

Online Measurement of Urea Concentration in Spent Dialysate during Hemodialysis

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Background: We describe online optical measurements of urea in the effluent dialysate line during regular hemodialysis treatment of several patients. Monitoring urea removal can provide valuable information about dialysis efficiency.

Methods: Spectral measurements were performed with a Fourier-transform infrared 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-min intervals. Savitzky–Golay filtering was used to remove baseline variations attributable to the temperature dependence of the water absorption spectrum. Urea concentrations were extracted from the filtered spectra by use of partial least-squares regression and the net analyte signal of urea.

Results: Urea concentrations predicted by partial least-squares regression matched concentrations obtained from standard chemical assays with a root mean square error of 0.30 mmol/L (0.84 mg/dL urea nitrogen) over an observed concentration range of 0–11 mmol/L. The root mean square error obtained with the net analyte signal of urea was 0.43 mmol/L with a calibration based only on a set of pure-component spectra. The error decreased to 0.23 mmol/L when a slope and offset correction were used.

Conclusions: Urea concentrations can be continuously monitored during hemodialysis by near-infrared spectroscopy. Calibrations based on the net analyte signal of urea are particularly appealing because they do not require a training step, as do statistical multivariate calibration procedures such as partial least-squares regression.

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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 usually be removed from the blood stream by the kidneys build up to harmful concentrations. Hemodialysis removes toxins from the blood by a closed-loop process in which the blood of the patient is continuously withdrawn, dialyzed, and returned to the patient.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 2.5–4.5 h.

The amount of dialysis that patients receive has a strong impact on their general health and mortality. Unfortunately, the optimal dose required to maintain good health is not precisely known. Dialysis dose is most commonly quantified by use of the unitless expression Kt/V , where K is the clearance of the dialyzer (milliliters of plasma cleared of urea per min), t is the duration of the dialysis treatment, and V is the patient's urea distribution volume. The unitless expression Kt/V is thus the effective fraction of the patient's 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 postdialysis weight.

The standard procedure for monitoring the delivered dialysis dose is to collect pre- and postdialysis blood samples, which are analyzed for urea content in a clinical laboratory. The single-pool or equilibrated Kt/V is calculated from the change in urea concentration by use of one of several approximations or by an iterative calculation. This evaluation is typically performed once a month. If the delivered dose is less than the prescribed dose, the conditions for the dialysis treatment are adjusted. To avoid unnecessary corrections attributable to a single erroneous dose estimation, corrections are usually not made until the inadequacy of the dose is confirmed with at least one additional measurement (1).

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The traditional once-per-month blood-based Kt/V estimation has several recognized limitations. The first is a consequence of the infrequent monitoring: underdialysis can persist for up to 2 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 substantial burden on the accuracy of the measurement, which is sensitive to factors such as the precise timing of the postdialysis blood draw. Moreover, it is assumed that the delivered dose is consistent throughout the month and that the day on which the 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 during the rest of the month (2). 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 nonlinear function of dose, occasional treatments during which a patient is substantially underdialyzed may have a disproportionate effect on patient health, although the average dose that the patient receives is adequate (3).

There are several potential benefits to a sensor that would allow real-time, online monitoring of urea removal. If online monitoring can be used every session, systematic underdialysis of an individual can be identified and corrected on a much shorter time scale than with blood-based measurements (i.e., in two sessions rather than 2 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 who will not receive an adequate dose by the end of the prescribed session. For example, problems affecting dialysis efficiency, such as poor dialyzer performance, access recirculation, fistula obstruction, or low patient perfusion, 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.

We have previously demonstrated that urea can be measured reagentlessly in samples of spent dialysate by use of near-infrared spectroscopy under controlled laboratory conditions (4, 5). The goal of this work was to explore the possibility of performing online measurements of urea during actual clinical dialysis sessions.

Materials and Methods

Measurements were performed during 10 regular dialysis sessions of volunteers at the outpatient dialysis unit of the University of Iowa Hospital and Clinics. The use of human subjects was approved by the University of Iowa Human Subjects Office. Spent dialysate was drawn continuously from the dialysis system after passing through the dialyzer. The dialysate was drawn through an optical cell at 30 mL/min by a peristaltic pump. The optical pathlength (1 mm) was set by stainless steel spacers between the sapphire windows of the optical cell. The

time for dialysate to reach the spectrometer from the dialyzer membrane was <10 s. Dialysate temperature was measured by a thermocouple probe located just downstream from the optical cell. No active temperature stabilization of the optical cell was used. The observed dialysate temperature range was 28–32 °C.

Samples of spent dialysate (20 mL each) were collected seven times during the course of each dialysis treatment at ~5, 15, 30, 60, 120, 180, and 240 min after the start of dialysis. The samples were immediately refrigerated for later measurement of urea, glucose, lactate, and creatinine concentrations in our laboratory. Glucose and lactate concentrations were measured with a YSI Model 2300 Stat Plus analyzer (Yellow Springs Instruments). Urea was measured by the Berthelot reaction (6) in conjunction with urease. The creatinine concentration was measured by a commercial assay (cat. no. 555-A; Sigma Chemical Company).

Spectra were collected on a Nicolet Model 670 Nexus Fourier transform infrared spectrometer (Nicolet Analytical Instruments) with a CaF_2 beam splitter and a liquid-nitrogen-cooled InSb detector. A K-band filter (Barr and Associates, Inc.) was placed before the detector to isolate the 5000–4000 cm^{-1} wavelength range. Spectra computed from 128 coadded interferograms (collection time ~50 s) 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 indicated that the spectral noise in the measurement was 0.5–1.5 microabsorbance units (μAU)³ over the 4800–4300 cm^{-1} range.

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

Results and Discussion

The measured single-beam spectra of the spent dialysate were ratioed to a single-beam spectrum of water that was collected before the beginning of the 14-day experiment. The transmission spectra were converted to absorbance spectra by taking the base-10 logarithm. The resulting absorbance spectra, some of which are shown in Fig. 1A, contained broad variations because of the differences in temperature between the water reference and the online samples. Because temperature variations are difficult to eliminate entirely in a flow-through system, in which

³ Nonstandard abbreviations: AU, absorbance unit(s); PLS, partial least-squares regression; and SEP, standard error of prediction.

Table 1. Observed concentration range for each of the analytes and correlation among the analytes.

Analyte	Concentration range, mmol/L	Correlation (<i>r</i>)			
		Urea	Glucose	Creatinine	Lactate
Urea	0–11		-0.2046	0.8912	0.2525
Glucose	9–12.5	-0.2046		-0.0869	-0.1001
Creatinine	0–0.35	0.8912	-0.0869		0.2072
Lactate	0–0.75	0.2525	-0.1001	0.2072	

there is temperature variation in the input stream, a robust calibration scheme must be able to operate in spite of such temperature variations. In principle, temperature variations can be accounted for in the multivariate calibration process (7). If possible, however, it is better to remove these broad variations by use of a filtering step before the multivariate calibration to improve model robustness.

Broad baseline variations can be removed by use of a Savitzky–Golay filter (8). Savitzky–Golay filtering is usually used for data smoothing (9). It has been noted elsewhere that there is often little benefit from data smoothing (low-pass filtering) before multivariate analysis (10). In this study, however, high-pass filtering is used to remove the broad spectral features attributable to temperature variations while preserving the higher-frequency analyte signal. A broad Savitzky–Golay smoothing filter is first applied to the measured spectra to identify the broad variations and smooth out the relatively narrow analyte features. This smoothed baseline is then subtracted from the original spectrum, leaving only

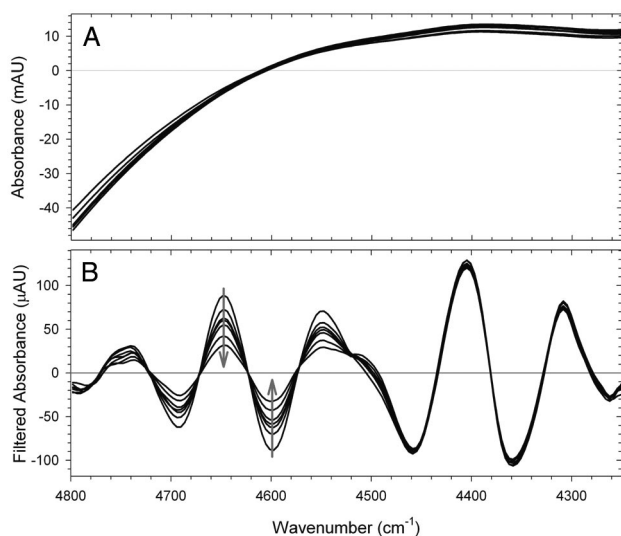


Fig. 1. Online absorbance spectra of spent dialysate recorded during a dialysis treatment.

(A), unfiltered spectra ratioed to water; (B), spectra after the application of a second-order Savitzky–Golay filter of width 200 cm^{-1} . The features between 4700 and 4500 cm^{-1} are primarily attributable to urea, whereas those between 4500 and 4300 cm^{-1} are primarily attributable to glucose. The arrows in B indicate the direction of change with time. The origins of the negative values in the filtered absorbance spectra are explained in the text.

the high-frequency components (8). Because of the subtraction of the smoothed baseline, local absorbance minima give negative absorbance features in the final absorbance spectra. Similar results have been obtained previously with use of a digital Fourier filter (11, 12). The advantage of filtering comes not from increasing the signal-to-noise ratio of the spectra by reducing high-frequency noise, but from excluding unimportant broad variations and reducing the number of factors required for a multivariate calibration model. Thus the benefit of filtering is to increase model robustness rather than accuracy.

Shown in Fig. 1B are the spectra from Fig. 1A after the application of a second-order, 200 cm^{-1} -wide Savitzky–Golay high-pass filter. The filter width was chosen as the minimum width that preserves at least 80% of the primary spectral features of urea, as measured by the difference between the urea absorbance at 4650 and 4600 cm^{-1} . Two passes with the filter were required to eliminate broad variations in the online spectra (13). Note that the magnitudes of the spectral features in the filtered spectra (Fig. 1B) are more than two orders of magnitude smaller than the broad variations attributable to sample temperature (Fig. 1A).

Filtered spectra of the near-infrared active dialysate components considered in this study are shown in Fig. 2. The urea absorption spectrum is distinct because it is dominated by features in the 4700 – 4500 cm^{-1} range.

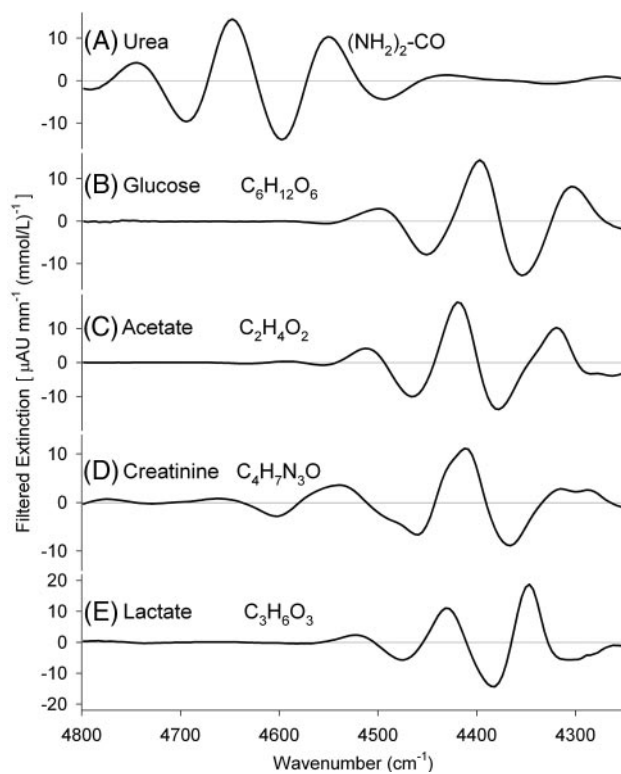


Fig. 2. Filtered pure-component extinction spectra of the five principal solutes found in spent dialysate.

These features are attributable to nonlinear combinations of vibrational modes of the N–H bond in the urea molecule, whereas the features between 4500 and 4300 cm^{-1} are attributable to combinations of C–H bond modes. Of the chemicals shown here, only urea and creatinine contain N–H bonds, although the creatinine absorption spectrum is dominated by C–H features. The shapes of the filtered online spectra, shown in Fig. 1B, are dominated by absorption of urea and glucose. From Fig. 1B, it can be seen that the glucose concentration remains relatively constant, whereas the urea concentration changes (decreases) by a factor of 3 during the treatment.

CALIBRATION USING PARTIAL LEAST-SQUARES REGRESSION

A calibration model for urea was generated from the filtered online spectra using partial least-squares regression (PLS). Factor-based approaches such as PLS do not require knowledge of all chemicals in the sample under investigation; instead, they rely on a statistical determination of the principal systematic variations observed in the calibration data set. However, factor-based methods must be used with care because of the possibilities of overfitting and the incorporation of secondary correlations (14). Care must be taken to ensure that calibration models generated with PLS contain significant spectral features of the analyte and are effectively orthogonal (linearly independent) to the spectral signature of other analytes present.

To generate a PLS calibration model, we divided spectra corresponding to the dialysate samples whose urea concentrations were measured by standard techniques into calibration and prediction sets. The spectra from the first eight treatments (57 spectra) were used as the calibration set. The spectra from the final two dialysis treatments (18 spectra) were used as the prediction set to gauge the extrapolation capabilities of the calibration. The calibration set was further subdivided into training and monitoring subsets (35 and 22 spectra, respectively) to optimize the number of factors used (15). The optimum number of factors was chosen as the number for which the root mean square error of the monitoring set was minimized after 50 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.

The standard error of prediction (SEP) for urea with use of the Savitzky–Golay-filtered spectra was 0.30 mmol/L (0.84 mg/dL urea nitrogen) based on a three-factor model. Predicted vs actual urea concentrations are shown in Fig. 3 for both the calibration and prediction spectra. A Bland–Altman plot of the difference between the optical and chemical assay results vs their mean is shown as an inset in Fig. 3 (16, 17). The SEP for the PLS calibration built with the unfiltered spectra was 0.38 mmol/L; however, the optimum number of factors in this

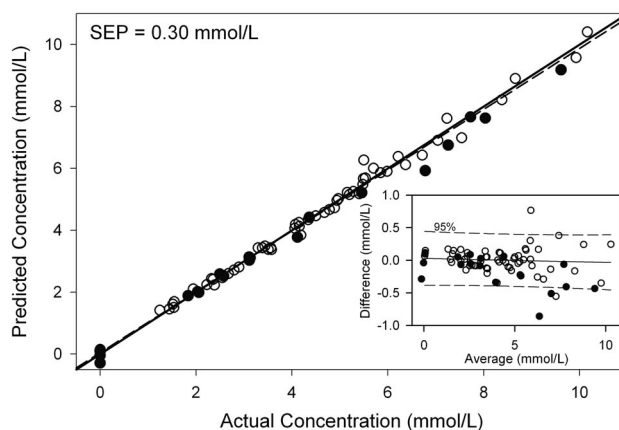


Fig. 3. Predicted vs actual urea concentration based on PLS regression with Savitzky–Golay filtering.

○, points used to build the calibration model; ●, values from the final two dialysis sessions, which were not included in the model-building process. The solid line has a slope of 1 and passes through the origin. The dashed line is a linear regression of the calibration and prediction points. (Inset), Bland–Altman plot of the difference between the optically predicted and actual urea concentration as a function of the mean of the concentrations from the two methods. The solid line on the inset is a regression line; the dashed lines represent the 95% confidence limits.

case was 10, which is substantially larger than for the model for the filtered spectra.

It is important to verify that the predictive 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 (14). This is especially important in this experiment because of the inherent correlation among the analytes (the correlation coefficients among the components are listed in Table 1). The calibration spectra generated by the PLS model for urea are shown in Fig. 4A for both the filtered (solid line) and

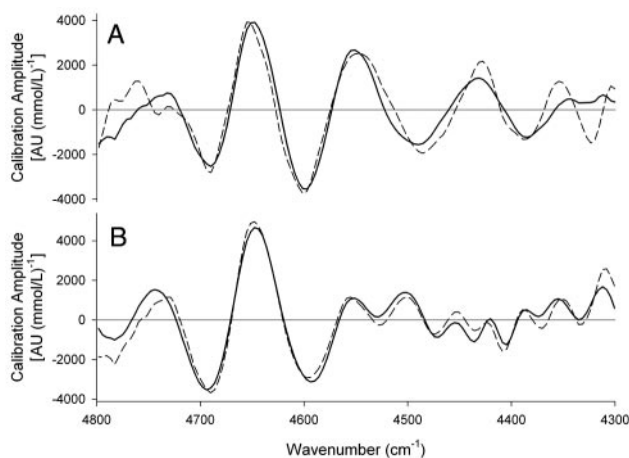


Fig. 4. Urea calibration spectra.

(A), PLS calibrations generated with filtered (solid line) and unfiltered (dashed line) spectra. (B), calibration spectrum constructed using the net analyte signal of urea (solid line) and the PLS calibration spectrum from A after it was forced to be orthogonal to pure-component spectra of glucose, acetate, creatinine, and lactate (dashed line).

unfiltered (dashed line) calibrations. The calibration spectrum is the final product of the PLS procedure. The predicted concentration is the inner product of the calibration spectrum and the absorbance spectrum of the sample for which the concentration is to be determined. The dominant features of the calibration spectrum clearly reflect the urea absorption features in the $4700\text{--}4500\text{ cm}^{-1}$ range (see Fig. 2A). The calibration spectra with and without Savitzky–Golay filtering are very similar. Because the amplitudes of the calibration spectra were almost identical, we conclude that there is no loss of sensitivity attributable to the filtering process.

The potential for interference between urea and the other known analytes can be estimated by taking the inner product of the urea calibration spectrum with the pure-component spectra of the other analytes. This provides an estimate of the difference introduced into the predicted urea concentration by a change of 1 mmol/L in the interfering compounds. The values would be zero if the urea calibration were independent of all other analyte concentrations. For the PLS calibration, the values are small but nonzero. We found differences of -0.09 mmol/L for glucose, 0.14 mmol/L for acetate, 0.17 mmol/L for creatinine, and 0.20 mmol/L for lactate. The value for glucose is troublesome because of the 3.5 mmol/L variation seen in the glucose concentration over the course of the measurements, which will introduce an additive difference of ± 0.15 mmol/L in the predicted urea concentration. The maximum differences attributable to creatinine (± 0.03 mmol/L) and lactate (± 0.08 mmol/L) are smaller because of the low concentrations of these components in the spent dialysate. That the values are nonzero is primarily a consequence of the partial correlation among the analytes in the calibration data set, which is inherent in a data set such as this. The orthogonality of the calibration model could be enhanced in future experiments by randomly adding analytes to calibration samples to artificially increase the concentration ranges of creatinine, glucose, and lactate (18). It is also possible to mathematically force the calibration spectrum to be orthogonal to the interfering analytes by use of measured single-component absorbance spectra.

Having built and verified a successful calibration model using a subset of the spectra for the analytes whose concentrations are known, it was possible for us to calculate urea concentration with time. Results for the final dialysis treatment, which was not used in the model-building process, are shown in Fig. 5. Predicted urea concentrations from the optical measurement at 1-min intervals are shown as a solid line. The circles indicate the urea concentrations measured by the standard chemical assays. The agreement between the two is excellent. Predicted values from the noninvasive spectroscopic method follow the expected trend of approximately exponential decrease in urea concentration with time. However, there are visible discontinuities in the urea concentration at 20 and 140 min after the start of the treatment.

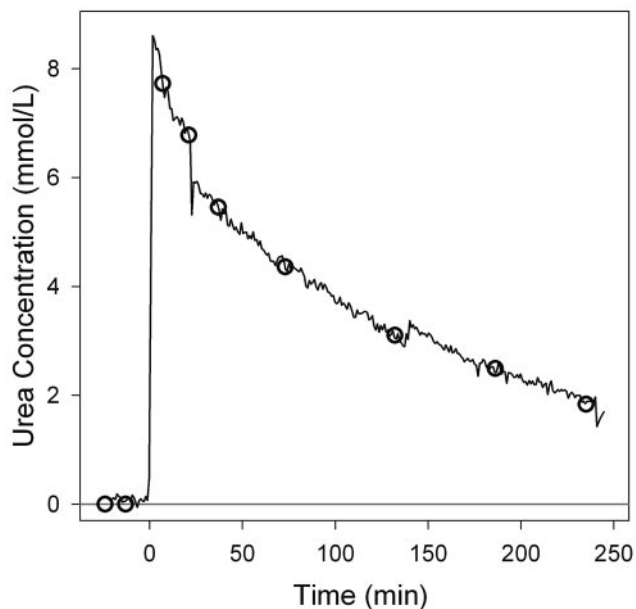


Fig. 5. Concentration of urea in the spent dialysate stream at 1-min intervals during the final dialysis treatment.

The solid line is the concentration determined by the PLS calibration model; \circ are the concentrations measured by standard chemical techniques. The discontinuities at 20 and 140 min in the urea concentration are real, as explained in the text.

These are attributable to modification of the rate of blood flow across the dialyzer. The clinical record indicates that the blood flow rate was reduced from 330 to 260 mL/min at 20 min and later increased to 300 mL/min at 140 min because of cramping in the patient.

CALIBRATION USING THE NET ANALYTE SIGNAL

Because of the relatively simple structure of the filtered absorbance spectra and the spectral isolation of urea, it is possible to construct a calibration spectrum directly from the urea absorption spectrum and those of the interfering compounds. The advantage of such an approach is that the calibration can be built using only pure-component spectra of the analytes involved. No multivariate calibration step is required, and the calibration spectrum generated is guaranteed to be based on spectral features of urea, which cannot be guaranteed with PLS. It is expected that the prediction ability of such a procedure might be less accurate but more robust than for PLS.

For a direct calibration procedure to work, however, all of the near-infrared-absorbing components should be included in the background (nonanalyte) set. The sufficiency of the analyte set used here can be estimated by performing a regression of the online spectra in terms of this basis and evaluating the spectral residuals. Fig. 6 shows a regression of a representative online spectrum in terms of the pure-component spectra of glucose, acetate, creatinine, and lactate. All of the dominant features are accounted for, although there are deviations at some of the peaks. The root mean square difference between the

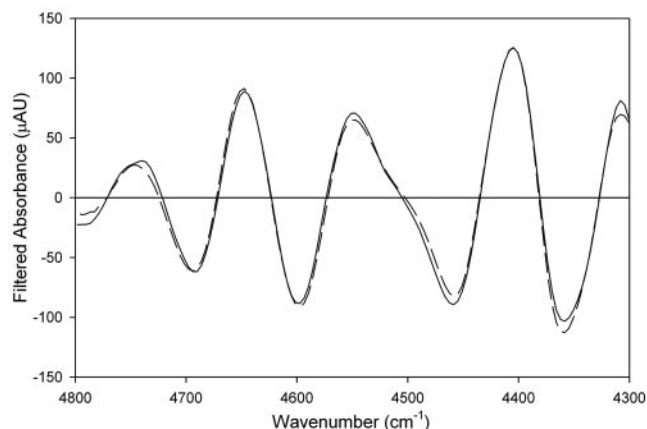


Fig. 6. Measured and regressed filtered absorbance spectra. The *solid line* is the measured spectrum; the *dashed line* is the regression in terms of the pure components shown in Fig. 2.

measured and regressed spectra can be used as an indicator for the presence of nonmodeled analytes or baseline variations. The root mean square difference for the spectra shown in Fig. 6 is $5.5 \mu\text{AU}$, which is $<10\%$ of the root mean square amplitude of the spectrum and a factor of 5 larger than the spectral noise. It is important to note that the regression procedure is not used here as a prediction tool; it is used only to gauge the completeness of using glucose, acetate, creatinine, and lactate as the principal component basis.

The desired calibration spectrum will be proportional to the net analyte signal (19, 20), which is the portion of the urea absorption spectrum that is orthogonal to all nonurea spectral variations. The constant of proportionality can be determined directly from the urea reference spectrum, for which the urea concentration is known. Thus the only information used in constructing the urea calibration model were five pure-component spectra, which were measured in a separate laboratory before moving the spectrometer to the clinic where the online measurements took place. In particular, there was no adjustment of the slope of the actual vs predicted concentration line to match the online spectra.

The results of this direct calibration are shown in Fig. 7, where the net analyte signal was computed by standard methods (19). The SEP is 0.43 mmol/L (1.2 mg/dL urea nitrogen), which is larger (by 60%) than the SE of the PLS calibration model, as expected. However, most of the error is attributable to a proportionality error, as is illustrated by the Bland–Altman plot shown in the inset of Fig. 7. This proportionality error is likely attributable to a combination of errors in the concentration of the reference urea solution, variations in the optical pathlength of the sample cell between collection of the pure component spectra and collection of the dialysate sample spectra, and unaccounted for solutes in the sample matrix. If a linear regression is used to correct for the proportionality error (11%) and a small offset (0.25 mmol/L), the SE is reduced

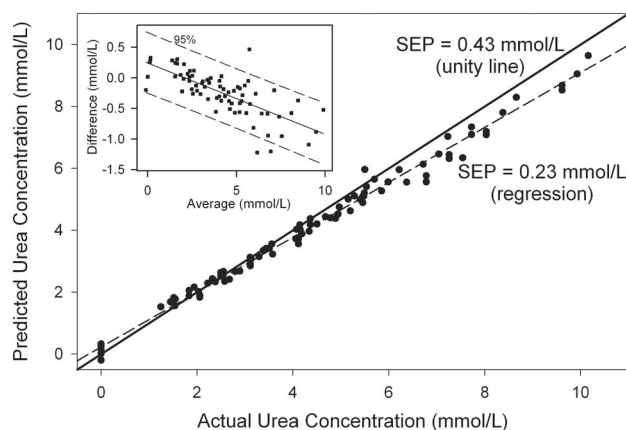


Fig. 7. Predicted vs actual urea concentration using the net-analyte-signal calibration.

The *solid line* has a slope of 1 and passes through the origin. The *dashed line* is a linear regression of the calibration and prediction points. (*Inset*), Bland–Altman plot comparing the optical and chemical assays, indicating a clear proportional error. The *solid line* in the *inset* is a regression line; the *dashed lines* represent the 95% confidence limits.

to 0.23 mmol/L (0.64 mg/dL urea nitrogen), which is lower than the SE for the PLS model.

The calibration spectrum generated from the urea net analyte signal is shown as the solid line in Fig. 4B. There is significant agreement between the net-analyte-signal calibration and the PLS calibration spectra (Fig. 4A) in the $4800\text{--}4550 \text{ cm}^{-1}$ range, which correlates with the dominant urea absorbance features (see Fig. 2A). However, the net-analyte-signal calibration differs from the PLS calibration between 4500 and 4300 cm^{-1} . The reason for this is that the PLS calibration is required only to be orthogonal to statistically distinct variations in the calibration set, whereas the net-analyte-signal calibration is forced to be orthogonal to all of the components in the background regression basis. The connection between the PLS and net-analyte-signal calibration spectra can be established by mathematically forcing the PLS calibration spectrum to be orthogonal to the pure-component spectra of glucose, acetate, creatinine, and lactate. The result is shown as the dashed line in Fig. 4B, which very closely matches the net-analyte-signal calibration. This implies that the primary difference between the PLS and net-analyte-signal calibrations is the additional orthogonality of the net-analyte-signal calibration to the interfering analytes whose concentration variations are too small to be included by PLS.

CONCLUSIONS

We have demonstrated that it is possible to accurately measure urea in the spent dialysate stream during hemodialysis sessions with a prediction accuracy of 0.43 mmol/L by use of a simple net-analyte-signal calibration. The model described here was constructed entirely from data available before the start of the first dialysis treatment. Its successful performance with no adjusted param-

eters gives us confidence that the calibration model is robust. When a slope and offset correction were included, the SE decreased to 0.23 mmol/L.

PLS regression was used to develop a more standard calibration model. Successful calibrations were built with a root mean square prediction error of 0.30 mmol/L for filtered spectra and 0.38 mmol/L for unfiltered spectra. These prediction errors were calculated from spectra from the final two dialysis sessions, which were not included in the model-building process. The three calibration spectra generated in this study show clear similarities in both shape and magnitude, which provides confidence that the calibrations are based on urea-specific information rather than over-fitting or chance correlations.

In the future, we hope to perform a longer-term study to investigate the stability of the generated calibration model. In addition, it might be useful to artificially increase the concentrations of components such as creatinine and lactate to decrease the potential for cross-analyte interference. 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.

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References

1. Kloppenburg WD, Stegeman CA, Hooyssuur M, van der Ven J, de Jong PE, Huisman RM. Assessing dialysis adequacy and dietary intake in the individual hemodialysis patient. *Kidney Int* 1999;55:1961-9.
2. Brimble KS, St Onge J, Treleaven DJ, Carlisle EJ. Comparison of volume of blood processed on haemodialysis adequacy measurement sessions vs regular non-adequacy sessions. *Nephrol Dial Transplant* 2002;17:1470-4.
3. Gotch FA, Levin NW, Port FK, Wolfe RA, Uehlinger DE. Clinical outcome relative to the dose of dialysis is not what you think: the fallacy of the mean. *Am J Kidney Dis* 1997;30:1-15.
4. Eddy CV, Arnold MA. Near-infrared spectroscopy for measuring urea in hemodialysis fluids. *Clin Chem* 2001;47:1279-86.
5. Eddy CV, Flanigan M, Arnold MA. Near-infrared spectroscopic measurement of urea in dialysate samples collected during hemodialysis treatments. *Appl Spectrosc* 2003;57:1230-5.
6. Ngo TT, Phan APH, Yam CF, Lenhoff HM. Interference in the determination of ammonia with the hypochlorite-alkaline phenol method of Berthelot. *Anal Chem* 1982;54:46-9.
7. Wülfert F, Kok WT, Smilde AK. Influence of temperature on vibrational spectra and consequences for the predictive ability of multivariate models. *Anal Chem* 1998;70:1761-7.
8. Bromba MUA, Ziegler H. Application hints for Savitzky-Golay digital smoothing filters. *Anal Chem* 1981;53:1583-6.
9. Savitzky A, Golay MJE. Smoothing and differentiation of data by simplified least squares procedures. *Anal Chem* 1964;36:1627-39.
10. Brown CD, Wentzell PD. Hazards of digital smoothing filters as a preprocessing tool in multivariate calibration. *J Chemometrics* 1999;13:133-52.
11. Hazen KK, Arnold MA, Small GW. Temperature insensitive measurements of glucose in aqueous matrices. *Appl Spectrosc* 1994;48:477-83.
12. Hu SY, Arnold MA, Wiencek JM. Temperature-independent near-infrared analysis of lysozyme aqueous solutions. *Anal Chem* 2000;72:696-702.
13. Rzhetskii AM, Mardilovich PP. Generalized Gans-Gill method for smoothing and differentiation of composite profiles in practice. *Appl Spectrosc* 1994;48:13-20.
14. Arnold MA, Burmeister JJ, Small GW. Phantom glucose calibration models from simulated noninvasive human near-infrared spectra. *Anal Chem* 1998;70:1773-81.
15. Baumann K, von Korff M, Albert H. A systematic evaluation of the benefits and hazards of variable selection in latent variable regression. Part II. Practical applications. *J Chemometrics* 2002;16:351-60.
16. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;i:307-10.
17. Dewitte K, Fierens C, Stöckl D, Thienpont LM. Application of the Bland-Altman plot for interpretation of method-comparison studies: a critical investigation of its practice. *Clin Chem* 2002;48:799-801.
18. Riley MR, Arnold MA, Murhammer DW, Walls EL, DelaCruz N. Adaptive calibration scheme for quantification of metabolites in insect cell media by near infrared spectroscopy. *Biotechnol Prog* 1998;14:527-33.
19. Lorber A. Error propagation and figures of merit for quantification by solving matrix equations. *Anal Chem* 1986;58:1167-72.
20. Lorber A, Faber K, Kowalski BR. Net analyte signal calculation in multivariate calibration. *Anal Chem* 1997;69:1620-6.