

Hemodialysis monitoring in whole blood using transmission and diffuse reflection spectroscopy: a pilot study

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Abstract. Visible and near infrared transmission and diffuse reflection spectroscopy were used to monitor changes in whole blood resulting from hemodialysis treatment for end-stage renal disease. Blood samples from 8 patients on chronic hemodialysis therapy were measured in the 500- to 1700-nm wavelength range immediately before and after a single treatment. Principal component scores characteristic of each spectrum were derived, and mean pre- and posttreatment scores of the first principal component indicated a significant treatment-dependent change in both optical transmission ($P=0.004$) and diffuse reflection ($P<0.001$). Significant treatment-induced change persisted ($P<0.05$) when the first four principal components were used to account for $>97\%$ of the treatment-dependent spectral variation. Some blood spectral changes expressed in terms of difference spectra (posttreatment – pretreatment) were consistent with standard clinical indicators of weight reduction, urea reduction, and potassium change, with probable origins at a molecular level. The results indicate the feasibility of using optical transmission and diffuse reflection spectroscopy to characterize clinically relevant blood changes for the future development of more comprehensive indicators of hemodialysis efficacy and long-term clinical outcomes. Moreover, the optical techniques employed are adaptable for potential online monitoring of blood changes during the hemodialysis treatment. © 2006 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2357611]

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1 Introduction

Hemodialysis is a medical treatment that involves diffusive and convective removal of solutes and water from the blood of patients with end-stage renal disease (ESRD), whose kidneys can no longer perform this task. Current standard measures of treatment adequacy and dose of hemodialysis are based upon the clearance of the low molecular weight compound urea from the blood, determined from pre- and postdialysis blood samples analyzed in a clinical laboratory. While measurement of urea clearance is the most widely used method to assess dialysis adequacy in ESRD, urea is only one of many metabolites that accumulate in ESRD and it may represent a surrogate marker rather than a principal toxin.¹⁻³ While the hemodialysis procedure filters the blood of low-molecular weight water-soluble molecules, a host of potentially toxic middle- and high-molecular weight molecules remain unfiltered. These include protein-bound molecules that may contribute to the development of complications such as

the uremic syndrome and vascular disease³ in ESRD patients. Investigation of uremic toxins is clearly of critical importance in developing treatment strategies that improve patient quality of life and longevity. One means of achieving this goal is to identify, classify, and characterize the clinical importance of as many candidate toxic molecules as possible, as is the mandate of the European Uremic Toxin Work Group (EUTox) initiated in 2000.⁴ As a consequence, however, of the complexity and our limited understanding of the chemistry of kidney disease and its treatment, it has proven difficult to find individual analytes (isolated from the rest of the blood chemistry) that can accurately and reliably describe the disease state or report the efficacy of treatment. An alternative approach could be to monitor whole blood as a complex structure and correlate any changes in this structure to the hemodialysis treatment and the patient's clinical status. The emergence of consistent patterns of change or absence of change in blood properties could lead to new candidate toxicity indicators and to the subsequent investigation of factors underlying the observed patterns. An advantage of this approach is the simultaneous inclusion of numerous molecules

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including their interactions and indirect effects as they contribute to the observed blood properties. In this regard, optical spectroscopy could be an effective tool to probe the complex response of blood to treatment in the clinic.

Several approaches using optical spectroscopy to monitor hemodialysis treatment have been reported. These studies utilize light as a noncontact tool for reagentless determination of urea and other solute concentrations in spent dialysate fluid.^{2,5-7} Although such approaches are useful for online monitoring of filtered analytes, the direct optical detection of analytes retained in blood (including unfiltered compounds) has to our knowledge not been reported. While whole blood optical monitoring during hemodialysis has been achieved, the reported methods have used a small number of discrete wavelengths to monitor blood parameters such as hematocrit, blood volume, oxygen saturation, and hemoglobin levels.⁸⁻¹⁰ These parameters, however, are not indicative of potential toxins within the blood nor do they provide a means to assess the efficacy of treatments.

Accordingly, as a first step toward investigating broader questions of toxicity and treatment efficacy using optical techniques, the purpose of this pilot study was to determine whether features in the optical spectrum of undialyzed versus dialyzed whole blood showed any significant difference as a result of the hemodialysis treatment. Additionally, to determine how changes in the spectrum of whole blood were related to accepted clinical measures, we compared the spectroscopic results to clinically measured analyte changes (as a gold standard) following dialysis treatment. While the optical monitoring techniques used are readily adaptable for online monitoring, the whole-blood approach could enable the future development of surrogate markers for toxicity or for patient prognosis through established disease pattern recognition techniques.¹¹⁻¹⁴

2 Materials and Methods

2.1 Clinical Design

A sample population of eight ESRD patients undergoing regular hemodialysis treatment (4-h sessions, three times a week) was recruited on a volunteer basis for the pilot study. The use of human subjects, extraction of blood samples, informed patient consent, and confidentiality requirements were all approved by the Research Ethics Board of the Ottawa Hospital. Candidate volunteers were identified by the Kidney Research Centre staff from the population of patients treated at the Hemodialysis Unit of the Ottawa Hospital General Campus. Volunteers represented a broad cross section of ESRD patients in terms of age and gender (6 male, 2 female; mean age 61.5 years; age range 39 to 75 years); time since initiation of dialysis (18 to 284 months); and the presence of other systemic conditions (3 hypertensives, 3 Type-II diabetics). Blood was extracted from the vascular access point and occurred immediately before and after a single hemodialysis treatment (<1 min). The day of blood extraction coincided with the monthly laboratory blood testing day for each volunteer, thereby allowing subsequent correlation of spectral data to clinical laboratory results.

2.2 Blood Sample Preparation

Samples of whole blood for the study were obtained at the same time the standard clinical blood samples were obtained. Blood was drawn into standard 3-ml purple-top collection tubes containing 5.4 mg K₂-ethylenediaminetetracetic acid as an anticoagulant. For spectroscopy measurements, blood samples were brought to the Visual Optics Laboratory of the University of Ottawa Eye Institute, colocated with the Ottawa Hospital General Campus. The collection tubes were manually agitated to provide a homogeneous suspension and 1 ml from each tube was transferred to an optical cell and sealed. The delay between sample collection and the start of optical measurements averaged 1 h, with a maximum delay of 2 h. To test the influence of the delay, optical spectra from a single blood sample were taken hourly over a 4-h period at room temperature, with no significant change observed in the spectra (data not shown). Clinical blood samples were sent to the clinical laboratory at the Ottawa Hospital for standard clinical evaluation. Serum urea and potassium levels were quantified using an automated Beckman Coulter LX20 analyzer. Manufacturer-supplied reagents were used and an indirect ion selective electrode method was used to quantify potassium, while a coupled enzymatic rate method was used for urea. The intra-assay variability of these techniques is nominally accepted to be approximately 2% (coefficient of variation).

2.3 Measurement of Transmission and Diffuse Reflection Spectra

Optical cells with a 2-mm optical path length in air were used, made from optical glass with >80% transmission over the wavelength range 365 to 2500 nm (Varsal Inc.). For measurements, the optical cells were placed in a custom sample holder ensuring both repeatable cell placement and minimal optical-mechanical interference to avoid stray light and spurious reflections. A focused spot (4-mm diameter) from a current-stabilized 20-W tungsten-halogen light source (model ASB-W-020, Spectral Products, Inc.) was used to illuminate the optical cell. Light transmitted through the cell was focused into a collection optical fiber (400- μ m core diameter low-OH fiber, Ocean Optics Inc.) connected to one of two spectrometers. Light backscattered in a cone over a 10- to 30-degree angle relative to the incident beam was captured by a large-aperture achromatic lens and focused to a second collection optical fiber (600- μ m core diameter low-OH fiber, Ocean Optics Inc.) connected to the other spectrometer. The off-axis geometry used for backscatter collection minimized the interference of both specular reflections and edge effects originating from the optical cell geometry.

Optical spectra were acquired over the 400- to 1700-nm region using two spectrometers spanning wavelength ranges of 400- to 1000-nm (model SD2000, Ocean Optics Inc., 2048-element silicon photodiode array; spectral resolution 0.33 nm) and 900- to 1700-nm (model InGaAs512, StellarNet Inc.; 512-element InGaAs photodiode array; spectral resolution 2.25 nm). Spectra were acquired through computer control with acquisition times of 8 ms and 800 ms for transmission and diffuse reflection, respectively, for the near infrared spectrometer, and 10 ms for both modes using the visible spectrometer.

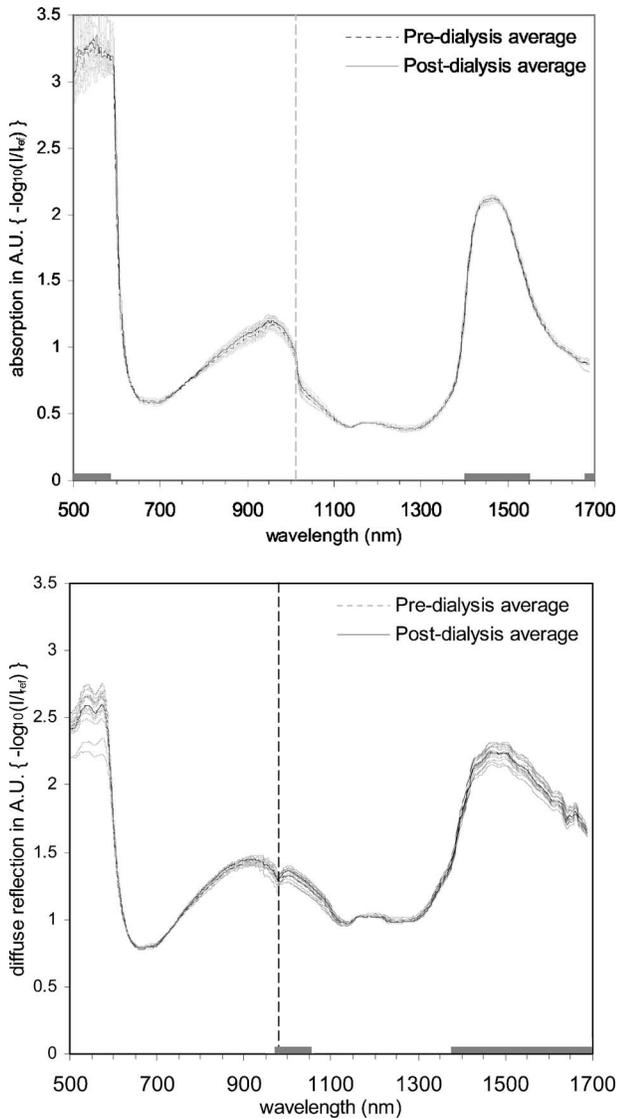


Fig. 1 Pre- and postdialysis whole-blood spectra in absorbance units for (a) transmission and (b) diffuse reflection modes. Blood spectra from individual patients are in gray, while average pre- and postdialysis values are indicated by dashed and solid black lines, respectively. Shaded regions of the abscissa indicate wavelength ranges with signal-to-noise ratio < 3 dB. The discontinuity around 1000 nm is due to a detected intensity mismatch between the two spectrometers, indicated by a vertical dashed line.

Blood samples were maintained at room temperature and sample heating was minimized by using a mechanical shutter to limit sample exposure by keeping it open only during the spectral acquisition period. Three data sets were obtained for each sample, where for each set the cell was removed, agitated, and replaced. The three spectra were subsequently area normalized and then averaged. Prior to averaging, the maximum coefficient of variation among any set of three normalized spectra was 2 and 9% for the visible and near infrared spectrometers, respectively. All subsequent analyses were performed using normalized mean spectra, smoothed with a 10-point moving average filter. Influences of the spectral properties of the light source, the optical cell, and optical elements

Table 1 Percent of the total variation in optical spectra among the 16 blood samples explained by a given principal component, for the different light interaction modes.

	Principal Component				
	1	2	3	4	First 4
Transmission	89.0	8.6	1.2	0.6	99.4
Diffuse reflection	76.9	11.4	4.5	4.3	97.1
Combined	86.0	8.4	2.6	1.0	98.0

in the light delivery and detection paths were removed by dividing the transmission and diffuse reflection spectra by a reference measurement taken with an empty optical cell (transmission path) and a broadband mirror placed behind an empty optical cell (diffuse reflection path).

About 1 W of focused optical power was delivered to the blood sample. Typically about 15% of the incident light was transmitted through a sample while about 5% was diffusely reflected in the direction of the detection cone. Although the transmitted and diffusely reflected light levels were high, signal-to-noise ratio was reduced due to manual attenuation of the delivered and/or detected light streams, which was necessary to accommodate both a limited photodetector dynamic range and a requirement for the simultaneous measurement of transmitted and diffusely reflected paths with differing light levels. Wavelength regions where optical signal-to-noise levels failed to exceed an imposed 3-dB minimum threshold were identified and excluded from subsequent analyses. Of particular note, strong water absorption in the 1400 to 1550-nm band resulted in a reduced signal-to-noise ratio.

3 Data Analysis and Interpretation

3.1 Whole Blood Spectra

32 mean spectra were obtained; these corresponded to pre- and postdialysis blood for eight patients in both transmission and diffuse reflection modes. The spectra are given in absorbance units in Figs. 1(a) and 1(b), defined as the base 10 logarithm of detected intensity relative to the reference intensity level. The spectra differed depending upon the light interaction mode, which is consistent with results reported by others.¹⁵ High absorption at wavelengths below 600 nm in Fig. 1(a) was a result of the relatively long 2-mm path length through blood, while in Fig. 1(b), diffusely reflected light originating from superficial blood layers underwent less attenuation thereby revealing the characteristic double-peaked absorption of oxyhemoglobin (peaks at 542 and 575 nm). In general, excellent agreement is observed between the spectrum in Fig. 1(b) and the published absorption spectrum of pure oxyhemoglobin.¹⁶ Hemoglobin (principally its oxygenated and deoxygenated forms) dominates whole blood absorption of wavelengths shorter than 1000 nm, whereas water generally dominates absorption above 1000 nm. The well-known absorption peak of water centered at 1440 nm and a minor peak around 1200 nm are also visible in the measured spectra in Fig. 1.¹⁷

Table 2 Principal component scores used to test the null hypothesis of no difference between pre- and postdialysis blood based on full-spectrum (500 to 1700 nm) analysis.

	Patient	Transmission		Diffuse Reflection		Combined	
		PC1	First 4	PC1	First 4	PC1	First 4
Predialysis	1	-1.08	4.33	0.08	0.92	0.99	2.64
	2	-0.88	3.73	0.00	0.90	0.78	2.14
	3	-1.24	4.53	0.11	0.86	1.16	2.90
	4	-0.86	3.62	0.07	0.89	0.78	2.07
	5	-0.92	4.10	0.00	0.90	0.82	2.46
	6	-1.04	4.08	0.08	0.91	0.95	2.47
	7	-1.22	4.61	0.11	0.92	1.14	2.96
	8	-1.18	4.58	0.10	0.93	1.09	2.92
Postdialysis	1	-1.16	4.31	0.13	0.97	1.08	2.65
	2	-0.99	3.83	0.07	0.94	0.90	2.19
	3	-1.40	4.84	0.15	0.91	1.32	3.24
	4	-1.11	4.09	0.14	0.90	1.04	2.47
	5	-0.92	3.93	0.03	0.93	0.82	2.32
	6	-1.26	4.44	0.16	0.95	1.19	2.82
	7	-1.27	4.79	0.13	0.94	1.19	3.11
	8	-1.32	4.82	0.15	0.94	1.24	3.18
Mean predialysis		-1.05	4.20	0.07	0.91	0.96	2.57
Mean postdialysis		-1.18	4.38	0.12	0.94	1.10	2.75
Paired <i>t</i>		4.25	-2.48	-6.21	-4.67	-4.45	-2.62
<i>P</i>		0.004	0.04	0.0004	0.002	0.003	0.03

3.2 Principal Component Analysis

To assess the significance of spectral changes observed as a result of hemodialysis, quantitative analysis was performed using the principal component analysis (PCA) method.¹⁸ Briefly, in the PCA method, a group of spectra are mathematically decomposed into a small set of uncorrelated, orthonormal variables (the principal components) that account for the major sources of variation across the group. For a given spectrum, it is then possible to derive a set of principal component "scores" or "weights" representing the contribution of each principal component to the linear decomposition of that spectrum in terms of the principal components. By limiting the analysis to only the most significant sources of variation among the spectra, an entire optical spectrum can be represented by one or a few variables.

PCA was performed three times (for transmission, diffuse reflection, and concatenated transmission + diffuse reflection),

in each case using the 16 spectra (pre- and posttreatment for 8 patients) shown in Fig. 1. In each analysis, the first four principal components accounted for nearly all the variation (>97%) among the spectra, with the first principal component representing the dominant source of variation (Table 1). Scores for the first principal component for each patient sample are given along with an aggregate score for the first four principal components in Table 2. The aggregate score was computed as the sum of the squared scores of the first four principal components. Mean scores for each group (pre- and postdialysis) are shown along with the corresponding results of a paired *t*-test (two-tailed significance $\alpha=0.05$) for the null hypothesis (no spectral difference due to treatment). For transmission, diffuse reflection, and combined modes, the null hypothesis could be rejected using scores from both the first and the first four principal components, indicating that hemodialysis treatment induced significant whole-blood spec-

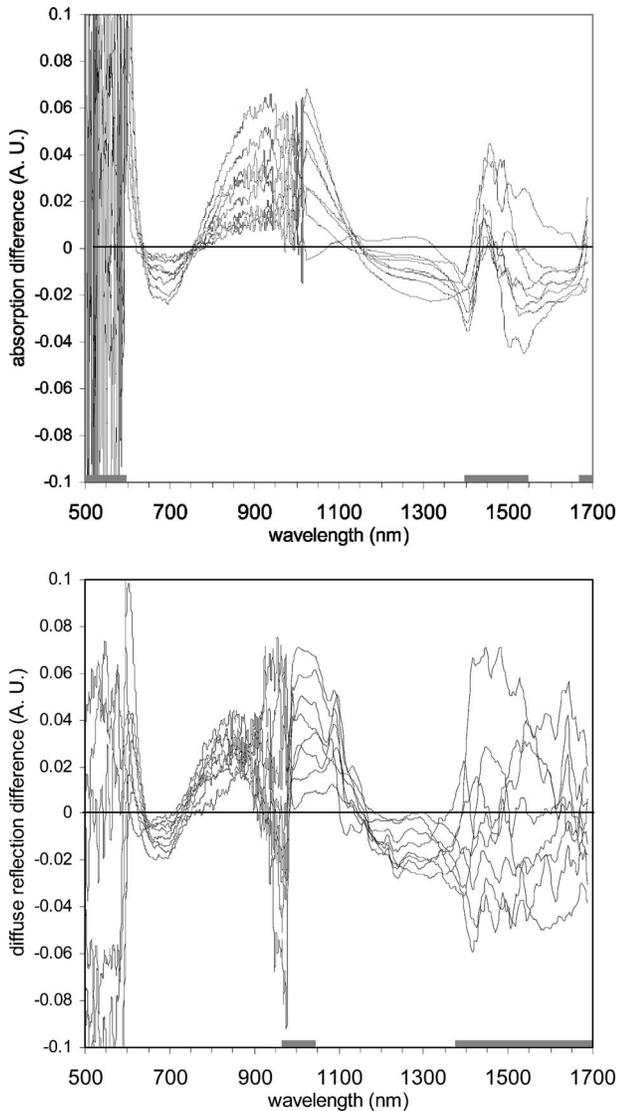


Fig. 2 Whole blood difference spectra (postdialysis – predialysis) for eight patients in (a) transmission and (b) diffuse reflection modes. A horizontal line at zero change is included for reference.

tral changes in the patient group. Moreover, significance at the high level of explained variance indicates that the observed spectral changes are almost completely due to the effect of the hemodialysis treatment alone.

3.3 Difference Spectra

Optical difference spectra (posttreatment – pretreatment) were calculated from the data shown in Fig. 1 and are given in Fig. 2. In the difference spectra, coinciding with local extrema are observed as well as isobestic wavelengths of near-zero change for all patients, which differ slightly in location depending on the light interaction mode. The magnitude of spectral change also differed among patients and was mode-dependent. For example, for first three local extrema in transmission [Fig. 2(a)], patient 6 exhibited the largest change while patient 4 exhibited the largest change for the following two extrema. In diffuse reflection [Fig. 2(b)], however, patient 2 shows the largest change at the first minimum while patient

3 shows the largest change at the following peak and patient 4 shows the largest change at the next minimum.

Periodic fluctuations observed in the diffuse reflection change at longer wavelengths are due to multiple reflections caused by the blood, optical cell, and air interfaces. Moreover, only a portion of the diffusely reflected light (20-deg solid angle) was captured in our setup resulting in a low absolute intensity level and greater relative noise contribution. It has been reported that increasing the solid angle of collection of diffusely reflected light using an integrating sphere significantly improves the quality of the whole-blood spectra.¹⁹

3.4 Correlation with Clinical Measures

We sought to investigate whether the observed whole-blood spectral changes (expressed as difference spectra) were consistent with known blood chemistry changes due to hemodialysis therapy. For each patient, the clinical record of the hemodialysis session was used to compile key measures of hemodialysis performance to allow a direct comparison of optical measurements and clinical outcomes. The analysis presented here focuses on a few key clinical measures of hemodialysis treatment: the urea reduction ratio ($URR=1 - \text{post-preblood urea concentration}$); a derived measure, the potassium reduction ratio ($KRR=1 - \text{post-preblood potassium ion concentration}$); a derived measure relating to fluid removal by filtration, the weight retention ratio ($WRR=\text{post-prebody weight}$); and Kt/V (dialysis dose, where K is urea clearance rate of the dialyzer in liters per minute, t is treatment time in minutes, and V is the urea distribution volume for the patient in liters).

The clinical values for the chosen measures along with the range of values among the patient group are shown in Table 3. The value of Pearson's correlation coefficient r among the clinical parameters has also been given in Table 3. A nearly perfect positive correlation was found between URR and Kt/V , which was expected given that Kt/V values are derived from the URR of a patient.²⁰ URR thus suffices to describe the behavior of Kt/V in the following analysis. Furthermore, while absolute potassium reduction is used as an accepted clinical measure, KRR was derived for the purposes of this study to provide a relative, unitless parameter for consistency in the analysis. Absolute potassium reduction and the KRR were highly correlated ($r > 0.97$) indicating nearly identical behavior.

Additionally in Table 3, correlations were observed between WRR and KRR as well as URR and KRR . To remove the effect of intervening parameters, the partial correlation coefficient $r_{xy,z}$ has been used to represent the correlation of x (the set of optical difference values at a given wavelength data point from Fig. 2) with y (the set of clinical parameter values), while removing the influence of z (a correlated clinical parameter). The result is a partial correlation spectrum for each clinical parameter with the influence of the most confounding clinical parameter factored out. The partial correlation spectra for the WRR , URR , and KRR are given in Fig. 3. Correlation spectra from the optical interaction mode (transmission or diffuse reflection) with the greatest degree of variation for each parameter are shown. While a detailed interpretation of wavelength regions of high correlation is precluded by the small sample size, general observations concerning the

Table 3 Hemodialysis parameter values for the patient group obtained from clinical blood laboratory results and patient charts. Minimum and maximum parameter values within the group (predialysis) are also given. Correlations among the parameter values are also shown. Parameter definitions are given in the text.

Patient	Hemodialysis Measure			
	WRR	URR	KRR	Kt/V
1	0.975	0.755	0.261	1.80
2	0.945	0.768	0.318	1.88
3	0.970	0.765	0.220	1.88
4	0.958	0.855	0.453	2.24
5	0.960	0.775	0.396	1.88
6	0.946	0.830	0.395	2.12
7	0.979	0.791	0.217	1.96
8	0.953	0.728	0.415	1.72
	Weight	Urea	Potassium	
Min value	65.6 kg	12.9 mM	4.1 mM	
Max value	100 kg	27.2 mM	5.3 mM	
	Parameter Correlation			
	WRR	URR	KRR	Kt/V
WRR	—	-0.171	-0.704	-0.190
URR		—	0.369	0.997
KRR			—	0.370
Kt/V				—

overall nature of the variations and their likely origins can be made.¹⁸ In particular, the presence of large correlation variations with wavelength indicates the influence of molecular absorption bands.¹⁸ Second- and third-order overtones of the N–H symmetric and asymmetric stretching vibrations in the urea molecule are broad bands centered at 960 to 1000 nm and 720 to 750 nm, respectively.⁶ First-order overtones of these stretches occur in the 1450- to 1500-nm region, but in blood samples these would be buried under the strong water absorption band in this region.⁶ Additionally, potassium ions in whole blood have been shown to exhibit a broad correlation with absorption in the 500- to 1000-nm wavelength region.²¹ Observed regions of high correlation may correspond to analyte levels directly or may be due to more subtle, indirect effects related to their removal. For instance, potassium is known to play a role in the intra- and extracellular fluid balance, potentially influencing blood cell volume and thereby the diffuse reflection spectrum. In addition, the WRR (or weight change, a measure of fluid removal during hemodialysis) reflects not only a hemoconcentrating effect in the blood, but relates indirectly to the removal of a number of water-

soluble solutes during treatment—effects that may contribute to the observed spectral changes.

4 Discussion

To our knowledge, in this pilot study we documented for the first time the change in the optical transmission and diffuse reflection spectrum of whole blood resulting from a hemodialysis treatment session. Using PCA, statistically significant whole-spectrum change due to treatment was found for transmission, diffuse reflection, and combined spectra at high levels of explained variance. The clear differentiation of pre- and postdialysis blood in this manner could serve as the basis for a novel approach to online hemodialysis monitoring. When calibrated with a larger spectral database from multiple treatments, an online measure such as the principal component score could be used to monitor the progress of a treatment session. As the score reflects multiple blood parameters including unfiltered analytes, it may provide an alternate means to determine the adequacy of treatment and could be a candidate for a comprehensive indicator of longer-term clinical patient outcomes.

Although the patients recruited in this study represented a cross section of age, gender, and clinical condition, the observed spectral shape changes due to treatment were similar among patients, though the magnitude of change differed. Follow-up studies using more homogeneous patient groups or, alternatively, following individual patients over the course of multiple treatments, could be used as a means to isolate spectral shape changes due to treatment and thereby investigate their properties and clinical relevance.

In this study, a few key clinical measures of uremic toxicity and hemodialysis adequacy were chosen and shown to have a probable molecular association with whole blood optical transmission and diffuse reflection change. Upon comparison of Figs. 2 and 3, however, it is evident that regions of large absorbance change due to hemodialysis do not necessarily correspond to similar regions of high correlation for the chosen clinical measures. Instead, direct changes in urea and potassium may only have minor effects on the optical spectrum compared to their indirect effects in altering the optical properties of blood. The bulk of the optical property changes seen are likely due to changes in the molecular and cellular environment of major absorbing and scattering components in whole blood, namely hemoglobin, oxygen, water, and red blood cells (RBCs). Besides the chosen clinical measures, a host of other factors and processes could potentially modify this environment. To exemplify this point, clinical observations indicate that prior to hemodialysis treatment, patients usually exhibit a mild metabolic acidosis, while the effect of bicarbonate in standard dialysate solution results in a mild alkalosis posttreatment.²² Using near infrared spectroscopy of whole blood, it has been reported that pH-induced changes in the hemoglobin molecule correlate with RBC size and oxygen saturation changes.^{23,24} Such changes would directly modify the optical absorption and scatter properties of whole blood. Moreover, in addition to the amplitude changes observed in Fig. 2, directional changes of extrema are also evident, where minima for some patients correspond to maxima for others. This indicates possible competing processes and patient-specific responses, further illustrating the complex nature of

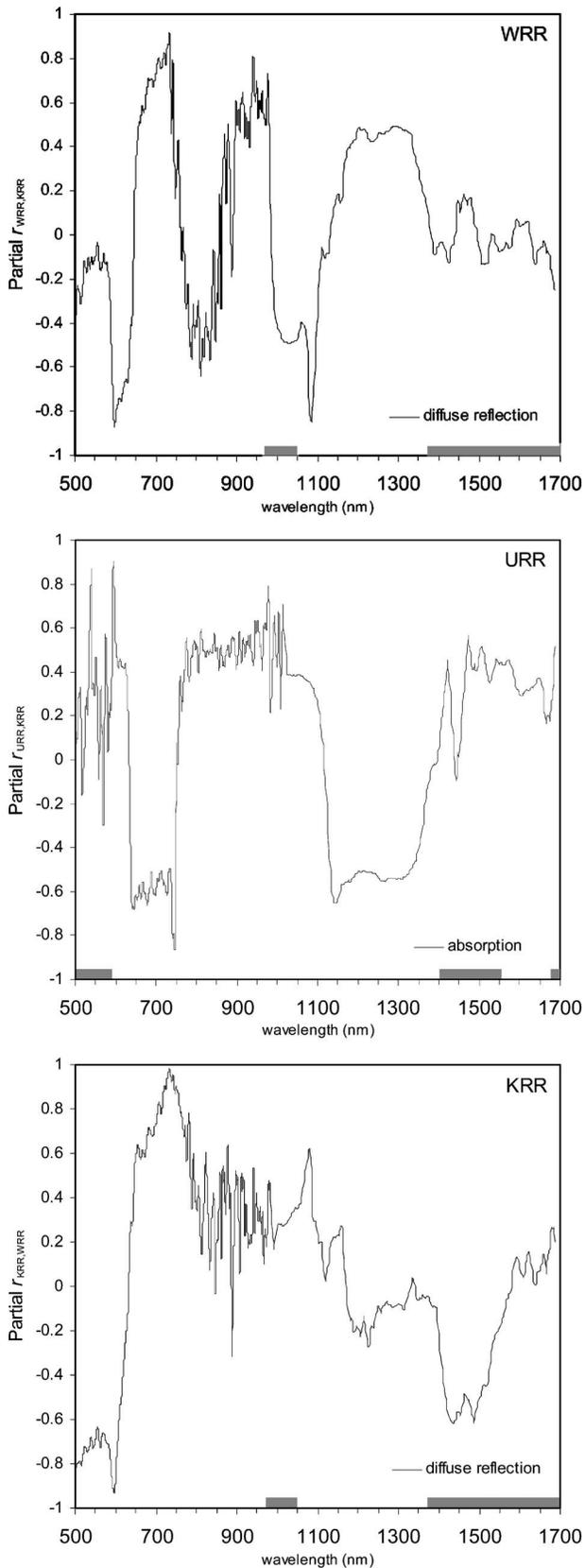


Fig. 3 Partial correlation spectra for (a) WRR with the effect of KRR removed, (b) URR with the effect of KRR removed, and (c) KRR with the effect of WRR removed. The spectra represent partial correlation with the measured transmission or diffuse reflection difference spectra from Fig. 2.

hemodialysis-induced changes in whole blood. The broad spectral effects observed are not easily accounted for by measuring the concentration of a few analytes. While certain correlations between optical properties in blood and clinical parameter levels may exist, the relation is unlikely a simple causal one. In this respect, the full optical spectrum measures reported from the PCA analysis may prove to be more useful in assessing broader factors such as disease status, treatment efficacy, or patient outcomes. It is worth mentioning that correlation between measured spectra and the clinical indicators chosen may also be affected by the use of serum-based analyte levels in the clinic. While analyte levels in hemolyzed blood may better correlate with whole-blood spectral changes, routine laboratory analysis procedures were followed in the study as these represented the clinical standard upon which patients were assessed and treatment was routinely delivered.

A potential confounding factor in this study was the possibility of oxygen saturation (O_2 -sat) changes in blood influencing optical properties primarily below 1100 nm. In this study, mixed arterio-venous blood was drawn following standard clinical protocol. Blood drawn in this manner typically has a high partial pressure of oxygen and a corresponding high O_2 -sat, as confirmed by the similarity and consistency of measured pre- and postdialysis spectra with that of pure oxyhemoglobin.¹⁶ The maintenance of O_2 -sat levels during hemodialysis has also been noted by others.¹⁰ Spectral features characteristic of a change in the high O_2 -sat level are also absent from the measured difference spectra. In particular, spectral shape changes in the 540- to 580-nm double-peak region and changes in the 760-nm region due to the presence of deoxyhemoglobin¹⁶ are absent in the spectra shown in Fig. 1. The absence of O_2 -sat change was confirmed by performing a linear fit of each measured spectrum to published oxy- and deoxy-hemoglobin spectra.¹⁶ In all cases the O_2 -sat level exceeded 98%, indicating that O_2 -sat changes were minimal and therefore had a negligible confounding effect upon the analysis.

Another potential confounder in the analysis was the change in hematocrit level due to treatment. As hemodialysis removes fluid while the blood cells remain, a hemoconcentrating effect is expected leading to an increased fraction of optically absorbing and scattering species within the blood. Because the standard clinical protocol used in this study excluded postdialysis hematocrit determination, the influence of hematocrit was investigated in a separate substudy. Hematocrit change due to treatment was determined by volumetric hematocrit measurement after centrifuging, followed by comparison of pre- to postdialysis levels. Hematocrit change due to treatment was found to vary from -17 to $+20\%$, reflecting both an increase due to hemoconcentration as well as a counteracting effect due to rapid plasma refilling in some patients. Hematocrit changes were compared with transmission and diffuse reflection changes in the samples and no significant correlation was found in the 500- to 1700-nm wavelength range (data not shown). The effect of hematocrit change on the observed spectral changes was therefore not significant in our measurements. Others have noted a linear change in absorption and diffuse reflection with hematocrit,²⁵ resulting in a variation of overall detected intensity with hematocrit

level—an effect that, if present, would have been removed by our spectral normalization procedure.

Although in this study no attempt was made to preprocess the spectral data, such schemes can be useful in extracting meaningful spectral information for molecular identification^{2,7,19} or disease pattern recognition.¹¹ With a larger patient group and a broader set of clinical parameters, multivariate methods such as PCA, linear discriminant analysis, and the partial least-squares method could be used with the full optical spectrum to investigate the underlying mechanisms resulting in the observed blood changes and to potentially predict treatment outcomes.

Finally, although measurements in the present study were limited to wavelengths shorter than 1700 nm, it would be beneficial to use the entire near infrared wavelength range (up to 2500 nm). Fundamental overtones of molecular vibrations present at longer wavelengths would yield more distinct spectral features and thereby a more robust characterization of whole blood properties. While the absorption due to water increases at longer wavelengths, useful features in whole blood spectra throughout the near infrared region have been reported in the literature despite this interference.^{26,27}

5 Conclusion

In the present pilot study, new information pertaining to changes in whole blood resulting from hemodialysis treatment for ESRD has been presented. Using the PCA method, the optical spectrum of blood (500 to 1700 nm) from 8 patients was analyzed and it was found that a significant difference could be detected between undialyzed and dialyzed blood in the patient group at a level of $P < 0.01$ in both transmission and diffuse reflection modes. Difference spectra due to treatment were shown to have an association with clinical measures of hemodialysis efficacy likely originating at the molecular level. While the spectroscopic techniques presented may eventually provide a limited usefulness in monitoring specific molecular parameters, the complexity of hemodialysis-induced changes in whole blood indicate that a full-spectrum monitoring approach may be better suited to the investigation of macroscopic clinical questions relating to hemodialysis adequacy, disease progression, and overall toxicity. Wide-spectrum blood monitoring combined with a database of spectral patterns could enable complex relations among numerous parameters to be recognized as a pattern differing from an ideal or baseline. In this manner, an ensemble of physiologic and molecular changes in the retained blood are monitored together with an implicit weighting. The resulting patterns may correlate better with treatment-related complications, disease progression, and quality-of-life factors than current clinical parameters. With rapid, noncontact and nondestructive monitoring, both time- and wavelength-resolved information could be significantly more useful, and it is envisioned that extending the present technique to an online system would provide a valuable approach to investigating the provision of optimum hemodialysis for the ESRD patient population.

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