Online measurement of urea concentration in spent dialysate during hemodialysis

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ABSTRACT

We describe on-line optical measurements of urea concentration during the regular hemodialysis treatment of several patients. The spectral measurements were performed in the effluent dialysate stream after the dialysis membrane using an FTIR spectrometer equipped with a flow-through cell. Spectra were recorded across the 5000-4000 cm^{-1} (2.0-2.5 μm) wavelength range at 1-minute intervals. Optically determined concentrations matched concentrations obtained from standard chemical assays with a root-mean-square error of 0.29 mM for urea (0.8 mg/dl urea nitrogen), 0.03 mM for creatinine, 0.11 mM for lactate, and 0.22 mM for glucose. The observed concentration ranges were 0-11 mM for urea, 0-0.35 mM for creatinine, 0-0.75 mM for lactate, and 9-12.5 mM for glucose.

Keywords: Urea, hemodialysis, noninvasive, spectroscopy, online monitoring, creatinine

1. INTRODUCTION

Treatment options for end-stage renal disease are limited to kidney transplantation and hemodialysis, with hemodialysis currently being the primary option. In patients with inadequate renal function, toxins and fluids that would normally be removed from the blood stream by the kidneys build up to harmful levels. Hemodialysis removes toxins from the blood by a closed-loop process where the blood of the patient is continuously being withdrawn, dialyzed, and returned to the patient. Low- and middle-molecular weight toxins pass through the dialyzer membrane into a dialysate stream, which is discarded. The procedure is generally performed three times per week, with a typical dialysis session lasting from 2.5 to 4.5 hours.

Urea, although not particularly toxic itself, is used as a marker for the removal of toxins during hemodialysis. Because the generation rate of urea is not necessarily the same as the generation rate of other toxins, the absolute concentration of urea at the beginning or the end of dialysis are not particularly significant in themselves.[1,2] However, the relative reduction in urea concentration can be used as a measure of the relative removal of other low- and mid-molecular-weight toxins.

Dialysis dose is most commonly quantified using the unitless expression Kt/V, where K is the clearance of the dialyzer (milliliters of plasma cleared of urea per minute), t is the duration of the dialysis treatment, and V is the patients urea distribution volume. Kt/V is thus the effective fraction of the patients urea distribution volume cleared of urea during a single treatment. The dialysis dose for a given treatment can be determined from published expressions that relate Kt/V to the fractional decrease in the plasma urea concentration, the quantity of ultrafiltrate removed, and the patient's post-dialysis weight.

The amount of dialysis a patient receives has a strong impact on their general health and mortality. Unfortunately, the optimal dose required to maintain good health is not precisely known. The standard procedure for monitoring the delivered dose is to collect pre- and post-dialysis blood samples that are analyzed for urea content in a clinical laboratory. The single-pool or equilibrated Kt/V is calculated from the change in urea concentration using one of several approximations or by an iterative calculation. This evaluation is typically performed once per month. If the delivered dose is less than the prescribed dose, the parameters of the dialysis treatment are adjusted. In order to avoid unnecessary

corrections due to a single erroneous dose estimation, corrections are normally not made until the inadequacy of the dose is confirmed with at least one additional measurement.[3]

The traditional once-per-month blood-based Kt/V estimation has several recognized limitations. The first is simply a consequence of the infrequent monitoring: underdialysis can persist for up to two months before the dialysis prescription is modified, by which time the patient's health can be detrimentally affected. The infrequency of blood-based measurements also puts significant burden on the accuracy of the measurement, which is sensitive to factors such as the precise timing of the post-dialysis blood draw. Moreover, it is assumed that the delivered dose is consistent throughout the month and that the day where dose is quantified is representative of the month in general. There is evidence that the dose delivered on the days when blood measurements are taken are, on average, higher than the rest of the month.[4] This could be explained by greater attention being paid by both the patient and clinic staff when they know that the effectiveness of the session will be evaluated. Finally, because the relative effect of dialysis is a highly non-linear function of dose,[1] occasional treatments where a patient is significantly underdialyzed may have a disproportionate effect on patient health even though the *average* dose the patient receives is adequate.

There are several potential benefits to a sensor that allows real-time, on-line monitoring of dialysis dose. Because on-line measurement of the delivered dose can in principal be applied to every session, systematic underdialysis of an individual can be identified and corrected on a much shorter time scale (i.e., in two sessions rather than two months). Even more far-ranging possibilities exist for sensors that can quantify dose *during* the dialysis treatment. This would allow clinical staff to identify patients that will not receive an adequate dose by the end of the prescribed session. For example, problems affecting dialysis efficiency such as to poor dialyzer performance, access recirculation, fistula obstruction, or low patient perfusion rates can be identified during the treatment, and steps can be taken either to correct them or to compensate for them by increasing the rate of dialysis.

There have been a number of sensors developed for monitoring urea removal. Most of these sensors are based on quantifying the concentration of urea in either the spent dialysate stream or in a separate ultrafiltrate stream using urease-based chemistry. Another strategy for quantifying urea removal is to use ionic dialysance to estimate the urea clearance of the dialyzer during the treatment.[5-10] Measurement of the ionic dialysance can be performed using a pair of temperature-corrected conductivity probes at the inlet and outlet of the dialyzer. The procedure requires conductivity measurements with two different dialysate conductivities, which can be obtained by varying the ratio of dialysate concentrate to water. However, doing so in an automated manner requires customized integration with the dialyzer unit.

It is also possible to measure urea using near-infrared spectroscopy. The useful spectral regions are limited to those between water absorption bands where sufficient amounts of light can be transmitted through aqueous samples. The three water transmission windows in the near-infrared include the combination region (5000-4000 cm^{-1}, 2.0-2.5 μm), which corresponds to the first order combination of stretching and bending modes of C-H, O-H, and N-H bonds. The second is the first overtone region (6500-5500 cm^{-1}, 1.53-1.82 μm), which corresponds to the first overtone of C-H stretching modes. The third is the short-wavelength infrared (12500-8600 cm^{-1}, 0.8-1.6 μm), which contains a multitude of higher-order combinations and overtone modes.

The combination region has been demonstrated to be the most attractive for measuring urea in biological samples.[11] The absorption spectrum of urea in this range is relatively strong and distinct from other components whereas the spectrum in the short-wavelength near-infrared is broad and significantly weaker. The first overtone region provides no useful urea information. In general, it has been shown that prediction errors for optimized calibration models generated from short-wavelength near-infrared spectra are significantly larger than those obtained from analogous combination region spectra.[11,12]

In addition to urea, it would be clinically useful to monitor creatinine concentrations. Creatinine is used clinically as an indicator of muscle mass.[13] Creatinine kinetic modeling can be used to estimate the nutritional adequacy of a dialysis patient's diet.

It has been shown that urea can be measured in samples of spent dialysate using near-infrared spectroscopy under controlled laboratory conditions.[11] The goal of this work is to explore the possibility of performing online measurements of urea during clinical dialysis sessions. In addition, the ability to monitor creatinine is also investigated.

2. EXPERIMENT

Measurements were performed during 10 regular dialysis sessions of volunteers at the outpatient dialysis unit of the University of Iowa Hospital and Clinics. Dialysate was withdrawn continuously from the dialysis system after passing through the dialyzer and routed through a flow-through optical cell with sapphire windows. The optical path length (1 mm) was set by stainless steel spacers between the sapphire windows. Dialysate was drawn through the system at 30 ml/min using a peristaltic pump. The time for dialysate to reach the spectrometer from the dialyzer membrane was less than 10 seconds. Dialysate temperature was measured using a thermocouple probe located just downstream from the optical cell. No active temperature stabilization of the optical cell was employed. The observed dialysate temperature range was 28-32 ºC.

Samples of spent dialysate (20ml) were collected seven times during the course of each dialysis treatment at approximately 5, 15, 30, 60, 120, 180, and 240 minutes after the start of dialysis. The samples were immediately refrigerated for determination of urea, glucose, lactate, and creatinine concentration in our laboratory. Glucose and lactate concentrations were measured with a Yellow Springs Instruments analyzer (model 2300 Stat Plus). Urea concentration was determined using the Berthelot reaction.[14] Creatinine concentration was determined using a commercial assay kit (Sigma 555-A).

Spectra were collected using a Nicolet Nexus Fourier transform infrared spectrometer with a CaF_2 beam splitter and a liquid-nitrogen cooled InSb detector. A K-band filter (Barr Associates) was placed before the detector to isolate the 5000-4000 cm^{-1} wavelength range. Spectra computed from 128 co-added interferograms (approximately a 50 second collection time) were collected continuously before and during the treatment. Single-beam intensities were recorded over the 5000-4000 cm^{-1} range with a 16 cm^{-1} spectral resolution. Comparison of back-to-back spectra indicates that the spectral noise in the measurement was 0.5-1.5μAU (1 AU corresponds to a transmission of 10%) over the 4300-4800 cm^{-1} range.

The nominal composition of fresh dialysate used in the clinic was 10 mM glucose, 4 mM acetate, 33 mM bicarbonate, and various salts in a pH 7.8 buffer. Of these components, only glucose and acetate have significant near-infrared absorption. In addition, spent dialysate will contain urea, creatinine, lactate, and other small molecular weight toxins. Of these, urea is present in the highest concentration. The experimental concentration ranges during the course of the experiment are listed in Table 1.

3. RESULTS AND DISCUSSION

The measured single-beam spectra of the spent dialysate are ratioed to a single-beam spectrum of water collected before the beginning of the experiment and converted to absorbance (base-10 logarithm). Because of the strong temperature dependence of water, the dialysate absorbance spectrum shows significant broad variations. In principle, this variation can be accounted for in a multivariate calibration model.[15] However, it is advantageous to remove these broad variations using a filtering step in order to improve modeling robustness.

Broad baseline variations can be removed using a Savitzky-Golay filter. A second order filter with a 160 cm^{-1} width is used in this analysis. The width was chosen as a minimal width that preserves the major features of the analytes that will be considered. The effect of applying the Savitzky-Golay filter is shown in Figure 1, which shows a selection of unfiltered and filtered absorbance spectra from one of the dialysis treatments. Previously, similar results have been obtained using a digital Fourier filter.[16-18]

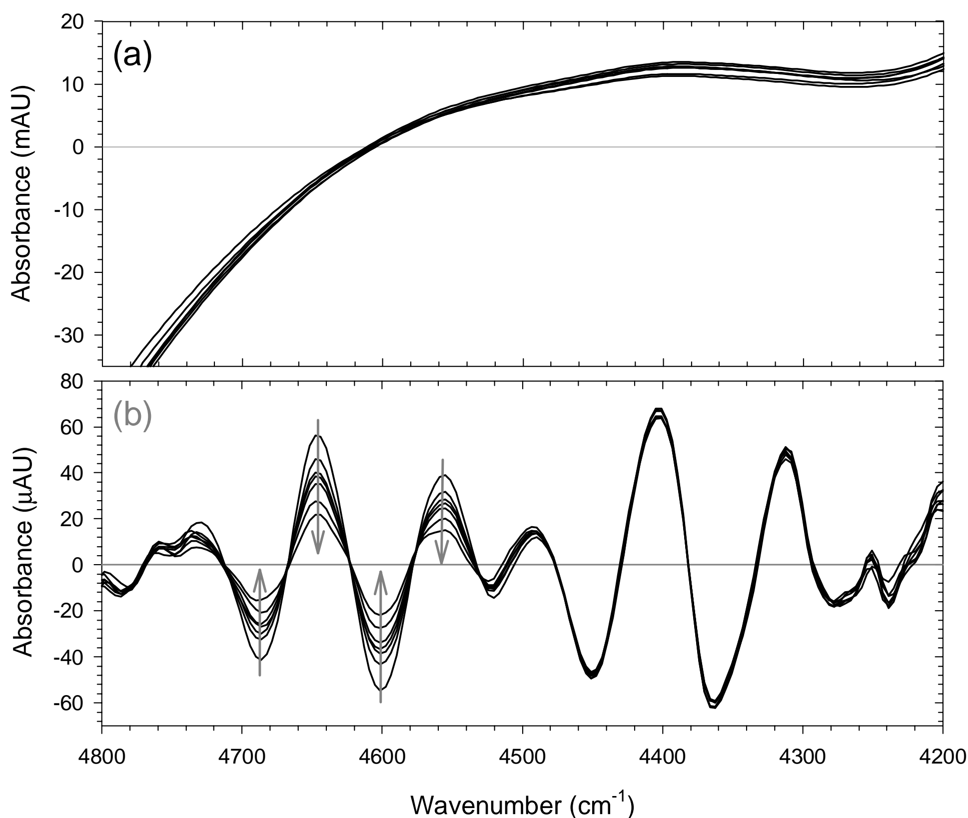

Figure 1: On-line absorbance spectra of spent dialysate recorded during a dialysate treatment. (a) Unfiltered spectra ratioed to water. (b) Spectra from (a) after the application of a second order Savitzky-Golay filter with width of 160 cm^{-1}. The features between 4700 and 4500 cm^{-1} are primarily due to urea, whereas those between 4500 and 4300 cm^{-1} are primarily due to glucose. The arrows shown in (b) indicate the direction of change with time.

The filtered spectra are dominated by absorptions due to glucose and urea. The features between 4500-4300 cm^{-1} are due primarily to glucose, while those between 4700-4500 cm^{-1} are primarily due to urea. Figure 1(b) indicates that the glucose concentration remains relatively constant while the urea concentration decreases with time during the treatment. The filtered absorbance spectra of several expected spent-dialysate components are shown in Figure 2.

Because of the rather simple structure of the filtered absorbance spectra and the partial isolation of urea, it is possible to extract the urea concentration using a simple regression of the measured spectra in terms of a set of pure component spectra. The pure component spectra used in this study are glucose, acetate, urea, creatinine, and lactate. Figure 3 shows a regression of a representative on-line spectra in terms of the above pure component basis. All of the dominant features are accounted for, although there are some deviations (particularly around 4500 cm^{-1}). In order to be a reliable means of predicting the concentration of all analytes, all of the near-infrared absorbing components should be present in the regression basis. However, urea is an exception in that its absorption features are relatively isolated from those of the other known components. In practice, the root-mean-square difference between the measured and regressed spectra could be used as an indicator for the presence of non-modeled analytes or baseline variations.

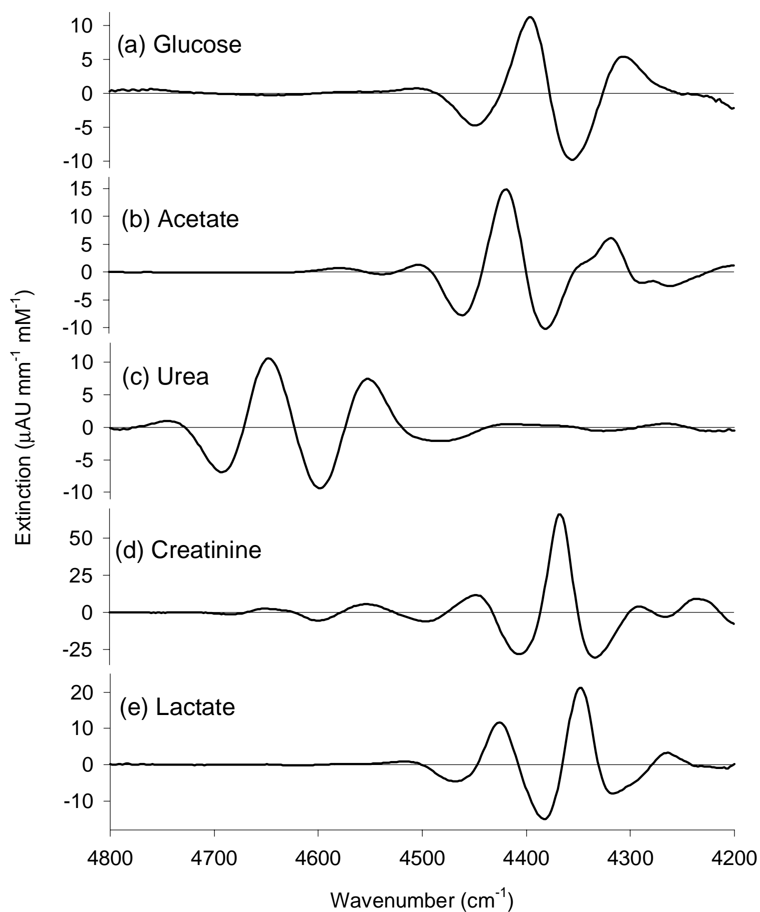

Figure 2: Filtered extinction spectra of five analytes found in spent dialysate.

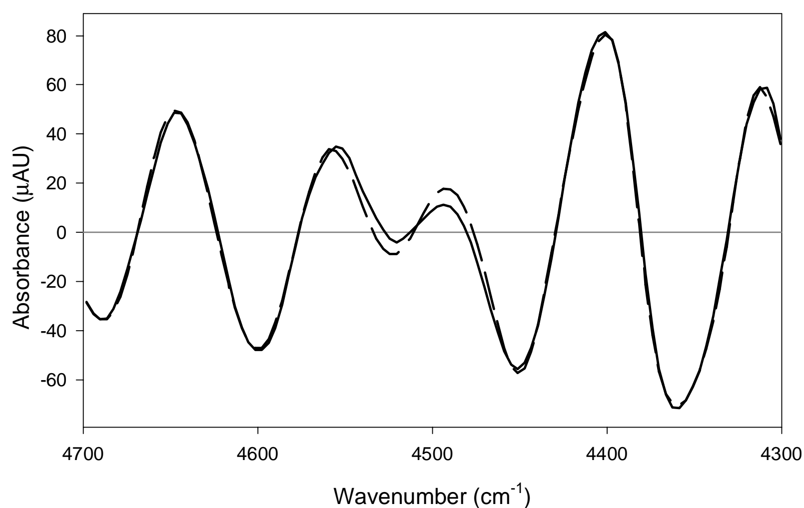

Figure 3: Measured and regressed filtered absorbance spectra. The solid curve is the measured spectrum and the dashed line is the regressed spectrum. The regression basis is shown in Figure 2.

For each dialysis treatment, there were seven spent dialysate samples collected whose urea concentration was determined using standard chemical techniques. The spectra concurrent with these samples were analyzed using a linear least-squares regression in terms of the five components shown in Figure 2 over the wavelength range from 4700-4300 cm^{-1}. The urea concentration extracted using the regression matched those determined using chemical techniques with a standard error of prediction of 0.33 mM (0.8 mg/dl urea nitrogen).

The advantage of this approach is that the calibration model can be constructed before any on-line data collection has taken place. The only information used in constructing the urea calibration model were a water reference spectrum and the five pure component spectra, which (with the exception of acetate) were measured in a separate laboratory before moving the spectrometer to the clinic where the on-line measurements took place. In particular, there was no adjustment of the slope of the actual vs. predicted concentration line to match the on-line spectra. Thus the entire experiment can be considered a study of the ability of this procedure to extract urea concentration from spectra that have not been encountered before.

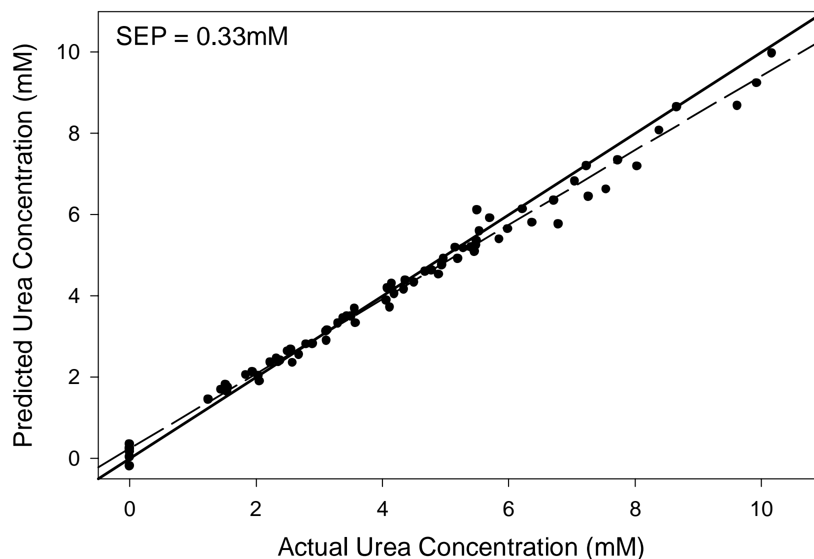

Figure 4: Predicted vs. actual urea concentration as determined by a linear regression using the basis shown in Figure 2. The solid line has a slope of one and a zero intercept. The dashed line is a least-squares fit of the data using a nonzero intercept.

One of the limitations of this type of modeling can be seen in the slight bias in Figure 4. This type of bias is likely due to incomplete baseline removal using the Savitzky-Golay filter. A slightly smaller SEP (0.31 mM) could be obtained by allowing a non-zero intercept and a slope adjustment, as shown by the dashed line in Figure 4. The regression strategy does not work for the analytes with significantly lower concentrations, such as creatinine and lactate, for which the intercept is large compared to the concentration range.

A more rigorous analysis can be obtained using a factor-based procedure such as partial least squares (PLS) regression. Factor-based approaches have the advantage of not requiring a complete basis set, relying instead on a statistical determination of the principal systematic variations observed in the actual data set. However, factor-based methods suffer from two dangers that make their use difficult: overfitting and incorporation of secondary correlations.[19] Care must be taken to ensure that calibration models generated with PLS regression contain significant spectral features of the analyte and are effectively orthogonal to the spectral signature of other analytes present.

In order to generate a PLS calibration model, spectra corresponding to the dialysate samples whose analyte concentrations are known were divided into calibration and prediction sets. The spectra from the final two dialysis treatments were used as the prediction set in order to gauge the extrapolation capabilities of the calibration.[19] The spectra

from the first eight treatments were used as the calibration set. The calibration set was further subdivided into training and monitoring subsets for the sake of optimizing the number of factors used. Preliminary calibrations were generated using the training set for models incorporating 1-15 factors. The optimal number of factors was chosen as the number for which the standard error of the monitoring set was minimized after multiple shuffles of the training and monitoring sets. After the number of factors was determined, a final model was generated based on all of the spectra in the calibration set. This model was then evaluated using the prediction set.

Table 1: Observed concentration range for each of the analytes with estimated error of the standard methods. Standard errors of calibration (SEC) and prediction (SEP) for urea, creatinine, lactate, and glucose for calibration models built using PLS regression with the specified number of factors.

Analyte	Concentration Range (mM)	Accuracy of Standard Method (mM)	PLS Factors	SEC (mM)	SEP (mM)
Urea	0 - 11	0.16	8	0.17	0.29
Creatinine	0 - 0.35	0.014	7	0.019	0.031
Lactate	0 - 0.75	0.022	13	0.064	0.108
Glucose	9 - 12.5	0.09	15	0.11	0.22

The results for the PLS regression analysis are listed in Table 1. The standard error of prediction for urea was 0.29mM (0.8 mg/dl urea nitrogen). With the exception of lactate, the estimated uncertainty in the standard methods is nearly as large as the standard error of the calibration process, which means that the quality of the prediction model may be limited by the standard methods themselves. Predicted vs. actual urea concentrations are shown in Figure 5 for both the calibration and prediction spectra. It should be noted that smaller SEP values were obtained for different choices of the numbers of factors; however, the number of factors was chosen based on minimizing the root-mean-square error of the monitoring set to avoid being misled by fortuitous cancellation of errors in a limited number of prediction spectra. The prediction set was not used until after the calibration had been built. Creatinine can be measured with much higher accuracy, although the range of creatinine in the dialysate samples is significantly smaller. Predicted vs. actual concentration values for creatinine are shown in Figure 6.

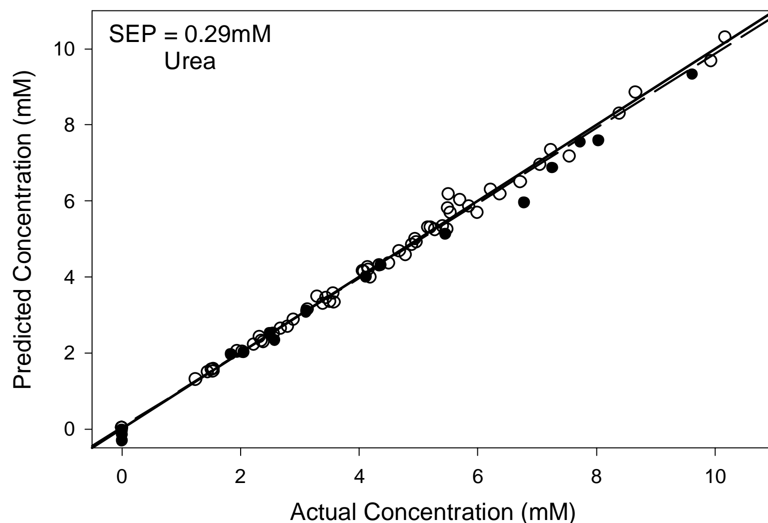

Figure 5: Predicted vs. actual urea concentration using PLS regression. The open circles are the points used to build the calibration model. The filled circles represent values from the final two dialysis sessions, which were not included in the model building process. The solid line has a slope of one and passes through the origin. The dashed line is a linear regression of the calibration and prediction points.

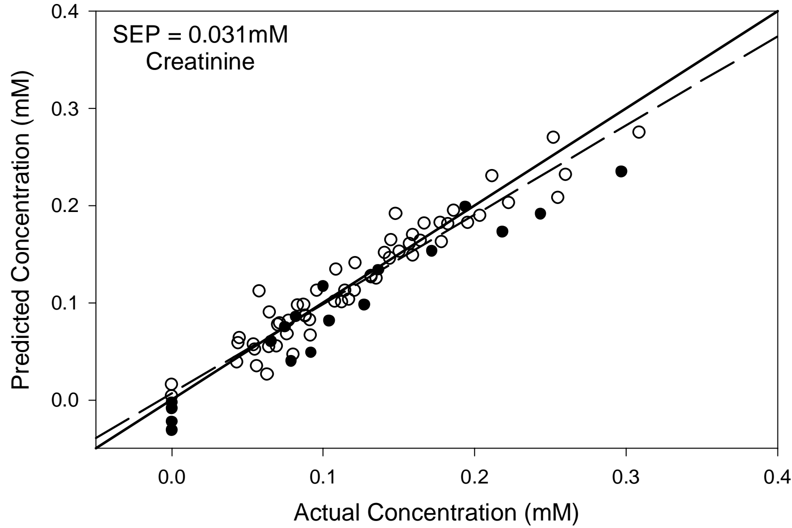

Figure 6: Predicted vs. actual concentration for creatinine as modeled using PLS regression. The open circles are the points used to build the calibration model. The filled circles represent values from the final two dialysis sessions, which were not included in the model building process. The solid line has a slope of one and passes through the origin. The dashed line is a linear regression of the calibration and prediction points.

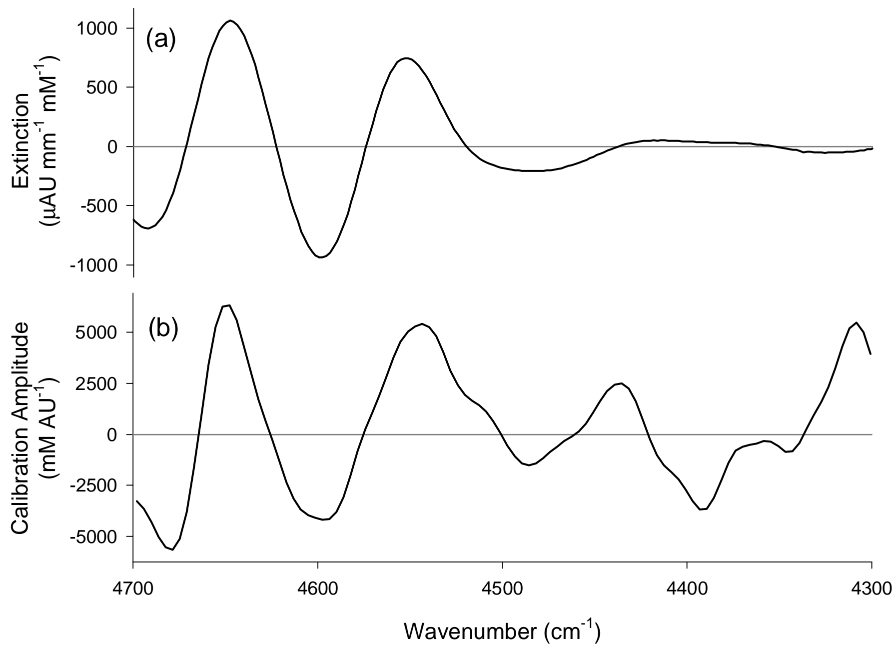

Figure 7: Comparison of (a) the urea pure component spectrum and (b) the PLS calibration vector for urea. The calibration vector contains significant urea-specific information in the 4700-4500 cm^{-1} range.

It is important to verify that the prediction ability of a PLS model is based on analyte-specific information. It is well known that apparently successful models can be built for a limited data set based on secondary or chance correlations with the analyte.[19] This is especially important in this experiment because of the a high degree of correlation between the different analytes (such as urea and creatinine), which is unavoidable. The calibration function generated by the PLS model for urea is shown in Figure 7 along with the pure component spectrum for urea. The dominant features of the

calibration function clearly reflect the urea absorption features in the 4700-4500 cm^{-1} range. The residual structure between 4500 and 4300 cm^{-1} do not appear to be correlated with any of the other known components (see Figure 2). Similar analysis of the creatinine calibration vector indicates that the calibration is based on creatinine spectral features.

The potential for interference between urea and the other known analytes can be estimated by taking the inner product of the urea calibration function with the pure component spectra of the other analytes. The results of this procedure are listed in Table 2. The values listed in the table represent the bias introduced into the predicted concentration of one analyte by a change of one mM in the interfering compounds. The values should ideally be much less than one. It is inevitable that there will be some bias among the different compounds given the limited concentration ranges in the current data set. The orthogonality of the calibration model could be enhanced in future experiments by spiking calibration samples to artificially increase the concentration range of creatinine, glucose, and lactate. It is also possible to mathematically force the calibration function to be orthogonal to the interfering analytes.

Table 2: Estimates of concentration bias due to other analytes.

	Interfering Analyte				
Prediction Analyte	Urea (mM/mM)	Creatinine (mM/mM)	Glucose (mM/mM)	Lactate (mM/mM)	Acetate (mM/mM)
Urea	-	0.112	-0.059	0.187	0.242
Creatinine	0.017	-	0.005	-0.037	-0.044

Having built and verified a successful calibration model using a subset of the spectra from each treatment, it is possible to calculate the concentration of the various dialysate components with time. Results for the final dialysis treatment, which was not used in the model-building process, are shown in Figure 8. Predicted values for all four components follow the expected trends. There are visible discontinuities in the curves for urea and creatinine at 20 and 140 minutes after the start of the treatment. These are due to modification of the rate of blood flow across the dialyzer during the treatment. The blood flow rate was reduced from 330 to 260 ml/min at 20 minutes and increased to 300 ml/min at 140 minutes.

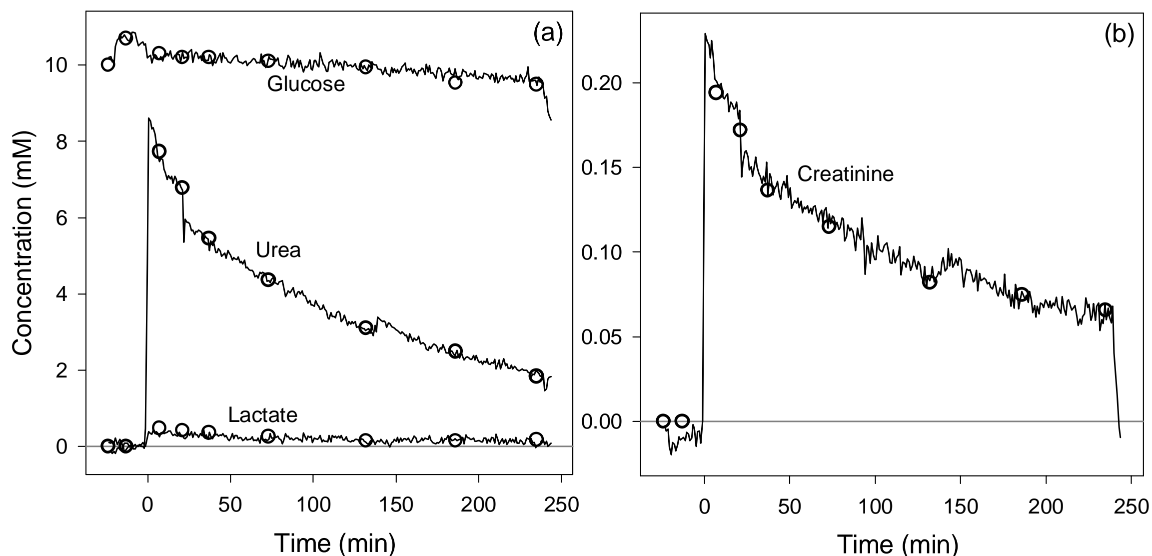

Figure 8: Concentration of urea, glucose, lactate, and creatinine in the spent dialysate stream at one-minute intervals during the final dialysis treatment. The lines are the concentrations using the appropriate PLS calibration model; the circles are the concentrations measured using standard chemical techniques. The discontinuities at 20 and 140 minutes in the urea and creatinine curves are real as explained in the text.

4. CONCLUSIONS AND FUTURE WORK

We have demonstrated that it is possible to accurately measure urea in the spent dialysate stream during hemodialysis sessions with a prediction accuracy of 0.33 mM using a simple regression model. The model described here was constructed entirely from data available before the start of the first dialysis treatment. Its successful performance with no adjusted parameters gives us confidence that the calibration model is robust. This approach is, however, not able to accurately measure lower-concentration components with less distinct absorption spectra, such as creatinine or lactate.

Partial-least-squares regression was used to develop more accurate calibration models for urea, creatinine, glucose, and lactate. Successful calibrations were built with a root-mean-square prediction error of 0.29 mM for urea, 0.031 mM for creatinine, 0.11 mM for lactate, and 0.22 mM for glucose. These prediction errors were calculated from spectra from the final two dialysis sessions, which were not included in the model-building process. Creatinine was measured with sufficient accuracy to permit evaluation of its removal rate independent of the removal rate of urea. The calibration function for urea was investigated to confirm that the modeled concentration was based primarily on the specific absorbance signature of urea.

In the future, we hope to perform a longer-term study in order to investigate the stability of the generated calibration model. In addition, it would be useful to artificially increase the concentration of components such as creatinine and lactate in order to decrease the potential for cross-analyte biasing. Finally, we are working to develop compact instrumentation that will allow spectral measurements to be made without the cost and size of a conventional research-grade Fourier-transform infrared spectrometer.

5. ACKNOWLEDGEMENTS

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