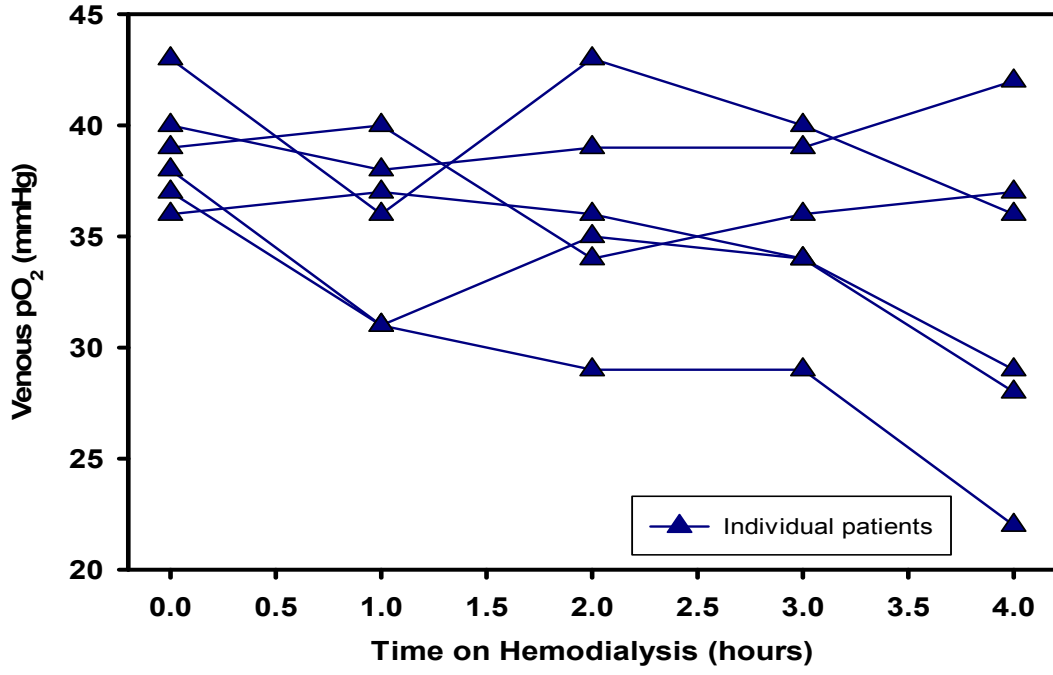
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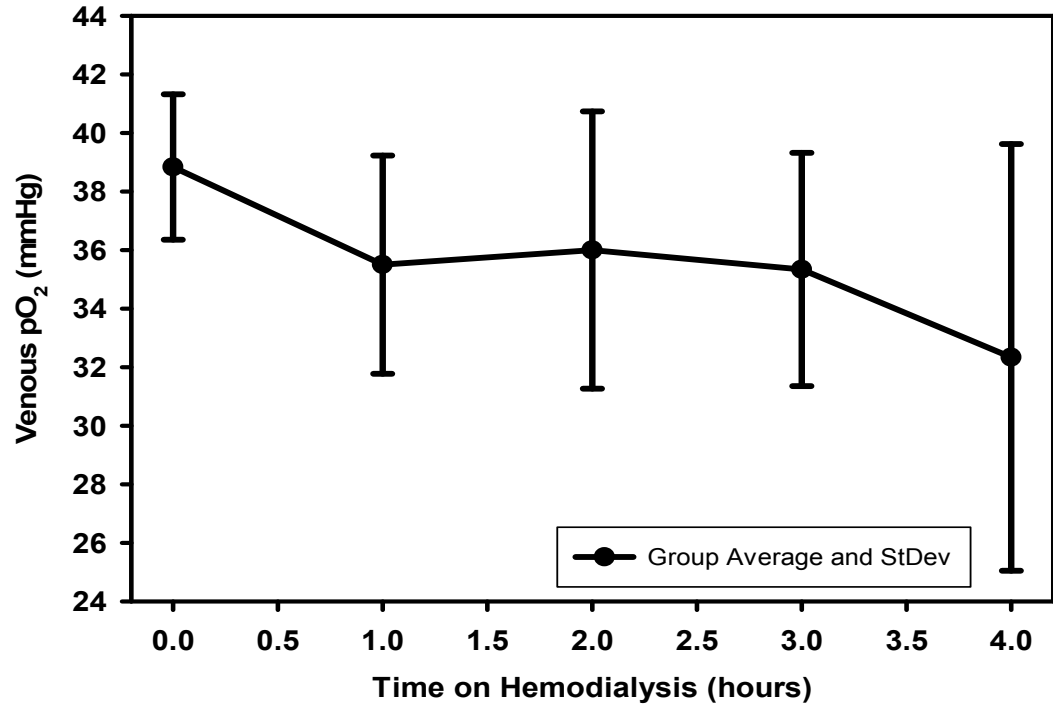
Figure 19. A) Variations in oxygen saturation (SO_2) of mixed-arterial blood (arteriovenous fistulae; AVF) during hemodialysis (HD) treatment for 9 individual patients with end-stage renal disease. B) The average and standard deviations (error bars) of the group. There is no change in SO_2 ($P = 0.949$) obtained from hemoglobin solution during the 4 hour HD treatment.

A
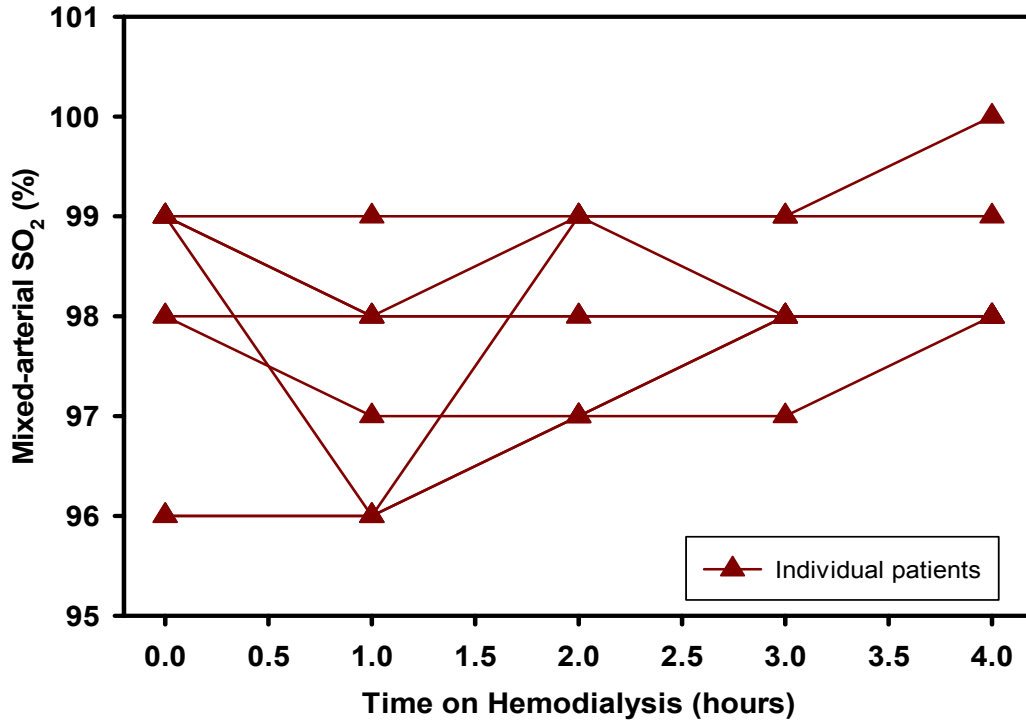
B
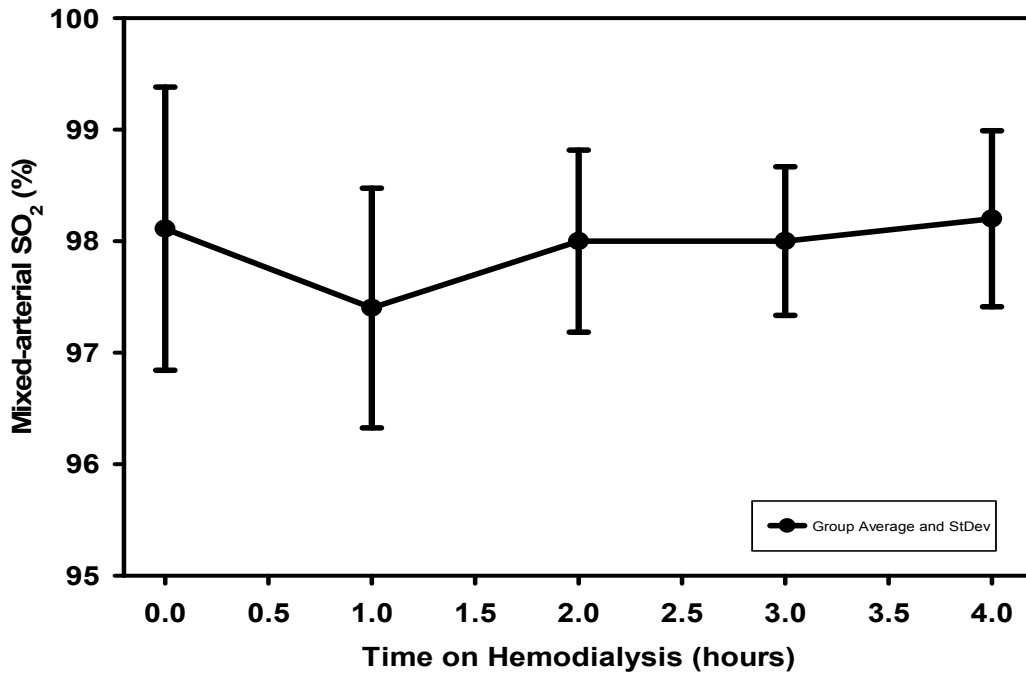
Figure 20. A) Variations in oxygen saturation (SO_2) of venous blood (central venous catheter; CVC) during hemodialysis (HD) treatment for 6 individual patients with end-stage renal disease. B) The average and standard deviations (error bars) of the group. There is no change in SO_2 ($P = 0.949$) obtained from hemoglobin solution during the 4 hour HD treatment.

A
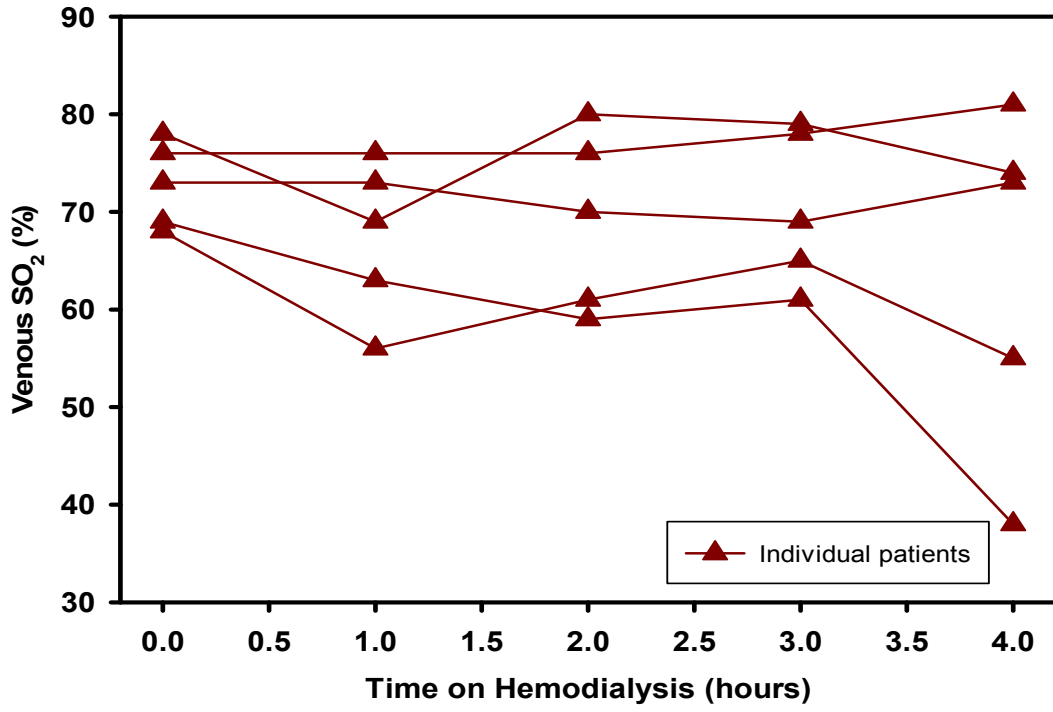
B
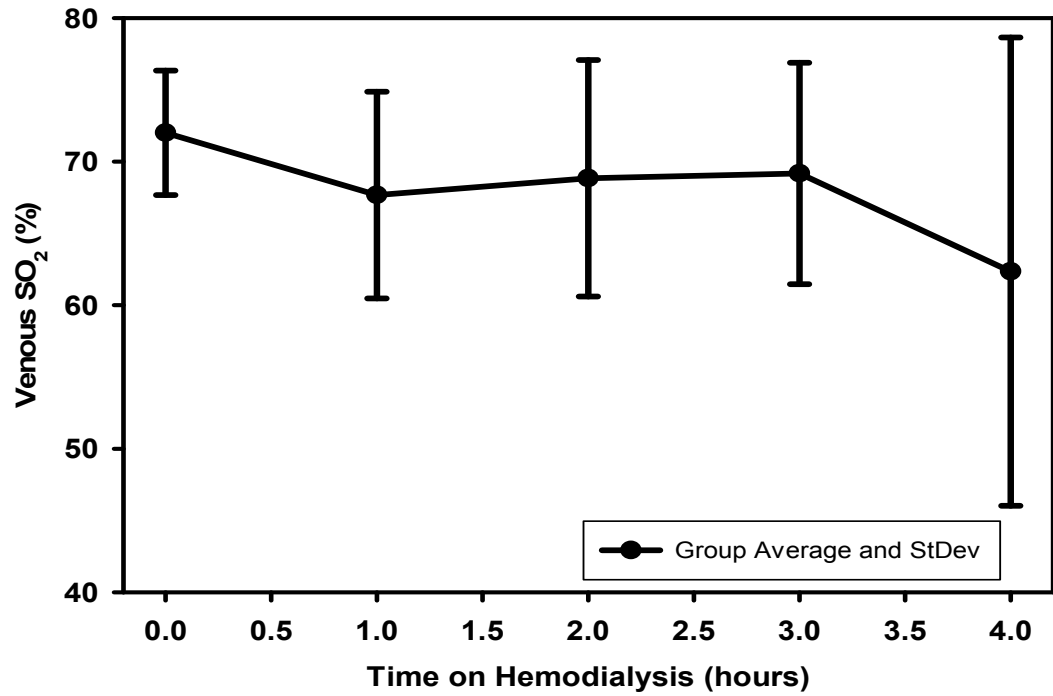
3.3.1.1 Whole Blood Oxygen Saturation (SO$_2$)

The SO$_2$ results discussed above were measured from hemoglobin solutions from the blood gas laboratory. However blood spectroscopy was performed on whole blood and because sample manipulation changed the oxygen saturation, the actual SO$_2$ was measured from whole blood. The comparison of samples that were manipulated under the same experimental condition was essential to further assess the time dependence of SO$_2$ during HD treatment. Given that this was a pilot study, we only chose 8 ESRD patients as it was the number of patients that could be recruited due to time constraints.

Spectral data measured using the cuvette with 100 μm path thickness for 8 ESRD patients were used for oximetry calculations. In the measurement of light passing through the sample, one obtains a mixture of forward scatter and transmitted light. The mixture of the two will depend on sample thickness: as samples grow thicker there are usually less transmittance (more absorbance) and more scatter (forward and back). The cuvette with 100 μm optical path thickness has a much higher light transmission than the 1000 μm optical path thickness and much less forward scatter and thus meets the conditions for applying the Beer-Lambert law for oximetry calculations more closely.

Whole blood normalized absorbance was calculated from the transmittance spectrum at all 5 time points during HD treatment as per section 2.5. Normalization allows easy visual comparison of the differences in the shapes of the absorbance spectra (figure 21), by factoring out differences in the average absorbance which are due to differences in hematocrit levels at each HD time point and between patients. Every

patient has the characteristic absorbance spectrum expected for hemoglobin dominated absorbance of blood at all 5 time points; however the spectral shapes are identical across all time point during HD treatment, which is consistent with the literature (Lagali et al., 2006; Lagali et al., 2007). The feature differences between the absorbance of O_2Hb and HHb are present at all 5 time points during HD treatment, across all patients, although the amplitude of the absorbance is patient-dependent, which is consistent with previous work in our laboratory (unpublished data). In order to extract SO_2 from whole blood, the fit between the measured absorbance and the Beer-Lambert absorbance model for a mixture of O_2Hb and HHb was assessed.

Figure 21. Normalized whole blood absorbance for, A) individual 8 ESRD patients and, B) average and standard deviations (error bars) of the group at 5 time points during hemodialysis treatment (HD) (100 μm cuvettes).

A
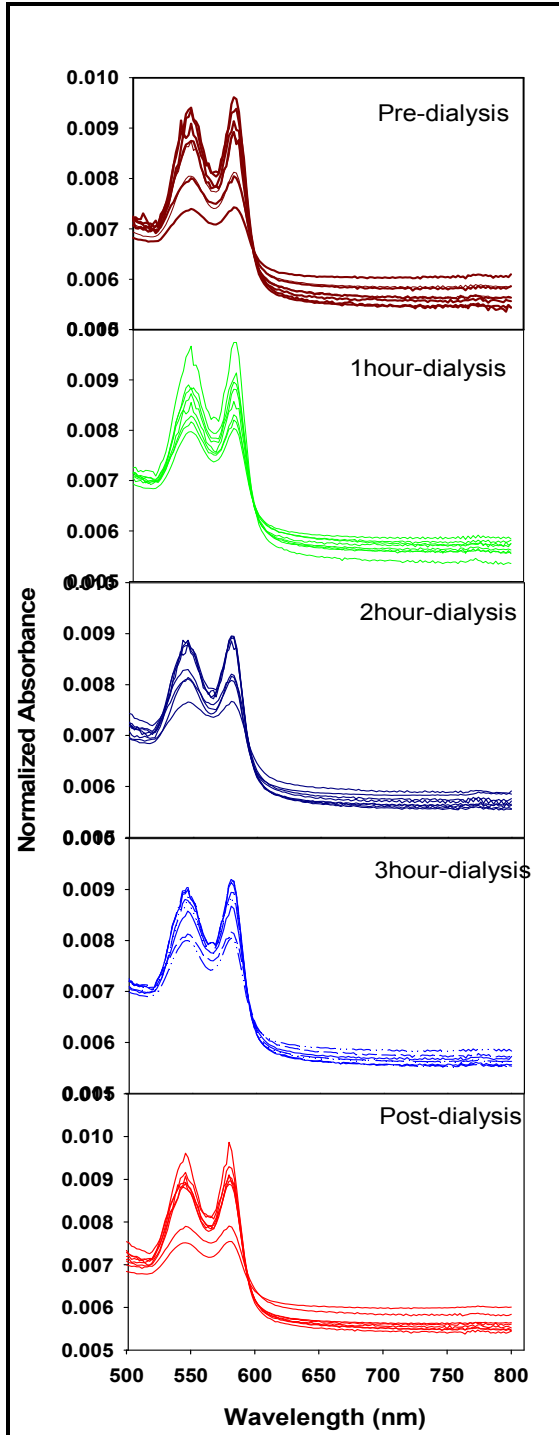
B
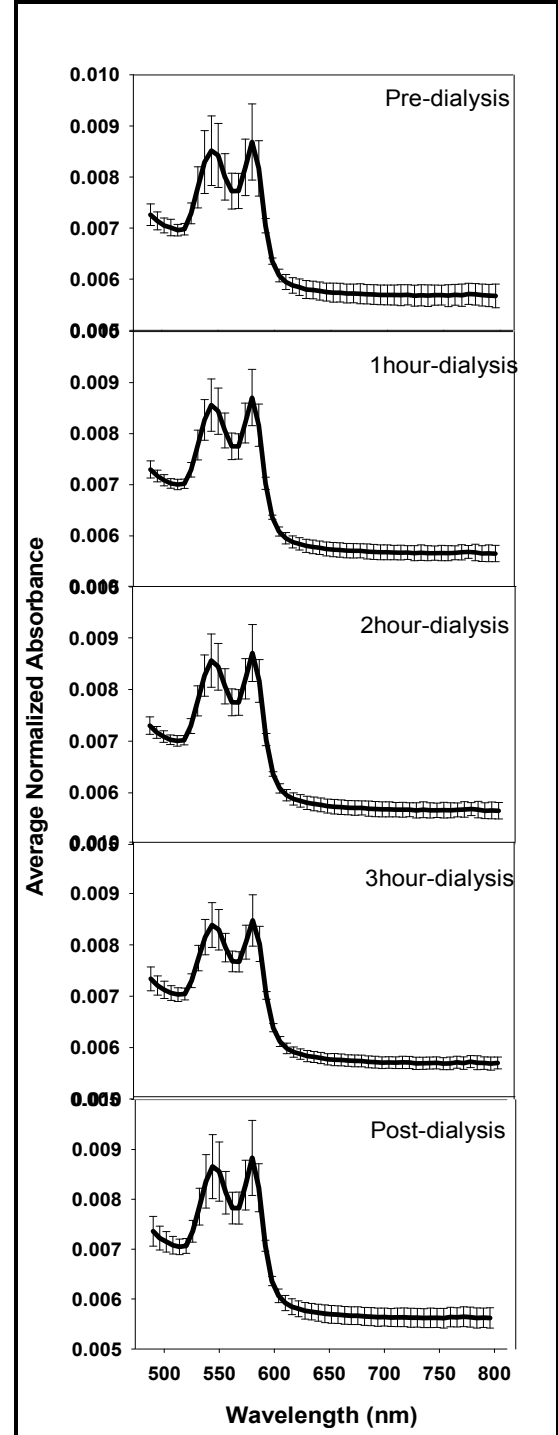
In order to quantify how closely the measured absorbance of whole blood matched the Beer-Lambert absorbance model for a mixture of O_2Hb and HHb, the measured absorbance spectra were fitted to the Beer-Lambert law using a multiple linear regression. The wavelength ranges of 515 nm to 595 nm were selected to ensure minimal chance of interference by other light absorbing substances in whole blood (Meinke et al., 2007). SO_2 values were calculated from the result of the fit between the measured absorbance and Beer-Lambert absorbance model. A representative example of the result of the fit between the measured absorbance spectra and Beer-Lambert absorbance model and the corresponding SO_2 values are illustrated in figure 22. With visual inspection, it can be seen that the fit between the measured absorbance and the model is not perfect and that differences from the model are not random.

The coefficient of determination (R^2) is a widely used method to assess the quality of the fit between the measured and theoretical absorbance (Diaconnu, 2009). The closer the R^2 value to 1, the more reliable the fit is, whereas a less reliable fit corresponds to a lower R^2 value. The group average R^2 values at each time point and their standard deviations across the 8 patients are shown in figure 23. There are no statistically significant differences between the R^2 values at each time point during HD treatment (ANOVA, Mann-Whitney Rank Sum Test, P = 0.28). The R^2 values were above 0.95 for all patients at all 5 HD time points, suggesting very high agreement between the model and the data. Although the R^2 values indicate very high match between the measured and model absorbance, it is not a good indicator of fit if small but systematic errors (due to red blood cell scattering) indicative of deviations from the Beer-Lambert absorbance model are present.

Figure 22. A representative example of the measured absorbance spectra fitted using multiple linear regression to the Beer-Lambert absorbance model of de-oxyhemoglobin and oxyhemoglobin for a single patient at 5 time points during hemodialysis treatment. Whole blood oxygen saturation (SO_2) and the corresponding coefficient of determination, R^2 shown on the right of the plot were calculated from the results of the fit. The red curve represents the measured absorbance and the blue curve represents the predicted absorbance.
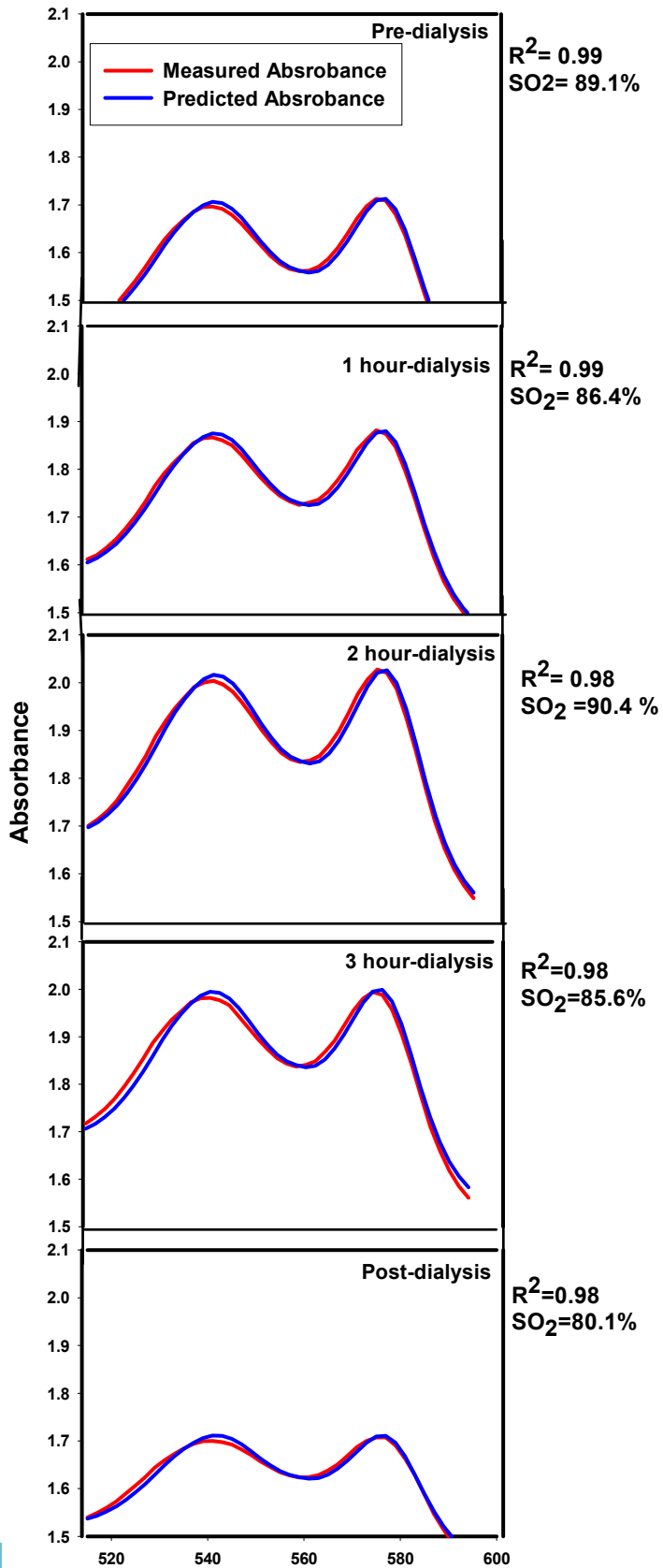
Figure 23. The average coefficient of determination (R^2) values and standard deviations (StDev) of 8 patients with end-stage renal disease at 5 time points during hemodialysis (HD) treatment. Average R^2 values are plotted as a function of time on HD to assess how closely the measured absorbance of whole blood matched the Beer-Lambert absorbance model for a mixture of de-oxyhemoglobin and oxyhemoglobin (100 μm cuvette).
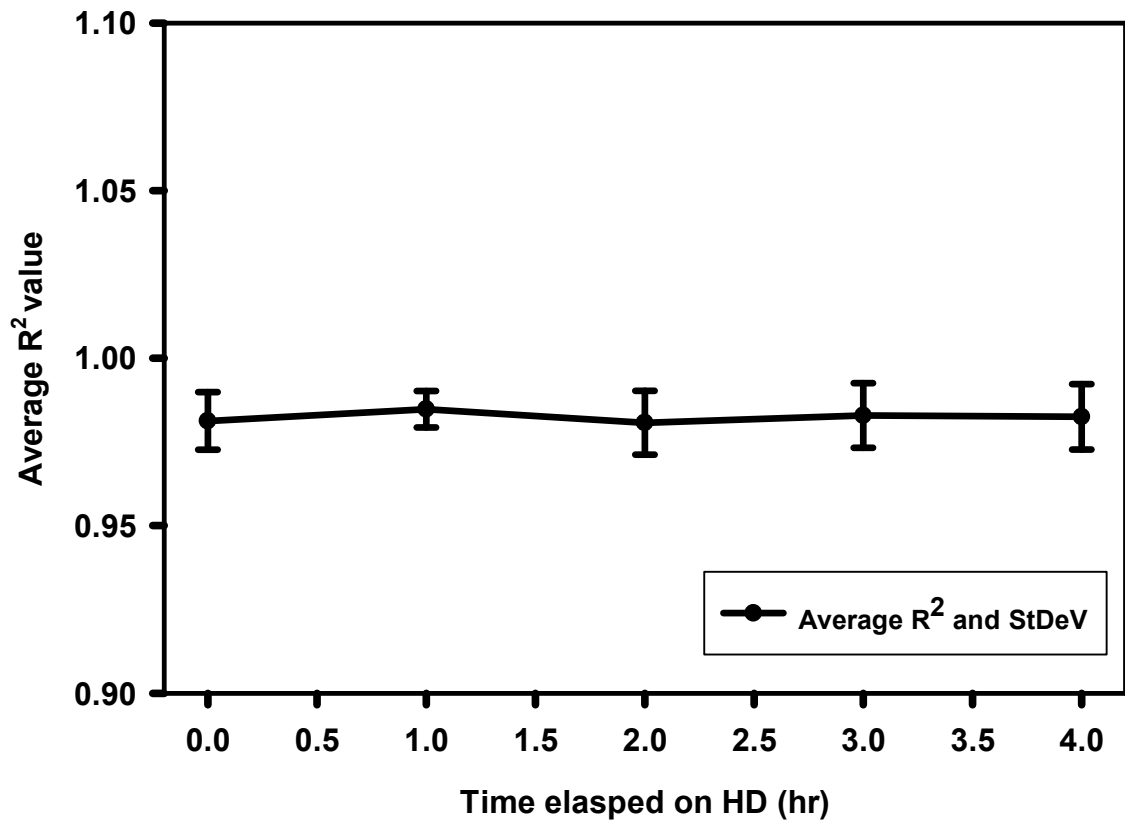
The deviations of the measured absorbance from the Beer-Lambert absorbance model were quantified in order to assess their possible impact on the calculated SO_2 from whole blood. The deviation differences between the measured spectra and the fitted absorbance spectra were calculated for each patient at each HD time point as a function of wavelength. These were calculated as the measured minus the predicted absorbance between 515 nm to 595 nm ranges (figure 24 A). The average deviation and standard deviations are plotted as function of wavelength in figure 24 B. Although the R^2 method reported reliable curve fits, there are consistent systematic errors at each HD time point across patients. These systematic errors are caused by red blood cell scattering that is present across every blood sample and patient. Consequently, these errors cause deviations of the measured absorbance data from the Beer-Lambert absorbance model for a mixture of O_2Hb and HHb, which could have an impact on whole blood SO_2.

Individually, patients show variations in the whole blood SO_2 (figure 25 A) during HD treatment. As a group, there is a large interpatient variability in whole blood SO_2 (figure 25 B) at each time point during HD treatment, as demonstrated by the large standard deviation for the population. Because of sample manipulation, both venous and mixed-arterial whole blood SO_2 were highly saturated (>80% on average) and do not compare with the blood gas SO_2 result for the same set of patients. On average whole blood SO_2 (ANOVA, P>0.05) values are constant across all 5 time points during HD treatment. This indicates that SO_2 may have a minimal or no impact on optical spectroscopy. In order to assess the potential effect of all the acid-base and oxygen parameters on optical spectroscopy, blood spectroscopy measurements were performed at

each HD time point (next section, 3.1.5) and analysis outcome of variance in blood transmittance was correlated to variations in the blood gas parameters (section 3.1.6).

Figure 24. Systematic deviations from the Beer-Lambert absorbance model at 5 time points during hemodialysis for, A) 8 individual patients with end-stage renal disease and, B) average and standard deviations (error bars) of the group. The colours in the plot on the right represent individual patients (100 μm cuvettes).
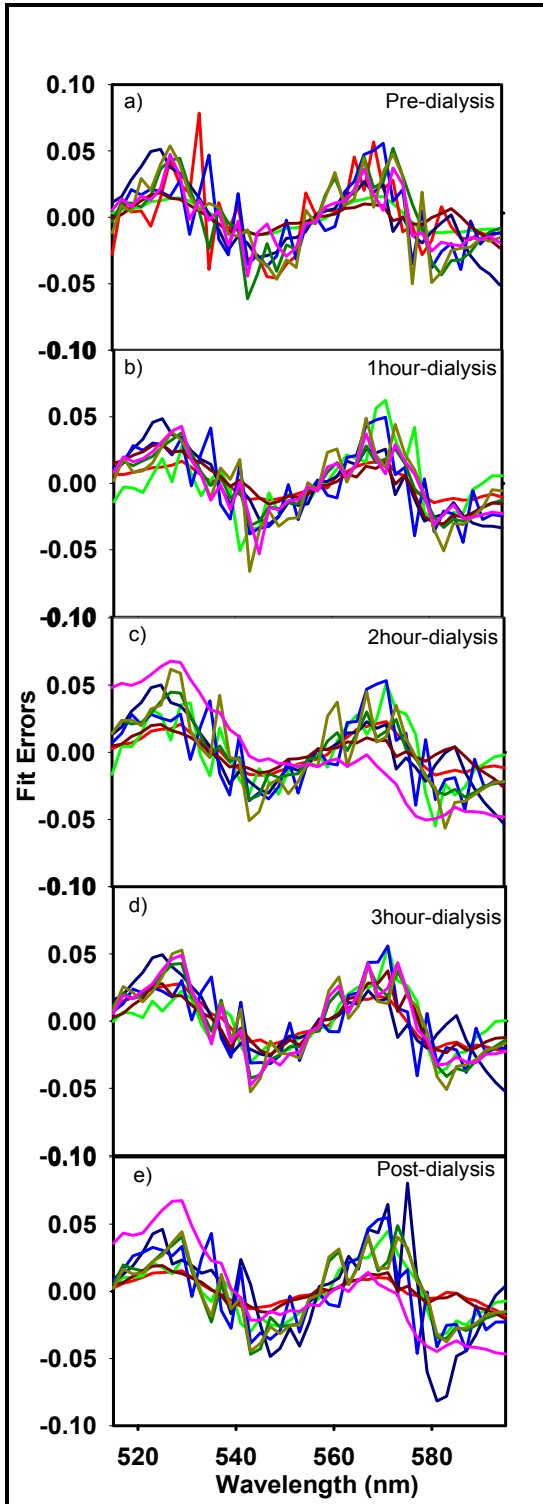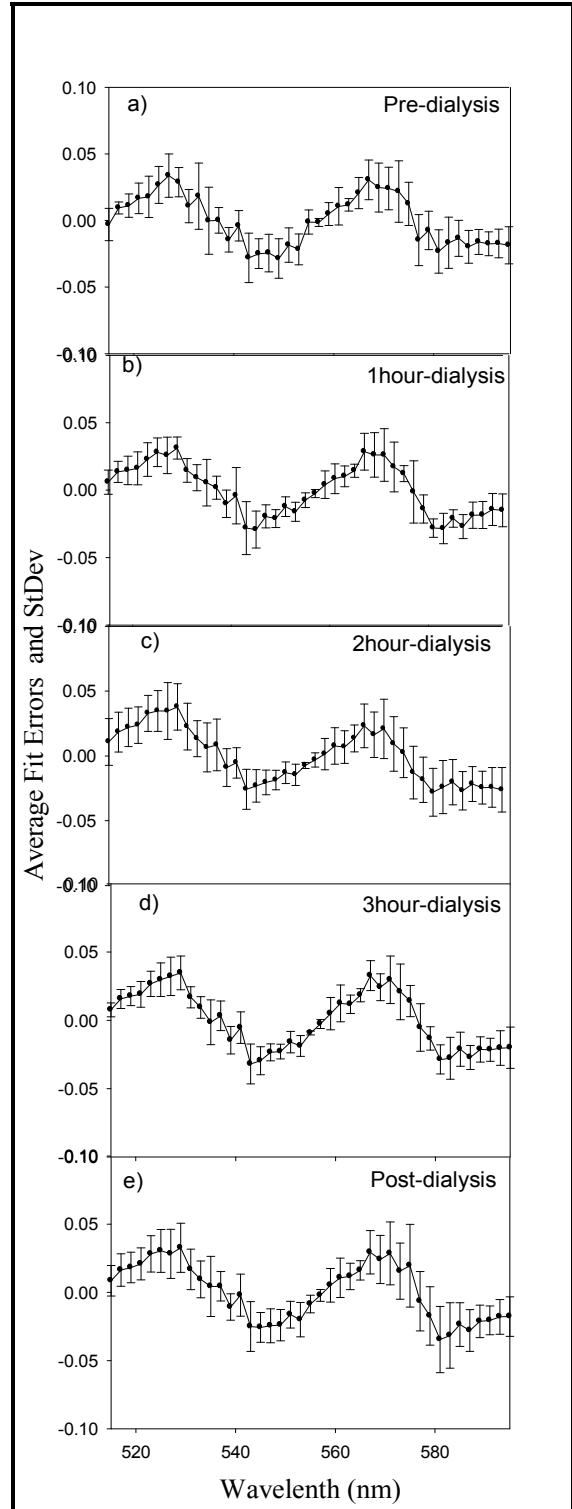
Figure 25. A) Variations in whole blood oxygen saturation (SO_2) of venous and mixed-arterial blood (central venous catheter; CVC and arteriovenous fistulae; AVF) during hemodialysis treatment for 8 individual patients with end-stage renal disease. B) The average and standard deviations (error bars) of the group. There is no change in the calculated whole blood SO_2 (P = 0.798) during the 4 hour HD treatment.

A
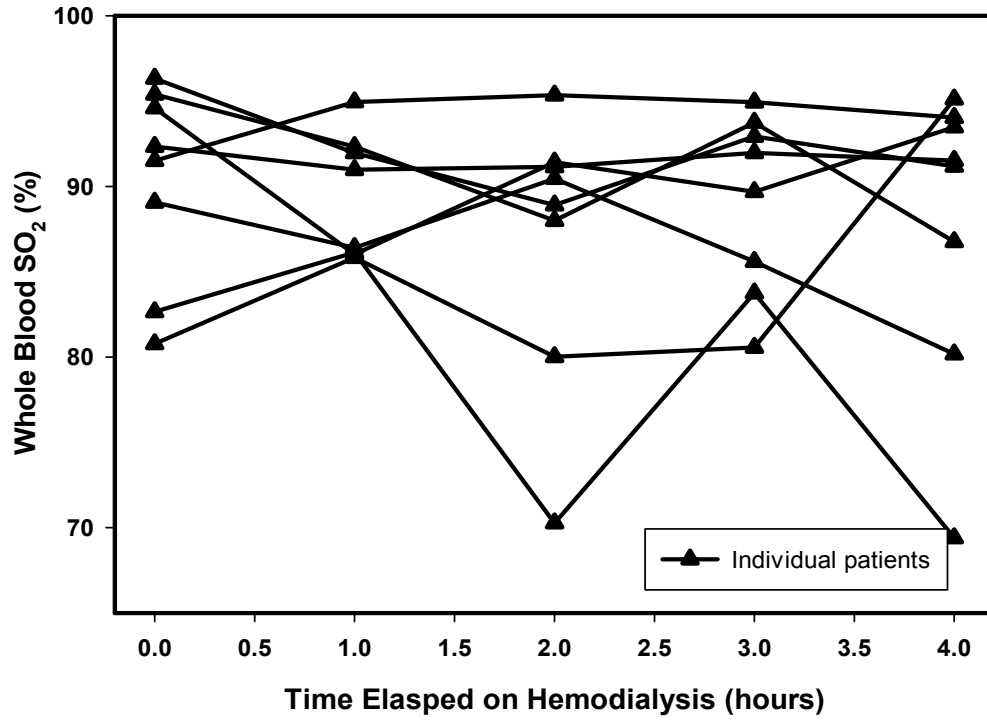
B
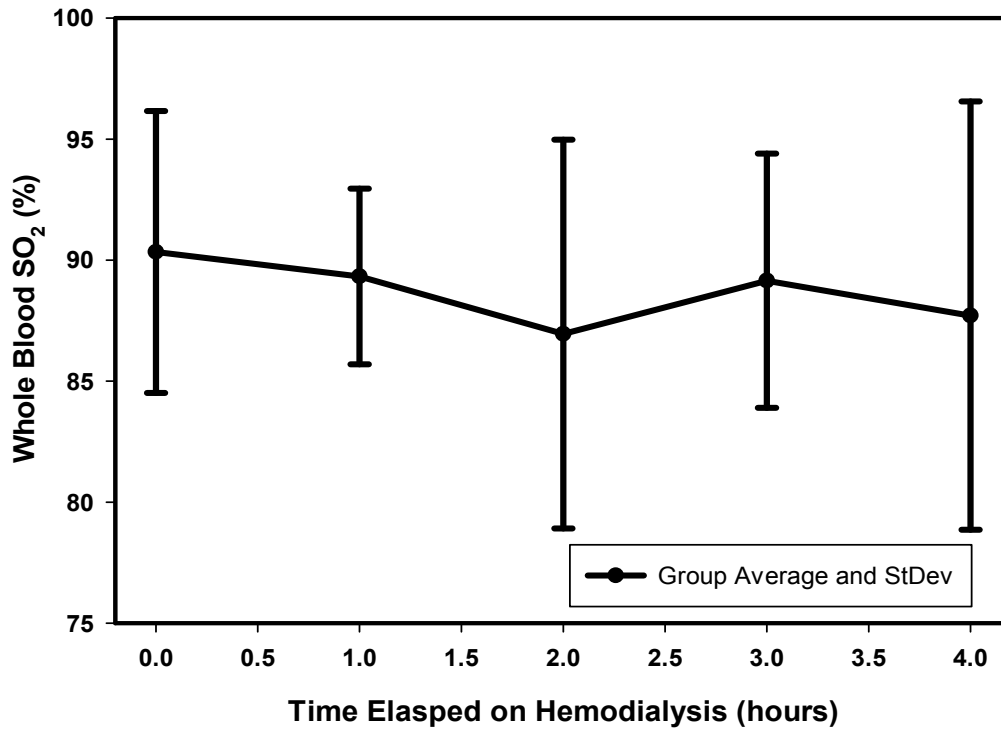
3.4. Whole Blood Transmittance Spectra

As discussed earlier (figure 12), 14 complete spectral data were obtained from 1000 µm cuvette and 8 complete spectral data were obtained from 100 µm cuvette. The normalized transmittance and changes in normalized transmittance during HD treatment were calculated for each patient and for each cuvette type. The VIS and NIR data are presented separately as the two ranges were obtained using different parameters and setup (see section 2.4.2 for details). A representative example of the blood transmittance spectra is shown for a single patient using 1000 µm cuvette data (figures 26 A and 26 B), VIS and NIR wavelength and using 100 µm cuvette (figure 28), VIS wavelength. For all patients and for all cuvette sizes we see changes in normalized transmittance in both the VIS and NIR wavelength (figures 27 A, 27 B, and 29). The regions where the largest changes occur are consistent across patients but the amplitude of the effect is patient-dependent which is consistent with the earlier published works of Lagali and colleagues (Lagali et al., 2006; Lagali et al., 2007). This suggests that we can proceed and assess the correlation between the clinical outcome measures discussed above and the analysis outcome of variance in the blood transmittance spectra during HD treatment.

Figure 26. A representative example of the blood transmittance spectra at 5 time points during hemodialysis for a single patient for, A) visible and, B) near-infrared wavelengths (1000 μm cuvettes).

A
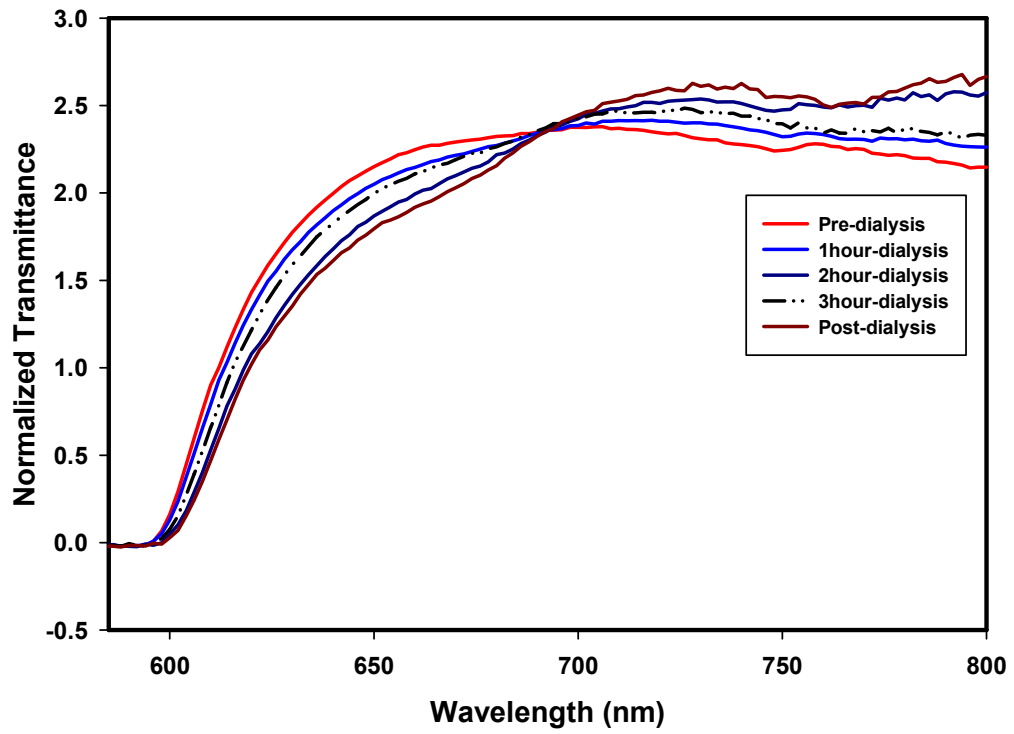
B
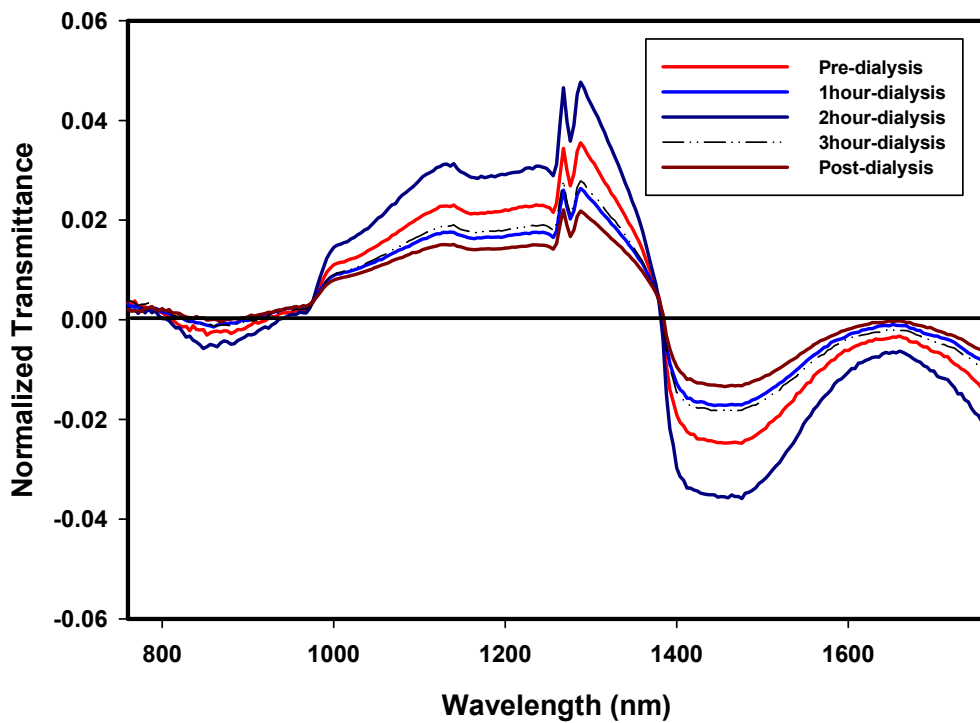
Figure 27. Changes in the blood transmittance spectra during hemodialysis treatment for 14 end-stage renal disease patients for, A) visible and, B) near-infrared wavelengths (1000 μm cuvette).

A
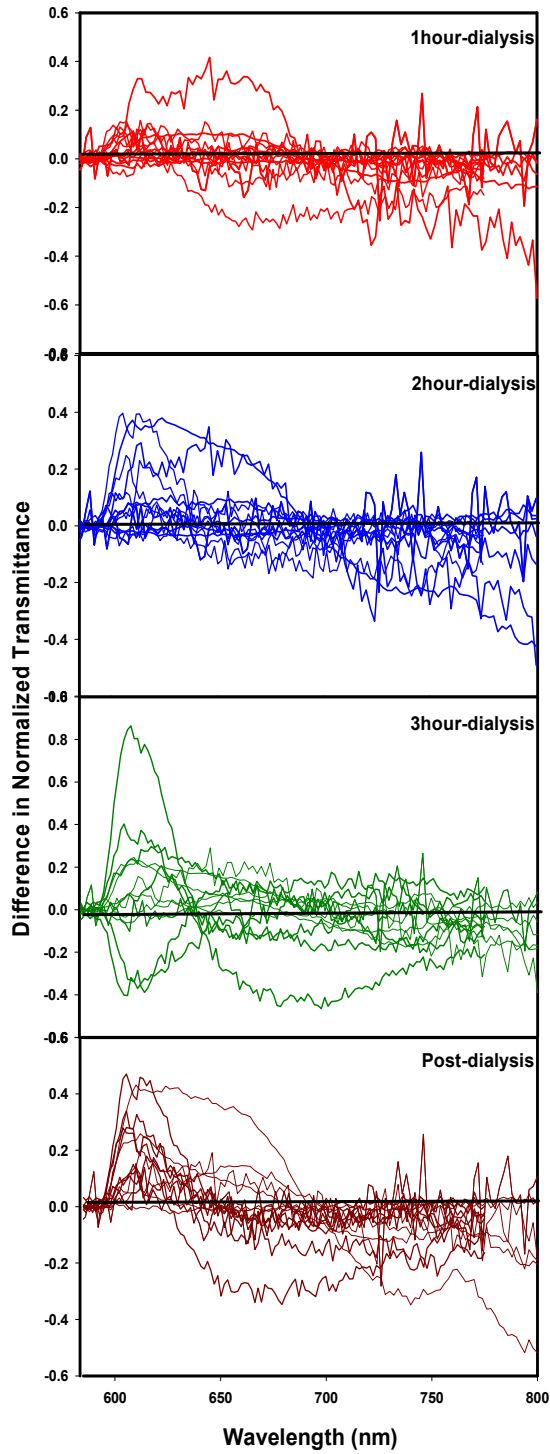
B
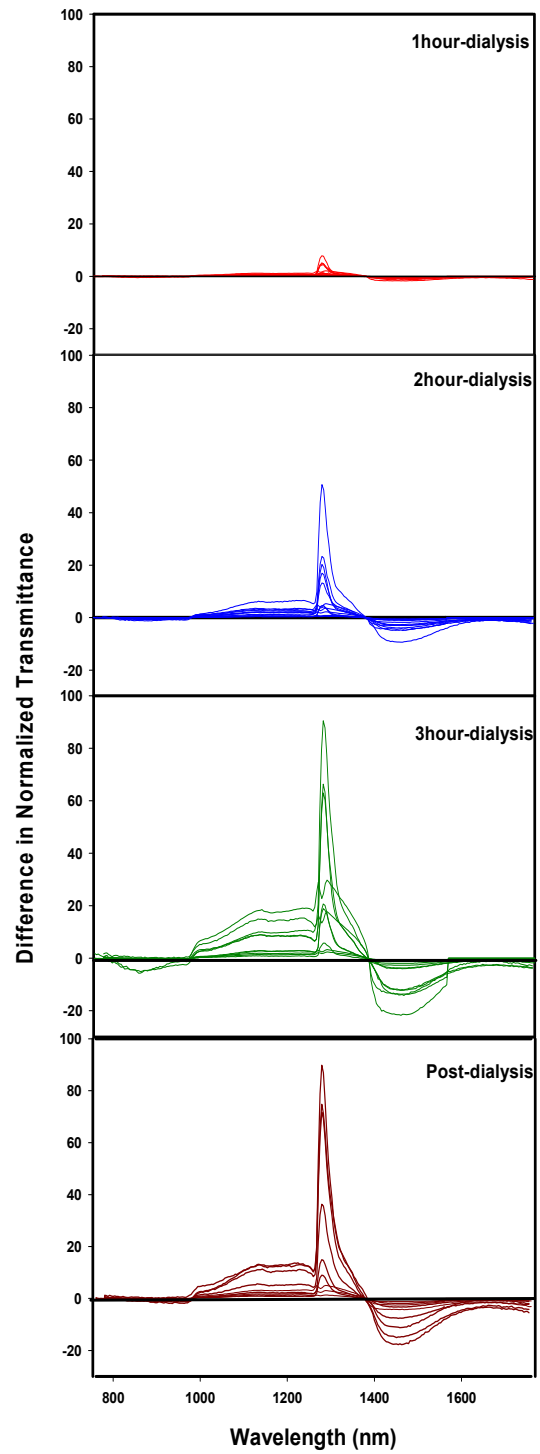
Figure 28. A representative example of the blood transmittance spectra in the visible wavelengths at 5 time points during hemodialysis for a single patient (100 μm cuvettes).
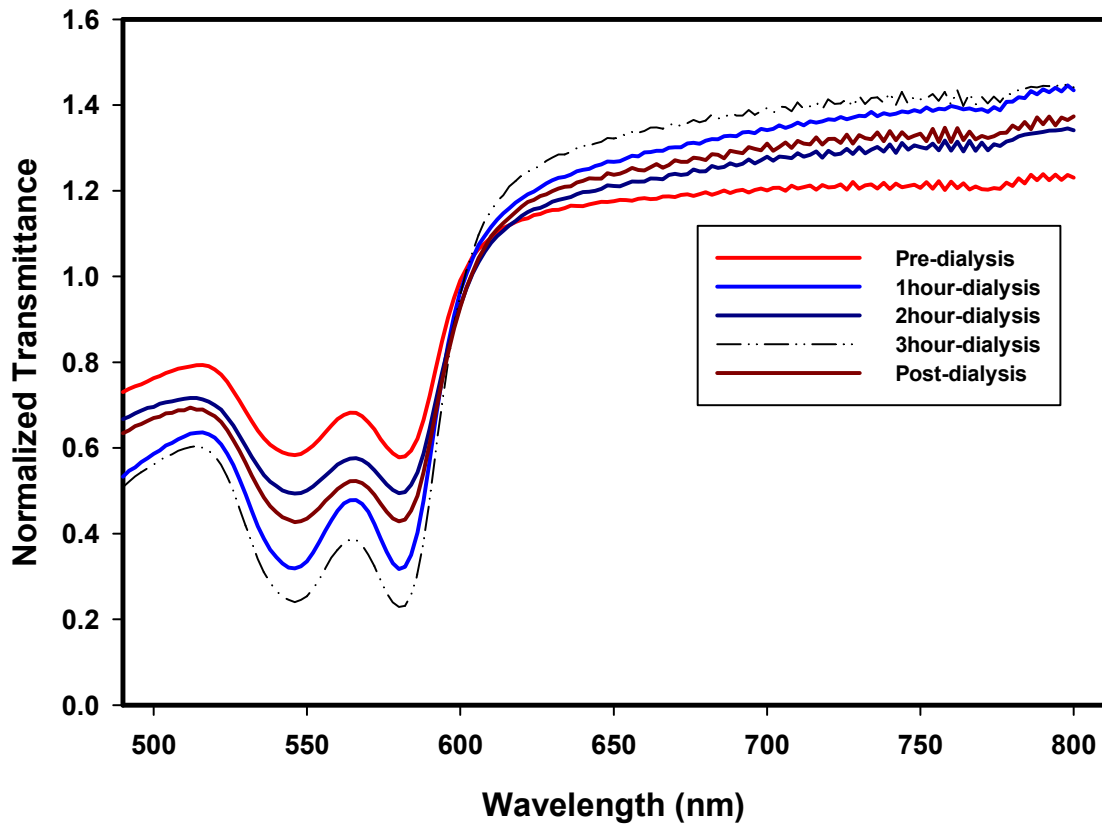
Figure 29. Changes in the blood transmittance spectra in the visible wavelengths during hemodialysis treatment for 8 patients with end-stage renal disease (100 μm cuvette).
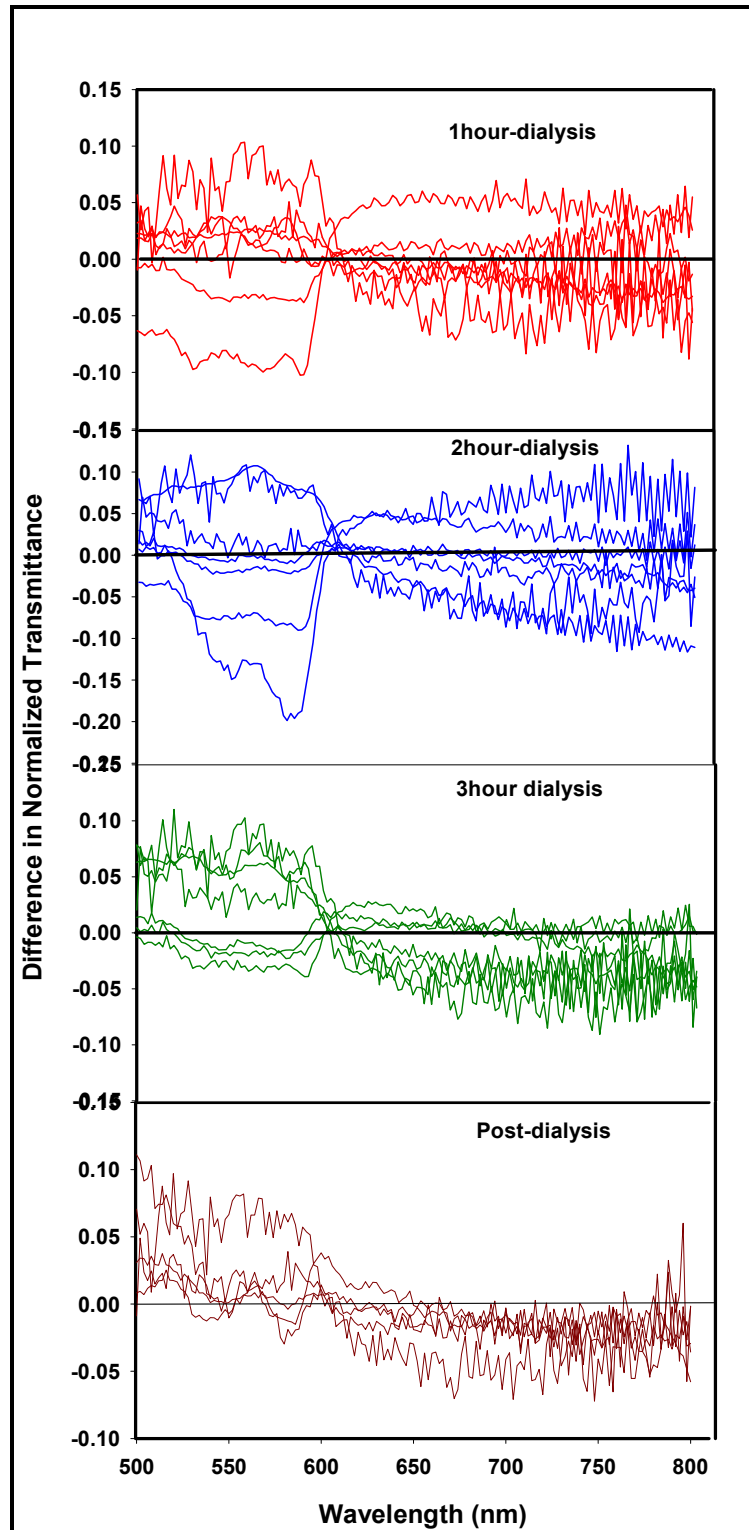
3.4.1. Correlation of the Variations and Changes in Acid-Base and Oxygen Parameters to Analysis Outcomes of Variance in Whole Blood Transmittance Spectra

The approach taken for the correlative analyses is to look at changes in the blood transmittance spectra at each wavelength and search for a correlation with each acid-base and oxygen parameter across individual patients. Given that the full spectrum (400 to 1700nm) has over 395 points, it is neither statistically advisable (there is always correlation with enough test variables) nor timely feasible to perform this analysis with every spectral point. Furthermore, as the changes are small, the data is noisy at each individual wavelength. The solution chosen was to reduce the number of variables by averaging the data over a wavelength range to, A) reduce the noise in the data by averaging, and B) reduce the number of test bands. The data was treated identically in the VIS and NIR creating 17 and 23 wavelength bands respectively in each range. Final values were calculated by using the area under the curve in the VIS range divided by bandwidth of 16 nm each and 40 nm each in NIR wavelength regions. Blood transmittance of every wavelength band was correlated to each acid-base and oxygen parameter. In order to evaluate the potential impact of the variations in acid-base and oxygen parameters on whole blood-base optical spectroscopy, blood transmittance spectra were correlated at every wavelength band to each acid-base and oxygen parameter across individual patients and across HD time points, for both the VIS and NIR wavelength regions, using the Pearson Product Moment Correlation analysis. Since the blood transmittance spectra of the 100 μm cuvette are dominated by absorbance and the 1000 μm cuvettes are dominated by forward scatter, there could be differences in the

spectral information obtained. For this reason correlations are performed on each data set separately in the analysis below.

The analysis outcomes of variance in blood transmittance spectra were correlated to variations and/or changes in each acid-base and oxygen parameter across 14 individual patients, at each time point during HD treatment. In order to facilitate comparisons between the 1000 μm and 100 μm cuvette spectral data, both results were plotted on the same graph with one graph for each acid-base and oxygen parameter. There were weak correlations found between the blood transmittance spectra and acid-base parameters ((|r| < 0.8 in all cases); pCO_2 (figure 30 A), HCO_3- (figure 30 B), pH (figure 31 A), pO_2 (figure 31 B), and oxygen parameters; blood gas SO_2 (figure 32 A) and whole blood SO_2 (figure 32 B) during HD treatment. The lack of strong correlation is consistent across both 1000 μm and 100 μm cuvettes, across VIS and NIR regions.

Although strong correlations were not found, certain regions of the blood spectrum had significant (P<0.05) correlations to all acid-base and oxygen parameters across patients. These regions are indicated by the solid lines in figures 30 to 32. However the correlation coefficients (r) for all the parameters tested were between 0.25 and 0.35. This indicates that perturbations in these parameters during HD treatment may have a minimal or no impact on the detection of HD-induced changes using optical spectroscopy.

Figure 30. Pearson Product Moment correlation of the whole blood transmittance spectra to variations in A) partial pressure of carbon dioxide (pCO_2) and B) changes in bicarbonate (HCO_3) in both the visible and near-infrared regions. Blue dotted line: 14 patients with 1000 µm cuvette data. Red dotted line: 8 patients with 100 µm cuvette data. The thick blue lines represent regions with significant correlation between analysis outcome of the variance in blood transmittance spectra and variations and/or changes in acid-base and oxygen parameters.

A
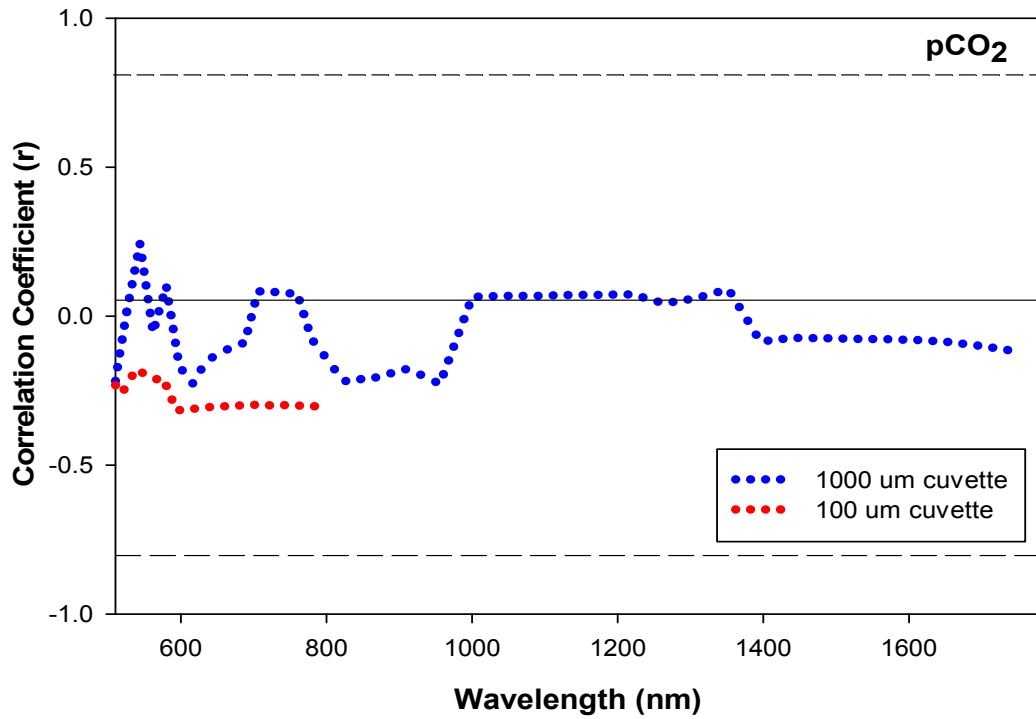
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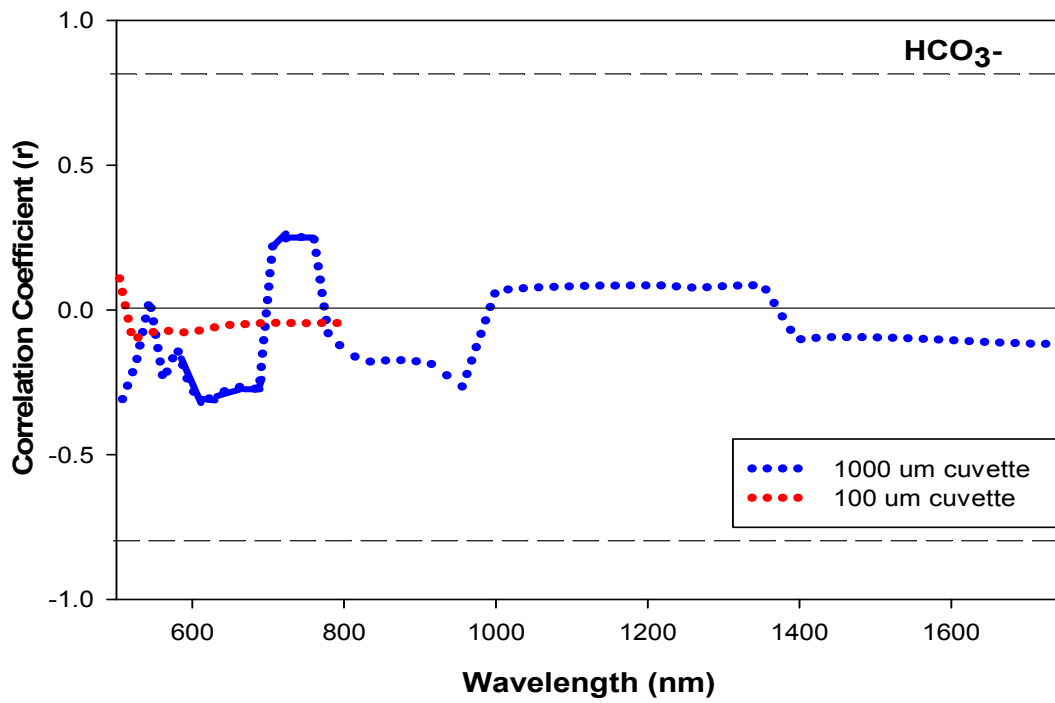
Figure 31. Pearson Product Moment correlation of the whole blood transmittance spectra to changes in A) pH and B) variations in partial pressure of oxygen (pO_2) in both the visible and near-infrared regions. Blue dotted line: 14 patients with 1000 μm cuvette. Red dotted line: 8 patients with 100 μm cuvette. The thick blue lines represent regions with significant correlation between analysis outcome of the variance in blood transmittance spectra and variations and/or changes in acid-base and oxygen parameters.

A
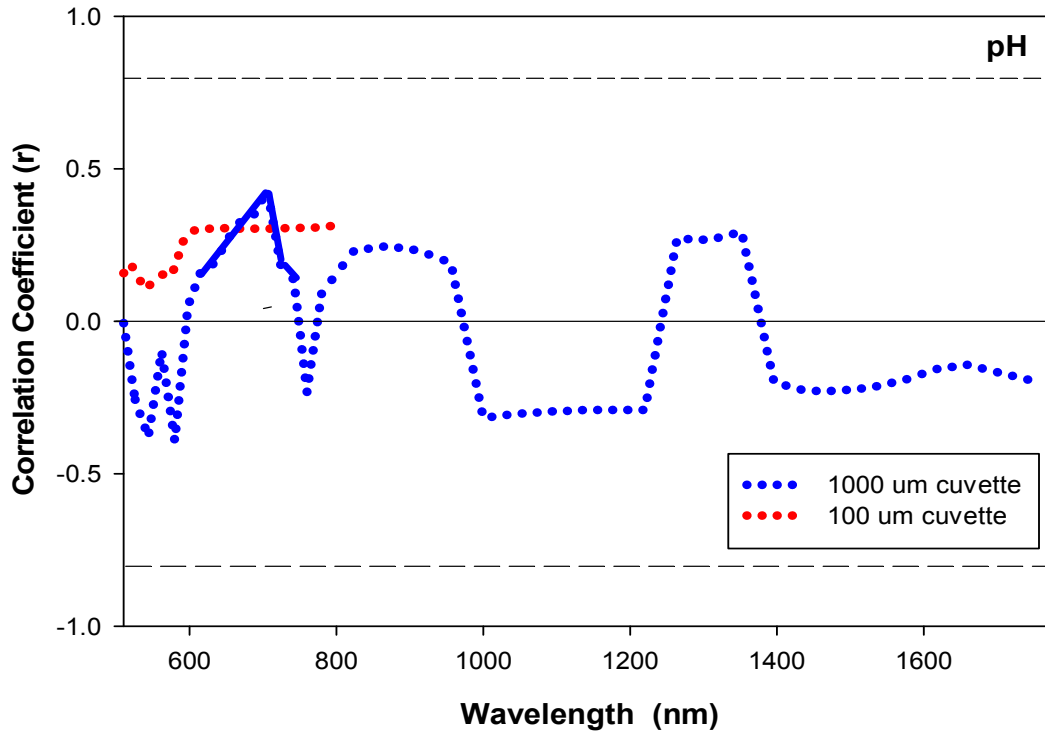
B
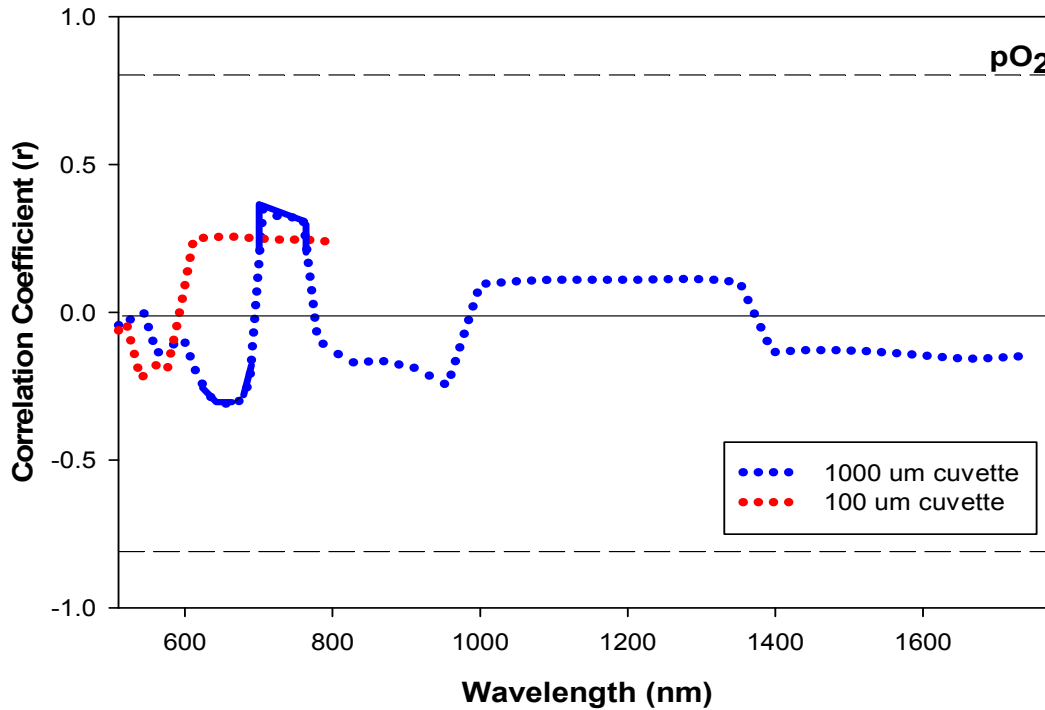
Figure 32. Pearson Product Moment correlation of the whole blood transmittance spectra of 8 patients correlated to variations in A) blood gas oxygen saturation (SO_2) and B) whole blood SO_2 in both the visible (VIS) and near-infrared (NIR) regions. Blue dotted line: 1000 μm cuvette data. Red dotted line: 100 μm cuvette data. The thick lines represent regions with significant correlation between analysis outcome of the variance in blood transmittance spectra and variations in oxygen parameters.

A
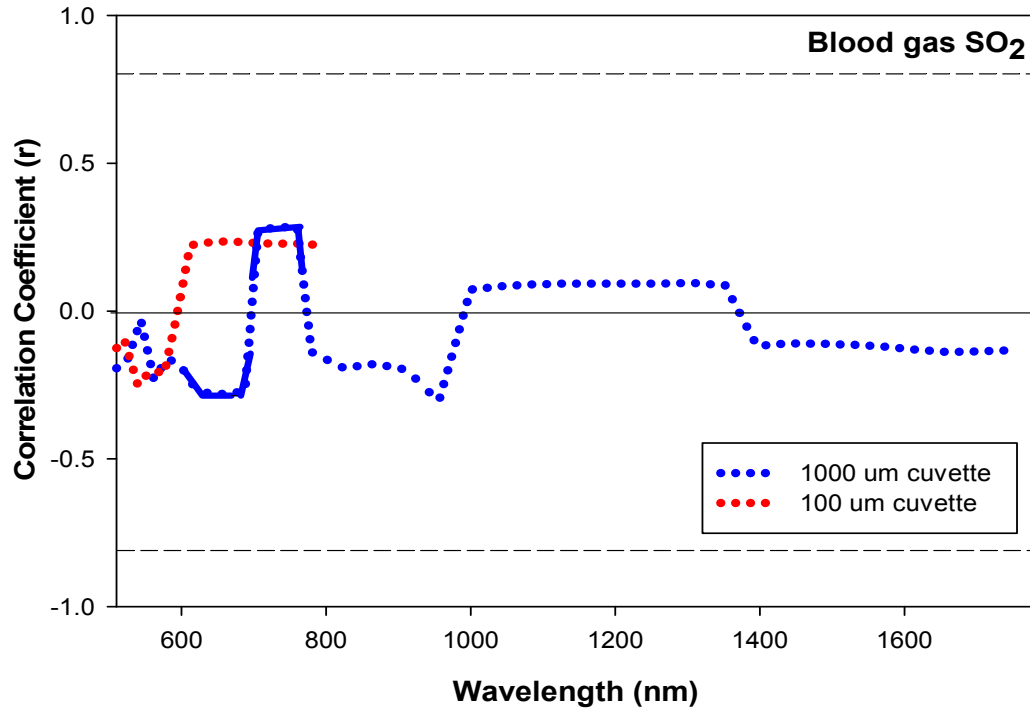
B
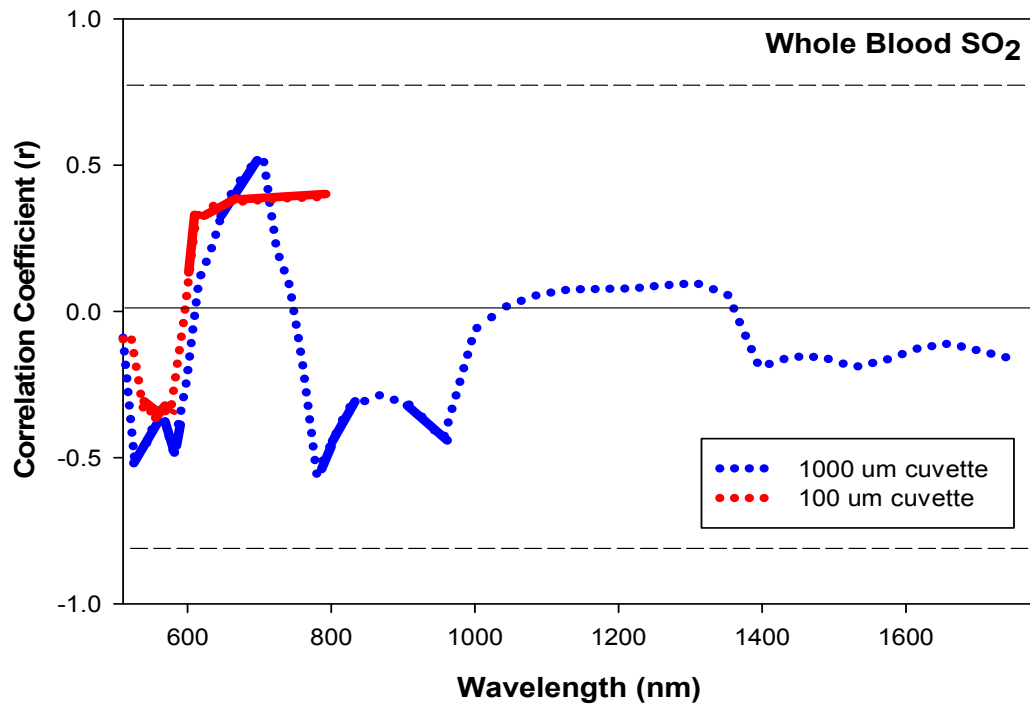
3.5. The Effect of Heparin in Modifying the Optical Properties of Whole Blood

In order to further improve the health assessment value of this novel method, the effect of heparin in modifying the optical properties of whole blood as a potential confounder was also investigated in a second pilot study as previously discussed.

3.5.1. Patient Demographics

A total of 5 ESRD patients on hemodialysis treatment were each monitored during a single HD treatment. Patient demographics are summarized in Table V. Enrolment included 4 females and 1 male, age ranged from 23 to 88 years old, 4 patients were hypertensive and a single patient was diabetic. Blood samples for spectroscopy testing (see section 2.4.2.3 for details) were collected from 5 ESRD patients at a single time before heparin administration.

Table V: Characteristics of the 5 end-stage renal disease patients recruited for the investigation of the effect of heparin in modifying the optical properties of whole blood and its impact on whole blood-based optical spectroscopy in the second pilot study.

Patient	Age	Sex	Hypertensive	Diabetic	Vascular access
1	23	F	No	No	CVC
2	75	M	Yes	Yes	AVF
3	68	F	Yes	No	CVC
4	88	F	Yes	No	AVF
5	82	F	Yes	No	AVF
	67				

3.5.2. The Concentration Dependence of the Optical Properties of Heparin in Solution

Once a heparin solution is added to a blood sample, there are two ways to possibly detect heparin, 1) heparin can be a free molecule in solution and we might detect its particular optical properties; 2) heparin can modify the chemistry of whole blood and thus its optical properties (absorption and transmission). In order to address the first scenario, the transmittance spectra of heparin solutions with 4 different concentrations were measured.

Since the solvent in a heparin solution is water, we need to separate the optical properties of water and heparin, for this reason we obtained the spectra of de-ionized water to use as a control. Transmittance spectra of heparin solutions with different concentrations are presented in the VIS (figure 33 A) and NIR (34 A) regions. There are differences in transmittance of heparin solutions across all 4 concentrations (heparin subtracted from pure water transmittance) for VIS (figure 33 B) and NIR (and 34 B) regions. These differences in transmittance are detectable within instrument repeatability level (0.01%) for all 4 concentrations of heparin solutions.

However the differences in transmittance of all 4 concentrations of heparin solutions are flat across the VIS region, suggesting that these differences are not likely due to the optical properties of heparin and if these differences are due to heparin, they will not be differentiated from changes in the overall transmittance. In the NIR region, the differences in transmittance spectra of heparin solution cross all 4 concentrations are

wavelength-dependent with a characteristic spectral shape. This indicates that in the NIR region, the differences observed across all 4 concentrations of heparin solutions are most likely due to the optical properties of heparin.

Nonetheless there are no features in the spectrum that are indicative of the dependence of the optical properties of heparin on its concentration in the VIS region. However, transmittance decreases as a function of heparin concentration in the NIR regions of the spectrum with pure heparin; 1.0 units/µl having the greatest reduction in transmittance and 1.0×10^{-1} units/µl with the least reduction in transmittance. Since heparin is detectable as a free molecule in solution (NIR region), its effect in modifying the optical properties of whole blood was evaluated.

Figure 33. A) The transmittance spectra of 4 different concentrations of heparin diluted with water. B) The difference in transmittance of heparin solutions from water plotted as a function of wavelength in the visible (VIS) region from 585 nm to 800 nm.

A
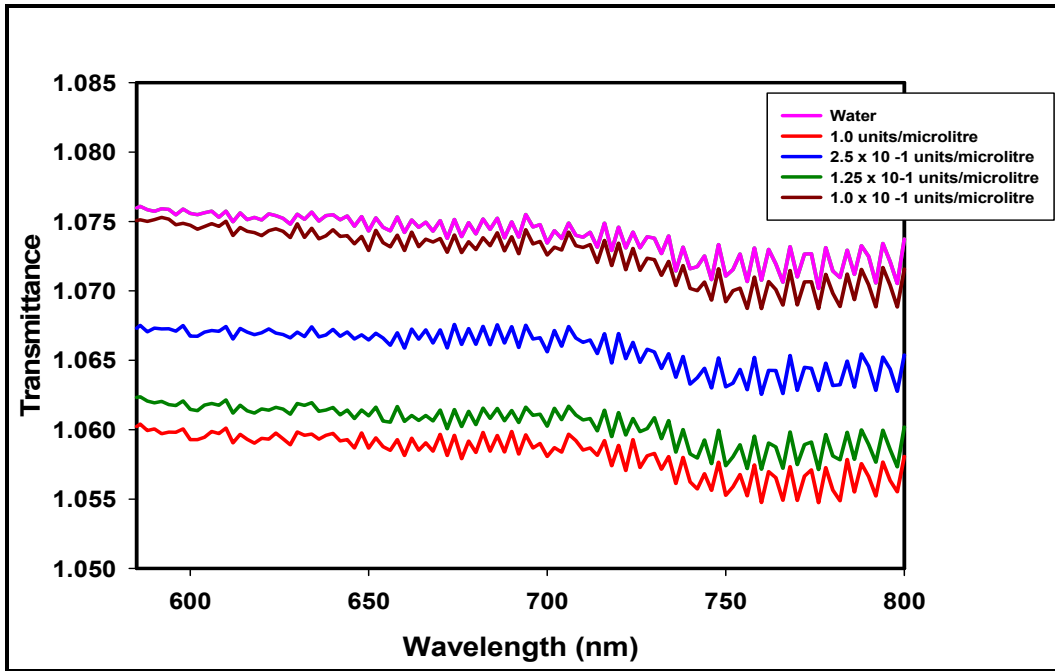
B
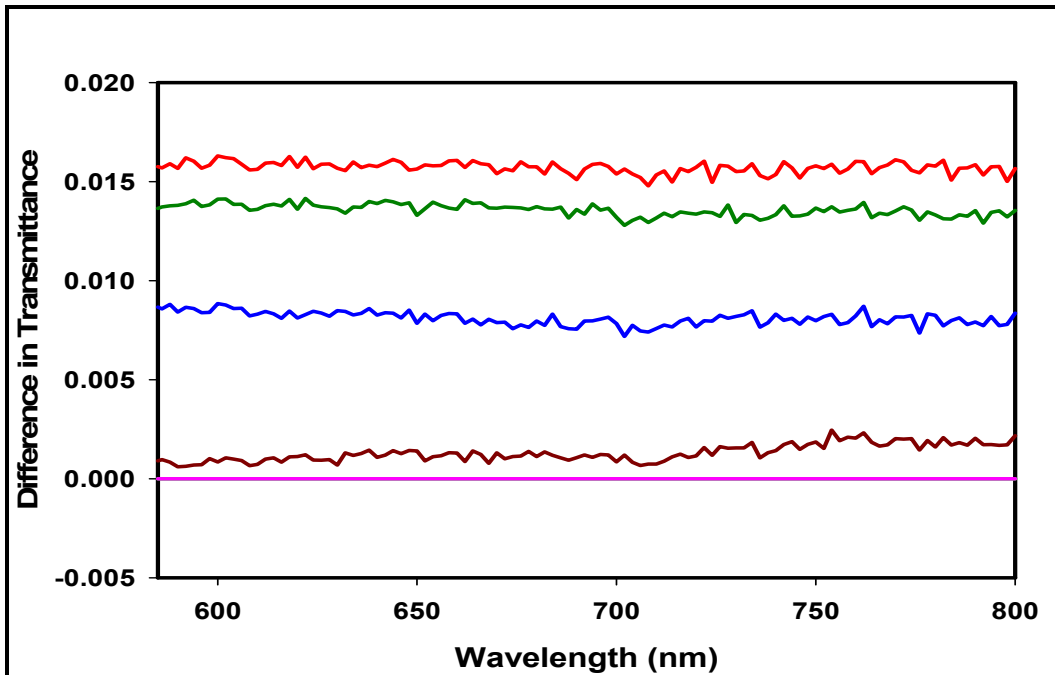
Figure 34. A) The transmittance spectra of 4 different concentrations of heparin diluted with water. B) The difference in transmittance of heparin solutions from water plotted as a function of wavelength in the near-infrared (NIR) region from 760 nm to 1760 nm.

A
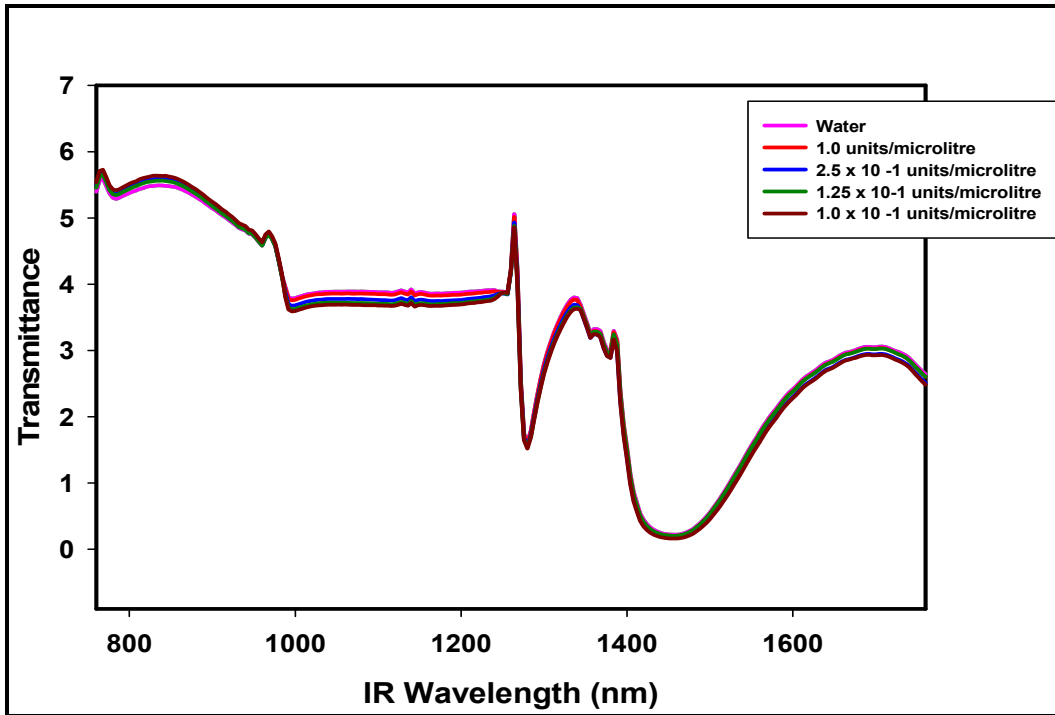
B
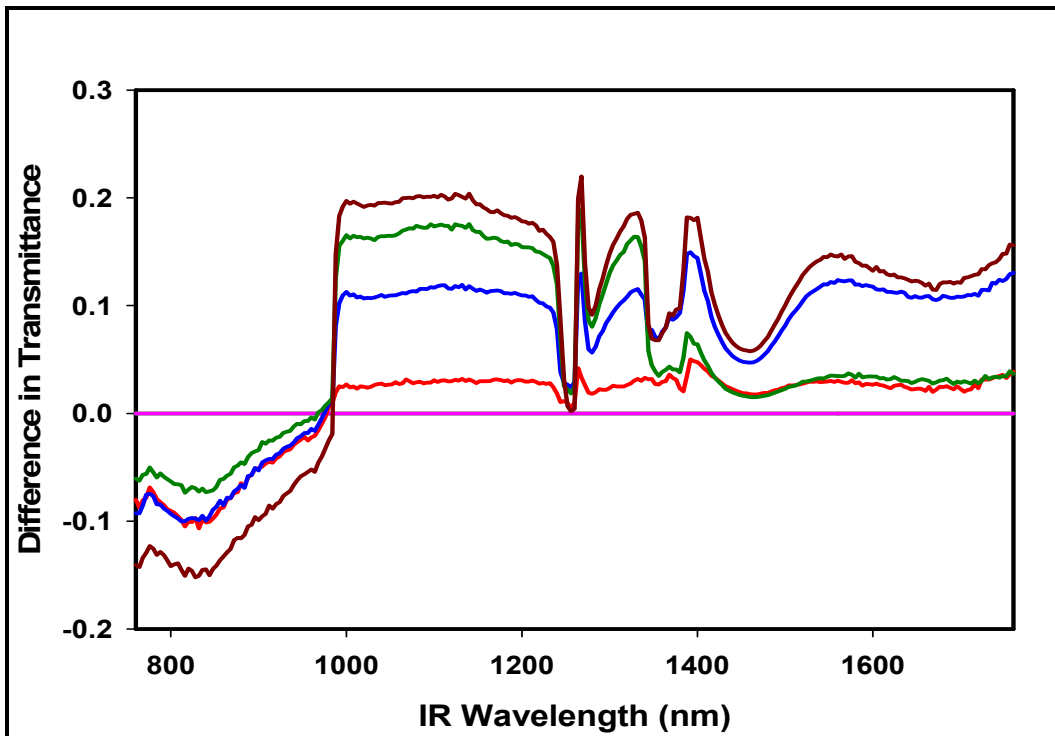
3.5.3. The Effect of Heparin in Modifying the Optical Properties of Whole Blood

In order to address the second scenario, the potential impact of heparin in modifying the optical properties of whole blood, transmittance spectra of blood diluted with 4 different concentrations of heparin were measured.

Pre-dialysis (not heparanized) blood sample from each patient was spiked with 4 different concentrations of heparin. The blood transmittance spectra for all 5 ESRD patients are presented for 4 different concentrations of heparin in the VIS (figure 35 A) and NIR (figure 36) wavelength ranges. Every patient respond differently to the same concentration of heparin, however the shapes of the spectra are identical for each patient although the amplitude varies slightly across patients. This indicates that the variations observed in the spectra are not concentration dependent because the change due to heparin is very small and dose variance is inconsistent across patients.

In order to highlight the effect of heparin in modifying the optical properties of whole blood, the difference in transmittance was determined by taking blood transmittance only (no heparin) as a control and the transmittance due to the 4 concentrations of heparin were subtracted from it. The differences in transmittance from blood samples with 4 different heparin concentrations are shown in the VIS (figure 34 B) and NIR (figure 37) wavelength ranges. There are differences in the effect of heparin in modifying the optical properties of whole blood that are detectable at all 4 concentrations of heparin using optical spectroscopy. However, these differences are not consistent

across patients. Blood samples from each patient show a unique response to heparin at every concentration that is detectable within instrument repeatability level (0.01%), in both the VIS and NIR regions.   However, these differences are very small and random across patients, and most importantly are insignificant relative to changes (300 times less) detected in the blood status of ESRD during HD treatment. This indicates that the effect of heparin in modifying the optical properties of whole blood does not confound the detection of HD-induced changes in the blood status of ESRD patients, using optical spectroscopy.

Figure 35. A) Transmittance spectra of pre-dialysis blood samples from 5 end-stage renal disease (ESRD) patients diluted with 4 different concentrations of heparin. B) The difference in blood transmittance plotted as a function of visible (VIS) wavelength from 585 nm to 800 nm. *Negative control is blood diluted with water.

A

Patient 1

Patient 2

Patient 3

Patient 4

Patient 5

Transmittance

Wavelength (nm)

Legend:
- Blood
- Negative control
- 5.3 x 10-3 units/microlitre
- 1.3 x 10-3 units/microlitre
- 6.6 x 10-4 units/microlitre
- 5.3 x 10-4 units/microlitre

B

Patient 1

Patient 2

Patient 3

Patient 4

Patient 5

Difference in Transmittance

Wavelength (nm)

Legend:
- Blood
- Negative control
- 5.3 x 10-3 units/microlitre
- 1.3 x 10-3 units/microlitre
- 6.6 x 10-4 units/microlitre
- 5.3 x 10-4 units/microlitre

Figure 36. Transmittance spectra of pre-dialysis blood samples from 5 end-stage renal disease (ESRD) patients diluted with 4 different concentrations of heparin plotted as a function of wavelength from 760 nm to 1760 nm in the near-infrared (NIR) region. *Negative control is blood diluted with water.
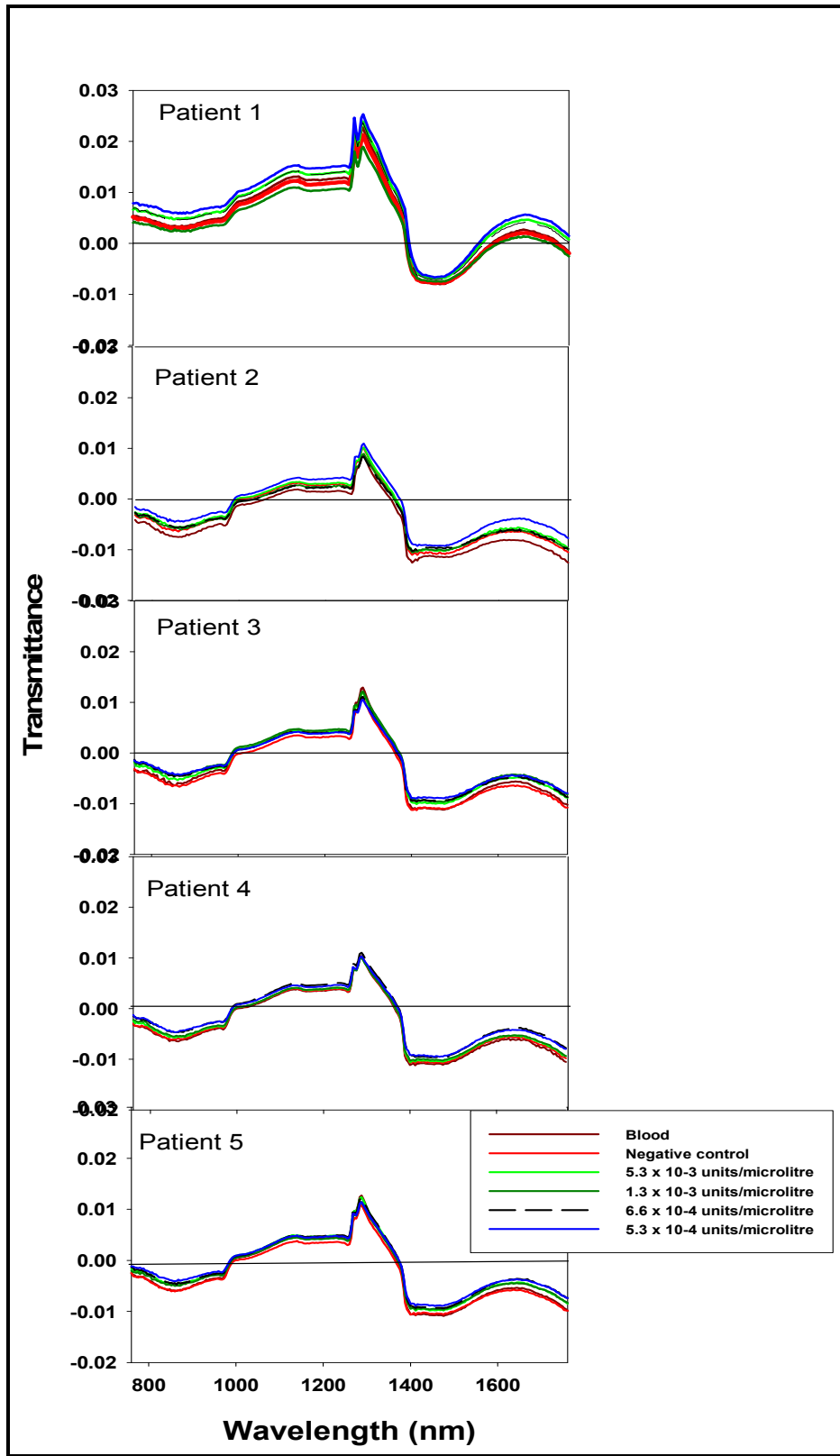
Figure 37. The difference in blood transmittance of 4 different concentrations of heparin from blood samples of 5 ESRD patients plotted as a function of wavelength from 760 nm to 1760 nm in the near-infrared (NIR) region. *Negative control is blood diluted with water.

The figure shows five stacked panels (Patient 1 through Patient 5) plotting Difference in Transmittance (y-axis, ranging from -0.006 to 0.006) versus Wavelength (nm) (x-axis, ranging from approximately 800 to 1600 nm).

Legend:
Blood
Negative control
5.3 x 10-3 units/microlitre
1.3 x 10-3 units/microlitre
6.6 x 10-4 units/microlitre
5.3 x 10-4 units/microlitre
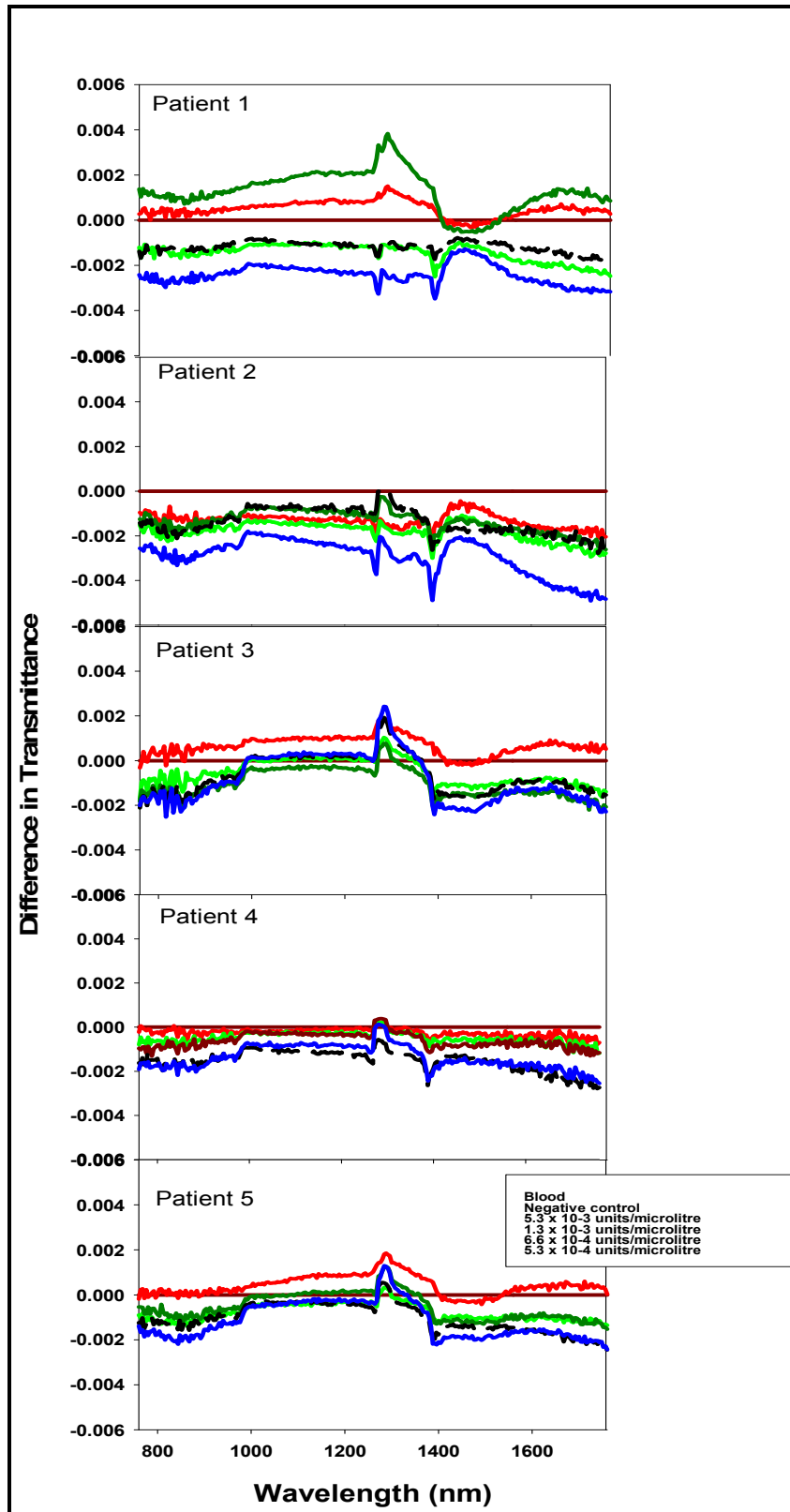
4. DISCUSSION

The first objective of this thesis was to investigate the effect of the perturbation of acid-base and oxygen parameters due to the hemodialysis procedures on whole blood-based optical spectroscopy.   More specifically, changes in the acid-base parameters (pCO_2, HCO_3-, and pH) and oxygen parameters (SO_2 and pO_2) for individual patients were correlated to variance in the blood spectroscopy measurements during HD treatment. Additionally, SO_2 was estimated from whole blood, correlated to blood transmittance measurements during HD treatment, and compared to SO_2 result obtained from hemoglobin solution (laboratory blood gas).   As a second objective, the effect of heparin in modifying the optical properties of whole blood as a potential confounding factor on the ability to monitor changes in ESRD patients during HD treatment via optical spectroscopy was also investigated.

4.1. Changes in Whole Blood Transmittance Spectra during Hemodialysis Treatment

The results indicate that significant measurable changes in whole blood spectra were detectable in a cross-section of ESRD patients during a 4 hour HD treatment using optical spectroscopy. This is consistent with the previous findings of Lagali and colleagues (Lagali et al., 2006; Lagali et al., 2007). However, these differences were not consistent between the spectral data obtained using 1000 and 100 μm cuvettes. For the spectral data obtained using the 1000 μm cuvette, significant spectral differences were detected in isolated regions of the VIS wavelengths (indicated by the red arrow in figure

37 A), across all 5 time points during HD treatment. However, significant measurable differences in the blood transmittance spectra were also detected only between pre-and post-dialysis in isolated regions of the VIS and NIR wavelengths (indicated by the blue arrows in figures 37 A and B). For the spectral data obtained in a thinner optical cell (100 μm cuvette), there were no significant ($P>0.05$) measurable differences found between the blood transmittance spectra at all 5 time points during HD treatment.

There is a lack of consistency between the blood spectral data obtained using 1000 and 100 μm cuvettes. This finding was not expected as one would suppose that light illuminates a sample in a thinner optical cell evenly without loss due to scattering effect of the cuvette. In previous experiments in our lab, it was found that a blood sample in a thinner optical can easily aggregate and dry off. Therefore detecting relative changes in the blood is difficult with 1000 μm cuvette compared to a thicker optical cell with 1000 μm.

The result from ANOVA revealed that each patient had a characteristic spectrum. Significant ($P<0.05$) differences in the blood spectra were found between patients in isolated regions of the VIS wavelength (1000 μm cuvette) and across the entire VIS wavelength (100 μm cuvette). In the NIR wavelength, no significant ($P>0.05$) differences were detected in the blood spectra between patients. The result in the VIS wavelength is consistent (1000 μm cuvette) with previous findings by Lagali and colleagues, whereby spectral differences were detected between the pre- and post-dialysis blood samples and each patient had a characteristic spectrum (Lagali et al., 2006; Lagali

et al., 2007). The advantage of this finding demonstrates the potential use of whole blood-based optical spectroscopy in identifying patients based on individual need and devising treatments to meet that need. In order to evaluate whether the changes detected in whole blood spectra during HD treatment were due to confounding factors, variance in the blood spectroscopy measurements were correlated to changes in both the acid-base and oxygen parameters across individual patients.

4.2 The Effects of the Perturbations of Acid-Base and Oxygen Parameters due to Hemodialysis Procedures on Whole Blood-Based Optical Spectroscopy

Correlations between the blood transmittance spectra and all the acid-base and oxygen parameters in both the VIS and NIR wavelength regions were all well below 0.8. This lack of strong correlations is consistent across spectral data obtained using both samples with the 1000 μm and 100 μm optical path thicknesses. In most of the spectral regions, these correlations are not statistically significant but some narrow regions of the blood spectrum did show significant ($P<0.05$) associations with both or either the acid-base and oxygen parameters in the 1000 μm cuvette data. Since the Pearson correlation coefficients (r) were all less than 0.4 even when statistically significant correlations were found for all the parameters tested, it is concluded that the variations and/or changes in these parameters do not impact the ability to detect HD-induced changes in the blood status of ESRD patients.

Because of sample manipulation, blood samples used for spectroscopy measurements were highly saturated and to further confirm the potential effect of the

variations and dominance of SO_2, oximetry was performed on whole to extract the real SO_2 and compared to outcomes in blood transmittance spectra.

4.2.1. Variations in Oxygen Saturation derived from Whole Blood

Whole blood SO_2 values were calculated using the Beer-Lambert absorbance model at 5 time points during HD treatment. ANOVA revealed no significant differences in whole blood SO_2 (both venous and arterial) across time points in the 8 ESRD patients studied.

Subsequently, these data were correlated to analysis outcome of variance in whole blood transmittance spectra obtained in both 100 and 1000 μm cuvette. For the spectral data obtained using 1000 μm cuvette, isolated regions in both the VIS and NIR wavelength regions were found to be significantly associated with whole blood SO_2. Interestingly, some regions also overlapped with the regions that were found to be significantly associated with SO_2 obtained hemoglobin solution from the blood gas laboratory (1000 μm cuvette).

For the blood transmittance spectral data obtained using 100 μm cuvette, wavelength regions (500nm to 600nm) that are normally dominated by SO_2 were found to have significant associations with whole blood SO_2 (Meinke et al., 2007). There is a consistency in the wavelength regions that were found to be significantly correlated to SO_2 obtained from both hemoglobin solution and whole blood, and both 100 and 1000

μm cuvette spectral data. This finding was not surprising since SO_2 is known to dominate these same regions (500 nm to 600) because of the distinct optical properties of the two hemoglobin derivatives; O_2Hb and HHb (Meinke et al., 2007; Diaconnu, 2009).

Although variations in SO_2 derived from whole blood had significant correlation to analysis outcome of variance in whole blood transmittance spectrum, the correlation coefficients were moderate (0.4-0.5). This result illustrates that although SO_2 is detectable in whole blood; its variation during HD treatment is not a confounding factor. Additionally, the whole blood SO_2 value was derived from a fit curve that demonstrated systematic deviations from the Beer-Lambert absorbance model for a mixture of O_2Hb and HHb, which could have an impact on whole blood SO_2 values.

Overall, our findings indicate that variations and/or changes in both the acid-base; HCO3-, pCO_2 and pH, and oxygen parameters; SO_2 and pO_2, are detectable using optical spectroscopy. However, the perturbations in these parameters during HD treatment do not confound the detection of HD-induced changes in the blood transmittance spectra of ESRD patients during HD treatment. In order to further improve the health assessment value of this novel method, the potential impact of heparin on whole blood signature was evaluated (next section).

4.3. The Effect of Heparin in Modifying the Optical Properties of Whole Blood

4.3.1. The Concentration Dependence of the Optical Properties of Heparin in Solution

The effect of heparin in modifying the optical properties of whole blood as a potential confounding factor was investigated. Spectral data obtained for heparin solutions at different concentrations revealed that the optical properties of heparin depend on its concentration only in the NIR region. The highest concentration of heparin contributed to transmittance reduction while the more dilute concentration had the least effect. However there were no changes in transmittance and the spectra were flat and was not dependent on heparin concentration in the VIS region. In the NIR region, differences in the transmittance between 4 different concentrations of heparin were wavelength dependent with a characteristic spectral shape and these differences were most likely due to the optical properties of heparin. Since heparin was detectable as a free molecule in solution (NIR region), its effect in modifying the optical properties of whole blood and its impact on our method was assessed.

4.3.2. The Effect of Heparin in Modifying the Optical Properties of Whole Blood

Nonetheless, heparin did not show the same behavior when added to whole blood as observed in free solution. There was no concentration dependence of the optical properties of heparin on whole blood in both the visible and NIR region. There were differences in the effect of heparin in modifying the optical properties of whole blood that were detectable at different concentrations. However, these differences were very small and random across patients as a function of concentration of heparin in both the VIS and

NIR regions. Although the effect of heparin in modifying the optical properties of whole blood was small and random across patients, the variations in the spectra could be due to the distinct blood chemistry between patients. In the circulation, heparin prevents thrombogenesis by interacting with antithrombin, the primary physiological inhibitor of blood coagulation (Desai et al., 1998). The variations observed in the blood transmittance spectra could be due to differences in two principle procoagulant proteases, factor Xa and thrombin among patients. The formation of antithrombin - heparin complex greatly increases the rate of inhibition of these two factors. Accelerated inactivation of both the active forms of proteases prevents the subsequent conversion of fibrinogen to fibrin that is crucial for clot formation (Rosenberg and Bauer, 1994). The coagulation cascade is affected by other factors such plasma proteins, calcium and EDTA in the blood which could have contributed to the variations in blood transmittance spectra for the same concentration of heparin among patients.

As well the variations in whole blood transmittance spectra across patients were perhaps due to the dependence of the optical properties of heparin on the acid-base status of the carboxyl group on the d-glucuronic acid (Park and Chakrabarti, 1977).

It is well known that acid-base status in ESRD patients fluctuates tremendously during HD treatment and patients are especially in acidic status before hemodialysis treatment commences (Uribarri et al., 1998). Furthermore, patients are not all in the same acid-base status and this can cause variations in just the blood spectra alone without the addition of heparin. Subsequently, when heparin is diluted with water to create different

concentrations, its optical properties can potentially change. After the addition of each concentration of heparin to the blood samples of ESRD patients with different acid-base status, the effect of heparin is no longer concentration dependent. Unfortunately, acid-base data of the 5 ESRD patients were not obtained at the time of this study. Therefore, a speculation on the potential effect of the acid-base status of patients on the optical properties of heparin is not possible at this point, perhaps in future work. Furthermore, volume change was not a factor contributing to the changes observed at the different concentrations of heparin since the volume of heparin solution was kept constant across all samples.

We have clearly shown that the effect of heparin in modifying the optical properties of whole blood is detectable at every concentration within instrument repeatability level (0.01%). However these differences are not significant relative to changes (300 times less) detected during HD treatment.

4.4. The Novelty of Whole Blood-Based Optical Spectroscopy in the Context of Current State of the Art Methods

This thesis has clearly demonstrated that optical spectroscopy can detect significant HD-induced changes in blood samples from ESRD patients undergoing HD treatment. HD monitoring in ESRD patients using whole blood fingerprinting that encompasses all markers of renal insufficiency simultaneously is a novel approach in the field of nephrology. This potentially new approach for monitoring HD adequacy and long-term patient outcome is unique because it is not based on monitoring of individual

molecules and thus it is not comparable to the current state of the art techniques such as mass spectrometry and gas chromatography (Niwa, 2009) which are based on identifying individual molecules. Although these techniques have been successfully applied for the identification of specific uremic toxins, studies have shown that no specific uremic toxin by themselves are good prognosticator of HD adequacy and long-term patient outcome (Vanholder et al., 2008; Eknoyan et al., 2002). The major difference between the whole blood-based optical spectroscopy and the current state of the art methods or strategies is its long-term potential application in detecting changes in the blood of ESRD patients over time and not uremic toxins which has been proven to be ineffective. Our novel strategy potentially overcomes the major challenges in finding uremic toxins that are effective prognosticator of HD adequacy and long-term patient outcome, thus reducing the high mortality rate in ESRD patients on HD treatment and improving long-term patient outcome.

4.5. Conclusions

The variations and changes in acid-base parameters during HD treatment were found not to be significant confounding factors in detecting the changes in the optical fingerprint occurring during HD treatment. Similarly, variation in oxygen saturation (both whole blood and laboratory blood gas data) during HD treatment was not an issue in using the new methodology for optically quantifying changes in the blood of ESRD patients using optical spectroscopy. The effect of heparin in modifying the optical properties of whole blood was very small but detectable; however the effect was approximately 300 times smaller than changes seen during HD and thus is not likely to be

a confounding factor. Significant measurable changes were detected in the blood spectra across ESRD patients during the 4 hour HD treatment however; each patient had a characteristic spectral signature. The lack of uniform spectral changes across ESRD patients indicates individual specific response which means it might be possible to correlate dialysis efficacy to individual changes in fingerprint and thus serve as an indicator of treatment efficacy.

4.6. Future Directions

We have shown that whole blood-based optical spectroscopy is able to detect HD-induced changes in the blood status of ESRD patients during HD treatment independent of confounding factors. However, the challenge right now is developing algorithm that can identify parameters with clinical significance from the blood spectrum. The ongoing long-term study (MISSION) in our laboratory should be able to address this challenge by identifying elements with clinical importance that can serve as indicators of HD adequacy and long-term patient outcome.

Further work is warranted to determine whether medical conditions such as diabetes can potentially affect the detection of changes in the blood status of ESRD patients during HD using optical spectroscopy.   Preliminary work can be performed with animal blood in order to establish the appropriate concentration range of glucose to be tested using optical spectroscopy. Based on this result, it can be determined whether or

not glucose is detectable in blood and this work can furthermore be extended to healthy subjects in a fast state and then to ESRD patients on HD treatment.

Additionally, since ESRD patients on HD treatment receive multiple drugs (i.e. hypertensive and cardiac drugs) at the same time, the effects of all these drugs as confounding factors should be investigated using the same approach as suggested for the glucose study.

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APPENDIX I

English Informed Consent form
Oxygen Study

Patient Information Sheet and Consent Form

Protocol Title: **A pilot study to evaluate whole-blood changes in end-stage renal disease patients as a result of dialysis treatment**

Principal Investigator: **Dr. Rejean Munger**
Co-Investigator: **Dr. Kevin Burns**

Managed by the Kidney Research Centre- Clinical Trials Group

INTRODUCTION:

We are asking you to take part in a research study at the Ottawa Hospital. Taking part in this study is completely voluntary. The quality of your care will not be affected by your decision to participate or not. The study is described below. This description tells you about the purpose, additional tests, and risks/discomforts, which you might experience. You should take the time to read this information sheet and discuss any questions you have about your potential participation in this study with the person who explains it to you.

PURPOSE OF THE STUDY:

You are being asked to participate in this research study because you have kidney failure and you are currently receiving hemodialysis. The purpose of this study is to determine if it is possible to monitor changes in your blood (using blood samples) resulting from your condition using a novel technique developed at the University of Ottawa Eye Institute. If this technique proves successful, it could, in the long term, result in a method to monitor your condition that requires much fewer blood samples.

We are planning on enrolling approximately 65 hemodialysis patients (and 10 normal controls) in this research study at the Ottawa Hospital.

STUDY PROCEDURES:

Currently you are receiving hemodialysis three times per week in hospital. We are asking your permission to draw 17 additional tubes of blood (approximately 3 tablespoons) at the time of a regularly scheduled treatment. Four 3ml tubes would be drawn before dialysis, three more after each hour on dialysis and four 3ml tubes after dialysis. The blood will be drawn from your hemodialysis line so there will be no need for additional lines to draw blood. The drawing of samples will be done on 1 visit only, while you are on dialysis, and therefore will not require any extra time or visits on your part.

Twelve blood samples will be sent for routine blood analysis and the remaining five will be taken to the Visual Optics Laboratory located at the Eye Institute of the General Campus for analysis. The results of the analysis will then be used to determine if there is a way to optically monitor changes in your blood during hemodialysis.

RISKS/BENEFITS of PARTICIPATION:

There are no potential risks involved with having this non-invasive procedure completed.

There may be no direct benefit to you as a result of participating in this research study. A potential benefit may be the development of a non-invasive method to better manage dialysis patients in the future.

VOLUNTARY PARTICIPATION:

Your participation in this study is completely voluntary. If you choose not to participate, you will receive standard care as prescribed by your physician.

CONFIDENTIALITY:

All results of the study will be kept confidential. Representatives of the Ottawa Hospital Research Ethics Board may review your relevant records for audit purposes. You will not be identifiable in any publications or presentations resulting from this study. No records bearing your name will leave the Ottawa Hospital. All blood samples sent to the Visual Optics Laboratory will be coded with a study number only.

INFORMED CONSENT

I have been given time to read this 3-page Patient Information Sheet and have had all my questions fully answered to my satisfaction. I voluntarily agree to be a participant in this research study. A signed and dated copy of this information sheet will be given to me to take home. I do not waive my legal rights by signing this consent form.

I voluntarily consent to participate in this study.

_____
Participant's Name

_____
    _____
Participant's Signature   Date/Time

_____
Name of Person Administering Consent
Investigator/Designate

_____   _____
Signature of Person Administering Consent   Date/Time
Investigator/Designate

OHREB Validation Date: Valid until June 10, 2011.
Version: June 14, 2010.

APPENDIX II

French Informed Consent form
Oxygen Study

Document d'information du patient et formulaire de consentement

Titre du protocole : **Une étude pilote afin d'évaluer les changements dans le sang chez les patients en stade terminal de maladies rénales résultant des traitements de dialyse.**

Chercheur principal : **Dr Réjean Munger**
Collaborateur : **Dr Kevin Burns**

Géré par : <u>Centre de recherche sur les maladies du rein</u>

INTRODUCTION :
Nous vous demandons de prendre part à une étude de recherche de l'Hôpital d'Ottawa. Votre participation à cette étude se fait sur une base volontaire seulement. Votre décision d'y participer ou non, n'aura aucune conséquence sur la qualité de vos soins. Vous trouverez la description de l'étude ici-bas. Cette description vous renseignera sur le but de l'étude, les examens additionnels et les risques et inconforts que vous pourriez ressentir. Veuillez prendre le temps de lire ce document d'information et posez à la personne qui vous l'explique toutes les questions que vous avez sur votre participation.

BUT DE L'ÉTUDE :
Nous vous demandons de participer à cette étude de recherche parce que vous avez une insuffisance rénale et que vous recevez des traitements hémodialyse. Le but de cette étude est de déterminer si nous pouvons mesurer les changements dans votre sang (employer des échantillons de sang) en utilisant une technique novatrice développée par l'Institut de l'œil de l'Université d'Ottawa. Si cette technique s'avère un succès, il est possible qu'à long terme, nous aillions beaucoup moins d'échantillons de sang à prélever afin de surveiller votre condition.

Notre objectif est de recruter environ 65 patients d'hémodialyse (et 10 sujets en santé) pour cette étude de recherche de l'Hôpital d'Ottawa.

PROCÉDURE DE L'ÉTUDE :

Actuellement, vous recevez des traitements d'hémodialyse 3 fois semaine à l'hôpital. Nous vous demandons la permission de recueillir 17 éprouvettes additionnelles de sang (environ 3 cuillères à soupe) au moment d'un traitement habituel de dialyse. Quatre éprouvettes de 3 ml seront recueillies avant et après la dialyse ainsi que 3 éprouvettes de 3 ml après chaque heure de dialyse. Le sang proviendrait de votre perfusion d'hémodialyse et aucune autre perfusion ne serait nécessaire. La cueillette d'échantillons s'effectuerait lors d'une seule visite, pendant votre dialyse, et ne nécessiterait donc pas de temps ou de visite supplémentaire de votre part.

Douze échantillons de sang seront soumis à une analyse routine de sang à l'Hôpital d'Ottawa tandis que les autres seront apportés au Laboratoire d'optique visuelle, au Campus général de l'Institut de l'œil, afin d'être analysés par méthode optique. Les résultats de nos analyses seront utilisés pour déterminer si votre sang change au cours du traitement.

RISQUES/AVANTAGES LIÉS À VOTRE PARTICIPATION :

Il n'y a aucun risque potentiel relié à cette procédure non invasive.

Il ne pourrait y avoir aucun avantage direct résultant de votre participation à cette étude de recherche. Un avantage potentiel pourrait être le développement futur d'une méthode non invasive de gestion des patients dialysés.

PARTICIPATION VOLONTAIRE :

Votre participation à cette étude se fait sur une base volontaire. Si vous choisissez de ne pas participer, vous recevrez les soins standards prescrits par votre médecin.

CONFIDENTIALITÉ :

Tous les résultats de cette étude seront confidentiels. Les représentants du Conseil d'éthique en recherches de L'Hôpital d'Ottawa peuvent revoir vos dossiers pour des fins de vérification. Vous ne serez identifiable dans aucune publication ou présentation résultant de cette étude. Aucun dossier portant votre nom ne quittera l'Hôpital d'Ottawa. Tous les échantillons envoyés au Laboratoire d'Optique Visuel seront codés avec un numéro d'étude seulement.

CONSENTEMENT ÉCLAIRÉ :

J'ai eu le temps de lire le document d'information de 3 pages et on a répondu à toutes mes questions de manière satisfaisante. Je consens volontairement à participer à cette étude de recherche. Une copie datée et signée du document d'information me sera remise pour apporter chez moi. Je ne renonce pas à mes droits légaux en signant ce formulaire de consentement.

Je consens volontairement à participer à cette étude.

_____
Nom du participant

_____
      _____
Signature du participant   Date/Heure

_____
Nom de la personne administrant le consentement
chercheur/désigné

_____   _____
Signature de la personne administrant le consentement   Date/Heure
chercheur/désigné

CÉRHO Date de validation : Valide jusqu'au 10 juin 2011.
Version: 14 juin 2010.

APPENDIX III

English Informed Consent form
Heparin Study

Patient Information Sheet and Consent Form

Protocol Title: **A pilot study to evaluate the effect of heparin concentrations on blood samples from end-stage renal disease patients using optical spectroscopy.**

Principal Investigator: **Dr. Réjean Munger**
Co-Investigator: **Dr. Kevin Burns**

Managed by the Kidney Research Centre- Clinical Trials Group

Consent Form presented to patient on _____ **at** _____
                                                                DD/MMM/YY   Time

INTRODUCTION:
We are asking you to take part in a research study at the Ottawa Hospital. Taking part in this study is completely voluntary. The quality of your care will not be affected by your decision to participate or not. The study is described below. This description tells you about the purpose, additional tests, and risks/discomforts, which you might experience. You should take the time to read this information sheet and discuss any questions you have about your potential participation in this study with the person who explains it to you.

PURPOSE OF THE STUDY:
You are being asked to participate in this research study because you have kidney failure and you are currently receiving hemodialysis. The purpose of this study is to determine if it is possible to monitor changes in your blood (using blood samples) resulting from your condition using a novel technique developed at the University of Ottawa Eye Institute. If this technique proves successful, it could, in the long term, result in a method to monitor your condition that requires much fewer blood samples.

We are planning on enrolling approximately 25 hemodialysis patients in this research study at the Ottawa Hospital.

STUDY PROCEDURES:
Currently you are receiving hemodialysis three times per week in hospital. We are asking your consent to draw 1 additional tube of blood (approximately 1/5 tablespoon) at the time of a regularly scheduled treatment. The blood would be drawn before dialysis, from your hemodialysis line so there will be no need for additional lines to draw blood.

The extra blood sample will be taken to the Visual Optics Laboratory located at the Eye Institute of the General Campus for analysis. The original sample is then divided into 4 different sub-samples. A different concentration of heparin, an anticoagulant used to prevent the clotting of blood when it moves through the blood tubing during dialysis will be added to each sub-sample.
The first blood sample will get the minimal dose of heparin and then we gradually add more to the remaining sub-samples. The last sub-sample will have a concentration ten times higher then the initial one. The results of the analysis will then be used to determine if there is a way to optically monitor heparin-related effects on the blood.

STUDY DURATION:
The drawing of your blood sample will be done during one visit only, while you are on dialysis, and therefore will not require any extra time or visits on your part.

RISKS:
There are no significant risks associated with this study, which involves the collection of an extra 3ml (1/5 tablespoon) of blood. The blood sample will be drawn before the dialysis from the hemodialysis line. An addition line will not be required to draw the blood needed for this study.

BENEFITS:

There may be no direct benefit to you as a result of participating in this research study. However, a potential benefit may be the development of a non-invasive method to better manage end-stage renal disease patients requiring dialysis in the future.

WITHDRAWAL FROM THE STUDY:

You have the right to withdraw from the study at any time without any impact to your current and future care at the Ottawa Hospital. Should you decide to withdraw your consent, we will not collect any new data from this point forward. However, you may not be able to withdraw your coded data if it has already been collected and used in a publication or presentation,

COMPENSATION:

In the event of a research-related injury or illness, you will be provided with appropriate medical treatment/care. You are not waiving your legal rights by agreeing to participate

in this study. The study doctor and the hospital still have their legal and professional responsibilities.

STUDY COSTS:

You will not be paid to participate in this research study.

CONFIDENTIALITY:

All personal health information will be kept confidential, unless release is required by law. Representatives of the Ottawa Hospital Research Ethics Board (OHREB), as well as the Ottawa Health Research Institute (OHRI), may review your original medical records under the supervision of study staff for audit purposes.

You will not be identifiable in any publications or presentations resulting from this study. No identifying information will leave the Ottawa Hospital. All information which leaves the hospital will be coded with an independent study number.

The link between your name and the independent study number will only be accessible by the study staff. The link and study files will be stored separately and securely. Both files will be kept for a period of 15 years after the study has been completed. All paper records will be stored in a locked file and/or office. All electronic records will be stored on a hospital server and protected by a user password, again only accessible by the study staff. At the end of the retention period, all paper records will be disposed of in confidential waste or shredded, and all electronic records will be deleted.

VOLUNTARY PARTICIPATION:

Your participation in this study is voluntary. If you choose not to participate, your decision will not affect the care you receive at The Ottawa Hospital at this time, or in the future.   You will not have any penalty or loss of benefits to which you are otherwise entitled to.

<u>Consent Form</u>

A pilot study to evaluate the effect of heparin concentrations on blood samples from end-stage renal disease patients using optical spectroscopy.

<u>Consent to Participate in Research</u>
I understand that I am being asked to participate in a research study about the effect of heparin concentrations on blood samples. This study has been explained to me by the study staff.   I have read this 4-page Patient Information Sheet and Consent Form (or have had this document read to me). All my questions have been answered to my satisfaction.   If I decide at a later stage in the study that I would like to withdraw my consent, I may do so at any time.

I voluntarily agree to participate in this study.
A copy of the signed Information Sheet and Consent Form will be provided to me.

<u>Signatures</u>

_____
Participant's Name (Please Print)

_____   _____
Participant's Signature   Date

<u>Investigator Statement (or Person Explaining the Consent)</u>
I have carefully explained to the research participant the nature of the above research study. To the best of my knowledge, the research participant signing this consent form understands the nature, demands, risks and benefits involved in participating in this study. I acknowledge my responsibility for the care and well being of the above research participant, to respect the rights and wishes of the research participant, and to conduct the study according to applicable Good Clinical Practice guidelines and regulations.

_____
Name of Investigator/Delegate (Please Print)

_____
Signature of Investigator/Delegate

 Consent process completed on 　　　　　　 **_____ at** 　　 **_____**
　　　　　　　　　　　　　　　　　　　　　 DD/MMM/YY   Time
(Valid until May 09, 2011)

APPENDIX IV

French Informed Consent form
Heparin Study

Feuille d'information et formulaire de consentement

Titre du protocole :   **Une étude pilote afin d'évaluer les effets de l'héparine sur la spectroscopie optique chez les patients en stade terminal de maladies rénales.**

Chercheur principal :   Dr Réjean Munger
Collaborateur :   Dr Kevin Burns

Géré par :   <u>**Centre de recherche sur les maladies du**</u>
<u>**rein**</u>

Formulaire de consentement présenté au patient le _____ **à** _____
                                                          JJ/MMM/AA

Heure
INTRODUCTION :
Nous vous demandons de prendre part à une étude de recherche à L'Hôpital d'Ottawa. Votre participation à cette étude se fait sur une base volontaire seulement. Votre décision d'y participer ou non n'aura aucune conséquence sur la qualité de vos soins. Vous trouverez la description de l'étude ici-bas. Cette description vous renseignera sur le but de l'étude, les examens additionnels et les risques et inconforts que vous pourriez ressentir. Veuillez prendre le temps de lire ce document d'information et posez à la personne qui vous l'explique toutes les questions que vous avez sur votre participation.

BUT DE L'ÉTUDE :
Nous vous demandons de participer à cette étude de recherche parce que vous avez une insuffisance rénale et vous recevez des traitements d'hémodialyse. Le but de cette étude est de déterminer si nous pouvons mesurer les changements dans votre sang (en employant des échantillons de sang) résultant de votre condition, en utilisant une technique novatrice développée par l'Institut de l'œil de l'Université d'Ottawa. Si cette technique s'avère un succès, il est possible qu'à long terme, nous ayons beaucoup moins d'échantillons de sang à prélever afin de surveiller votre condition.

Notre objectif est de recruter environ 25 patients d'hémodialyse pour cette étude de recherche de l'Hôpital d'Ottawa.

PROCÉDURE DE L'ÉTUDE :

Actuellement, vous recevez des traitements d'hémodialyse 3 fois par semaine à l'hôpital. Nous vous demandons la permission de recueillir 1 éprouvette additionnelle de 3 ml de sang (environ 1/5 cuillère à soupe) au moment d'un traitement habituel de dialyse. La prise de sang est faite avant le début de votre traitement d'hémodialyse, aucune autre perfusion ne serait nécessaire.

Cet échantillon additionnel de sang sera apporté au Laboratoire d'optique visuelle, situé à l'Institut de l'œil au Campus Général afin d'être analysé par spectroscopie optique. L'échantillon sera divisé en quatre portions égales (sous-échantillons). Chaque portion recevra une différente dose d'héparine, une drogue utilisée en clinique pour contrôler la coagulation du sang qui se déplace dans le tube lors de dialyse.

Le premier sous-échantillon de sang recevra la concentration la plus faible d'héparine, qui sera par la suite graduellement augmentée. Le dernier sous-échantillon aura une concentration d'héparine dix fois plus élevée que le premier. Les résultats de l'analyse par spectroscopie seront utilisés pour déterminer si nous pouvons évaluer l'effet de l'héparine sur le sang humain pendant la dialyse.

DURÉE DE L'ÉTUDE :

Le prélèvement de votre sang sera effectué lors d'une visite seulement, pendant votre traitement de dialyse, et ne requière donc pas de temps ou visites additionnels de votre part.

RISQUES :

Il n'y a aucun risque potentiel relié à cette étude qui consiste au prélèvement additionnel de 3 ml (1/5 cuillère à soupe) de sang. Le prélèvement est obtenu directement de votre ligne habituelle de dialyse et sera complété avant le début du traitement. Aucune intervention médicale supplémentaire ne sera effectuée.

AVANTAGES LIÉS À LA PARTICIPATION :

Il ne pourrait y avoir aucun avantage direct résultant de votre participation à cette étude de recherche. Un avantage potentiel pourrait être le développement futur d'une méthode non invasive de gestion des patients dialysés.

RETRAIT DE L'ÉTUDE :

Vous avez le droit de vous retirer de l'étude à n'importe quel moment sans que votre décision n'influe sur vos soins actuels ou éventuels à L'Hôpital d'Ottawa. Si vous décidez de retirer votre consentement, nous cesserons de recueillir de nouvelles données à votre sujet. Cependant, il pourrait s'avérer impossible de retirer les données qui auraient été recueillies précédemment et qui auraient été utilisées dans le cadre de publications ou de présentations.

INDEMNISATION :

En cas d'accident ou de maladie découlant de votre participation à cette recherche, vous bénéficierez de soins médicaux appropriés. Vous ne renoncez nullement à vos droits

légaux en participant à cette étude. Le médecin qui mène cette étude ainsi que l'hôpital seront toujours tenus de respecter leurs responsabilités légales et professionnelles.

COÛTS RELATIFS À L'ÉTUDE :
Vous ne recevrez aucune rémunération pour votre participation à cette étude.

CONFIDENTIALITÉ :
Tout renseignement personnel sur la santé sera gardé confidentiel, à moins que leur divulgation en soit requise par la loi. Des représentants du Conseil d'éthique en recherches de L'Hôpital d'Ottawa et de l'Institut de recherche en santé d'Ottawa, pourront procéder à l'examen de vos dossiers médicaux originaux, sous la supervision du personnel de l'étude, uniquement à des fins de vérifications.

Vous ne serez identifié dans aucune publication ou présentation résultant de cette recherche. Aucune information pouvant servir à vous identifier ne sera transmise à l'extérieur de L'Hôpital d'Ottawa. Toute l'information qui serait transmise à l'extérieur de L'Hôpital d'Ottawa sera codée à l'aide d'un numéro d'étude indépendant.

L'accès au lien entre votre nom et le numéro d'étude indépendant sera réservé uniquement au personnel de l'étude. Le lien entre les fichiers de recherche sera conservé séparément et entreposé en lieu sûr. Ces deux fichiers seront conservés pour une période de 15 ans suivant la fin de l'étude. Tous les dossiers papier seront entreposés dans un classeur ou un bureau verrouillé. Tous les dossiers électroniques seront entreposés sur un serveur de l'hôpital protégé par un mot de passe et seront accessibles uniquement au personnel de l'étude. Suivant la période de rétention, tous les dossiers seront éliminés dans une boîte à rebuts confidentiels ou déchiquetés, et tous les dossiers électroniques seront supprimés.

PARTICIPATION VOLONTAIRE :
La participation à cette étude s'effectue sur une base volontaire. Si vous choisissez de ne pas participer, votre décision ne changera pas la qualité des soins reçus à L'Hôpital d'Ottawa, ni maintenant, ni plus tard. Vous n'encourrez aucune pénalité ni perte d'avantages auxquels vous avez normalement droit.

Formulaire de consentement

Une étude pilote afin d'évaluer les effets de l'héparine sur la spectroscopie optique chez les patients en stade terminal de maladies rénales

Consentement à la participation à cette recherche

Je reconnais qu'on m'invite à participer dans une étude de recherche à propos du niveau de saturation d'oxygène au niveau de la rétine. Un membre du personnel m'a fourni des explications à propos de cette étude.
J'ai lu la feuille d'information du patient de quatre pages et le consentement (ou ce document m'a été lu). On a répondu à toutes mes questions de manière satisfaisante. Si je décide plus tard au cours de l'étude de retirer mon consentement, il me sera possible de le faire à tout moment.

Je consens volontairement à prendre part à cette étude.
On me remettra un exemplaire signé de cette feuille de renseignements et de ce formulaire de consentement.

Signatures

_____
Nom du participant (en caractères d'imprimerie)

_____   _____
Signature du participant   Date

Énoncé du chercheur (ou de la personne chargée d'obtenir le consentement)

J'ai expliqué soigneusement au participant la nature de l'étude susmentionnée. Pour autant que je sache, le participant apposant sa signature à ce consentement reconnaît la nature, les exigences, les risques et les avantages que comporte sa participation à l'étude. Je reconnais ma responsabilité envers le soin et le bien-être du participant susmentionné, le respect des droits et des désirs de ce dernier, et le déroulement de cette étude conformément aux directives et aux règlements de la bonne pratique clinique.

_____
Nom du chercheur/délégué (en caractères d'imprimerie)

_____
Signature du chercheur/délégué

Processus du consentement complété le : _____ à _____
(Valide jusqu'au 09 mai 2011)   JJ/MMM/AA   Heure

APPENDIX V

OLIS Spectrometer Repeatability Measurements

INSTRUMENT REPEATABILITY MEASUREMENTS

Three different measurements were done on three different occasions to assess the repeatability of the OLIS spectroometer.

First measurement

The spectrometer was turned on and 20 consecutive empty chamber measurements were performed. The beam ratio was plotted as a function of wavelength. The findings reveal that the instrument needs to be turned on and be allowed to warm up in order to obtain stable measurements. Figure 38 shows that the first 3 recordings are unstable but all recordings after the lamp warms up are stable.

Second measurement

The spectrophotometer was turned on and the lamp was left to warm up for 30 minutes after which 20 consecutive measurements were taken for 1 hour. A graph of beam ratio was plotted against wavelength and the results show all the recordings are stable following the lamp warm up (figure 39).
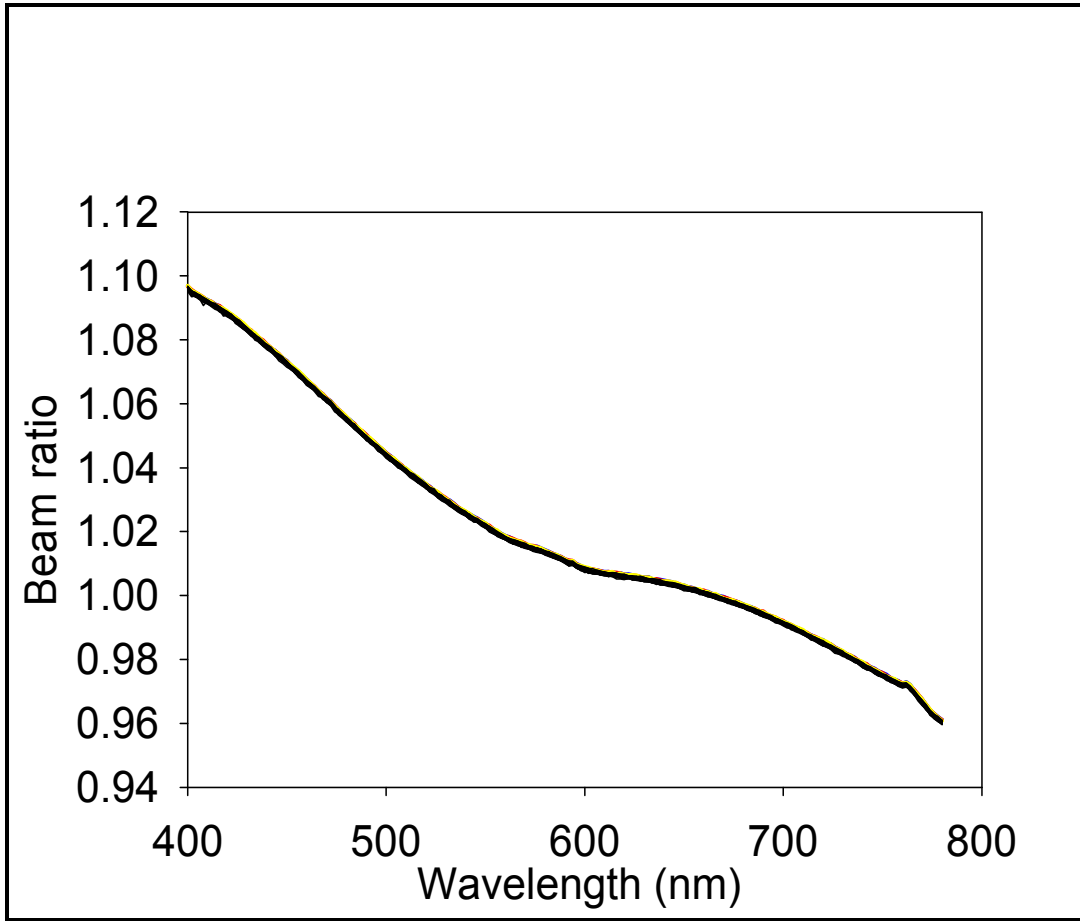
Third measurement

The spectrometer was turned on and the lamp was left to warn up for 20 minutes. Three consecutive measurements were taken every 2 hours for 6 hours. The data reveal that all the recordings are stable over the course of 6 hours, which show the long term stability of the spectrometer (figure 40). Collectively all three different sets of experiments show that the lamp needs to warm up at least 20 minutes before stable recordings can be obtained, after which reliable and repeatable data is obtained.

Figure 38. Beam ratio distribution for 20 empty chamber measurements. The coloured graphs represent the first 3 measurements after the instrument was turned on and the black lines represent 17 measurements after the lamp warmed up which show stability.

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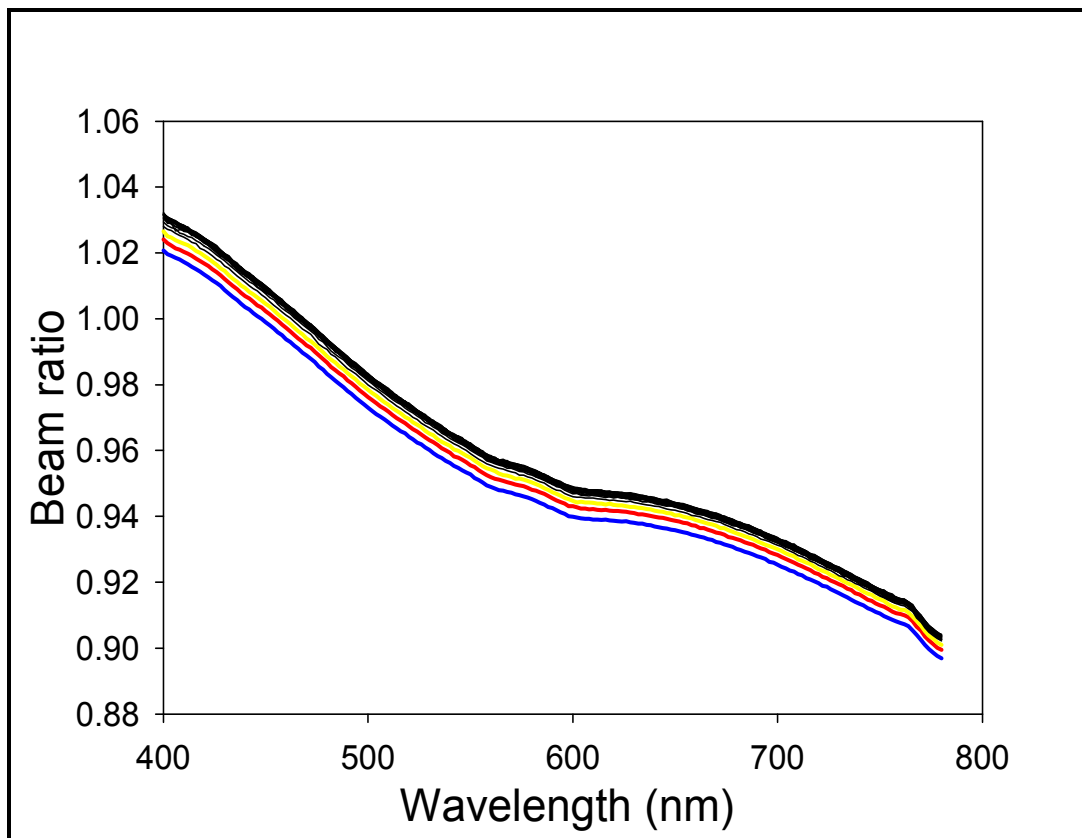
Figure 39. Beam ratio distribution for 20 empty chamber measurements and the lamp warmed up for 30 minutes and 20 consecutive measurements were taken for 1 hour. Stable measurements can be obtained when the lamp is left to warm for at least 20 minutes.

Figure 40. Beam ratio distribution after 2 hours of inactivity. The colored graph represents the first measurement after the lamp was warmed up for 20 minutes and the black lines represent 3 measurements taken every 2 hour of inactivity for 6 hours, which show stability.
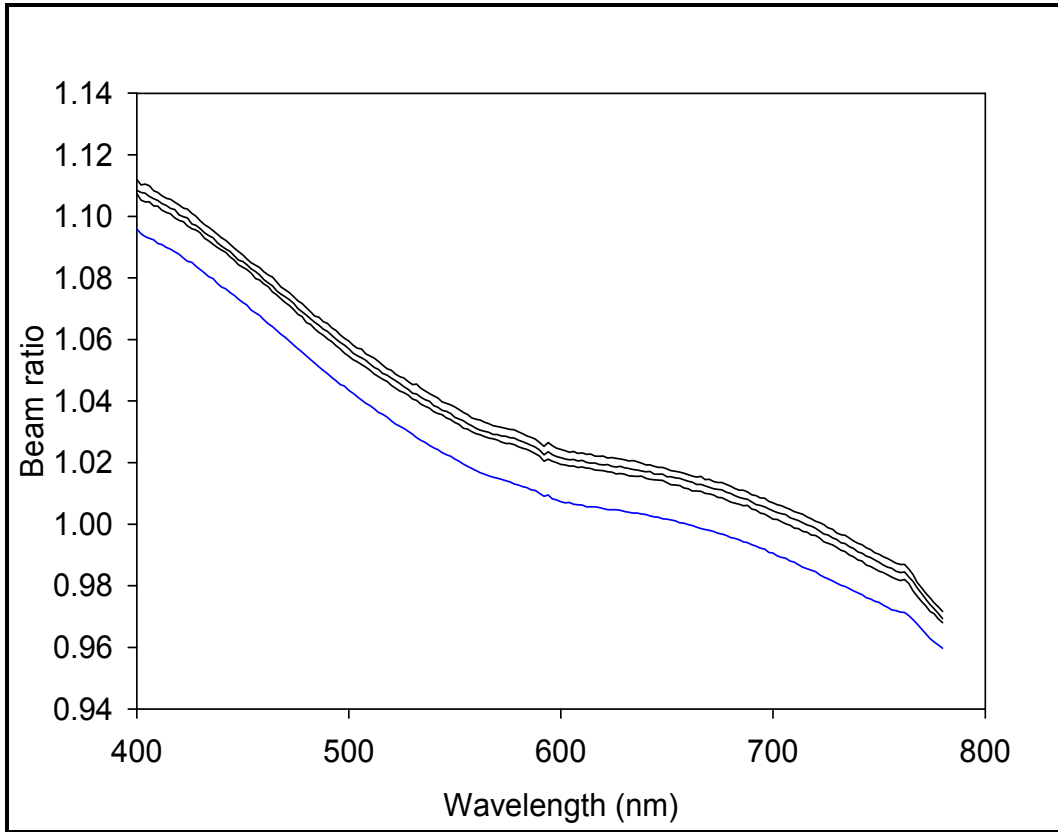
APPENDIX VI

Spectral Data Processing

SPECTRAL DATA PROCESSING

Transmittance due to empty cuvette

Cuvette Signal= $I_S(\lambda) * T_C * R_{sys}$ [**1**]

Reference signal= $I_R(\lambda) * R_{sys}$ [**2**]

Where I_S = intensity of incident light in the sample path

T_C = Transmittance of cuvette

R_{sys} = Response of the system

To get the transmittance due to empty cuvette, divide equation 1 by equation 2

Transmittance $_{Empty\ Cuvette}$ = $\dfrac{I_S(\lambda) * T_C * R_{sys}}{_R(\lambda) * R_{sys}}$ [**3**]

= T_C [4], transmittance due to empty cuvette only

Transmittance due to sample

Sample Signal= $I_S(\lambda) * T_C * T_S * R_{sys}$ (**5**)

Reference signal= $I_R(\lambda) * R_{sys}$ [**6**]

Where I = intensity of incident light

T_C = Transmission of cuvette

T_S = Transmission of measured sample

R_{sys} = Response of the system

To get the transmittance due to the sample in the cuvette, divide equation **5** by equation **6**

Sample Signal = $\dfrac{I_S(\lambda) * T_C * T_S * R_{sys}}{I_R(\lambda) * R_{sys}}$ [**7**]

= $T_C * T_S$ [**8**], transmittance due to both the empty cuvette and blood

Transmittance due to blood

To get the transmittance only due to blood, divide equation **8** by equation **4**

$T_S = TC * T_S/T_C$ **[9],** the transmittance due to just blood.

APPENDIX VII

Whole Blood Oximetry Spectral Data Processing

WHOLE BLOOD OXIMETRY SPECTRAL DATA PROCESSING

Instrument Noise level: White reflector (WR) measurement

WR Signal= $I_S(\lambda) * T_{WR} * R_{sys}$ [**1**]

Where I_S = intensity of incident light in the sample path

T_C = Transmittance of white reflector

R_{sys} = Response of the system

Empty chamber: Beam Ratio (BR) measurement

Sample Path Signal= $I_S(\lambda) * T_{SP} * R_{sys}$ [**2**]

Reference signal= $I_R(\lambda) * R_{sys}$ [**3**]

Where I_S = intensity of incident light in the sample path

T_{SP} = Transmittance in the sample path

R_{sys} = Response of the system

To get the Beam Ratio (BR), divide equation 2 by equation 3

$$\text{Transmittance }_{Beam\ Ratio} = \frac{I_S(\lambda) * T_{SP} * R_{sys}}{I_R(\lambda) * R_{sys}} \quad [\mathbf{4}]$$

BR = TSP [**5**], instrument beam ratio

Transmittance due to sample

Sample Signal= $I_S(\lambda) * T_{COP\ film} * T_S * R_{sys}$ (**6**)

Reference signal= $I_R(\lambda) * T_{COP\ film} * T_{de\text{-}ioned\ water} * R_{sys}$ [**7**]

Where I = intensity of incident light

$T_{COP\ film}$ = Transmission of COP film

T_S = Transmission of measured sample

T$_{\text{de-ionized water}}$ = Transmission of de-ionized water

R$_{\text{sys}}$ = Response of the system

To get the transmittance due to the sample, add equation **6** to **1**, divide by equation **7** and multiply every by equation **5**

$$\text{Sample Signal} = \left[\frac{[I_S(\lambda) * T_{\text{COP film}} * T_S * R_{\text{sys}}] + [I_S(\lambda) * T_{WR} * R_{\text{sys}}]}{I_R(\lambda) * T_{\text{COP film}} * T_{\text{de-ioned water}} * R_{\text{sys}}} \right] * T_{SP} \quad [\mathbf{8}]$$

$= T_S$ [**9**], transmittance due blood sample

APPENDIX VIII

Test Resolutions for the Acid-Base and Oxygen Parameters using the GEM Premier 4000
Analyzer

Table VI. **Measured Analytes-**measured directly and reported by the instrument

Measured Analyte	Units	Measured* Range	Tested* range	Resolution
pH	n/a	6.80 to 8.00	7.00 to 8.00	0.01
pCO$_2$	mmHg	0 to 150	6 to 125	1
pO$_2$	mmHg	0 to 800	5 to 690	1
O$_2$Hb	%	-10.00 to 110.00	0.0 to 98.0	0.1
COHb	%	-10.00 to 110.00	0.0 to 99.0	0.1
MetHb	%	-10.00 to 110.00	0.0 to 28.00	0.1
HHb	%	-10.00 to 110.00	0.0 to 96.00	0.1

Table VII. **Derived values**- calculated using equations applied to one or more measured analytes

Derived parameters	Unit of Measure	Resolution
sO$_2$	%	0.1
HCO$_3^-$ std	mmol/L	0.1
HCO$_3^-$ (c)	mmol/L	0.1

Note: The measuring ranges for the GEM Premier 4000 analyzer are the ranges that the instrument supports in terms of actual numeric values that the instrument can report. The tested ranges are those ranges that are tested during functional sensitivity and linearity testing.