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Online monitoring of urea concentration in dialysate with dual-beam Fourier-transform near-infrared spectroscopy

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Abstract. The robustness of a dual-beam, optical null, Fourier-transform near-infrared (FTNIR) spectrometer was investigated by means of online, near-infrared measurements and predictions of urea concentrations in spent dialysate during hemodialysis treatment. Simple multivariate calibration using a few factors based on a small number of prepared samples provided stable and accurate predictions over a period of 1 month. The calibration was robust when faced with adjustment of reference cell intensity and did not require a daily measured reference spectrum. The root-mean-square error of prediction of urea was 0.4 mM based on a two-factor partial least-squares regression model.
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1 Introduction

In hemodialysis treatment, the accepted measure of dialysis dose is based on the removal of urea from the blood pool.[1] The expression Kt/V, where K is the dialyzer urea clearance, t is the treatment time, and V is the patient's urea distribution volume, is used to quantify the dialysis dose. This expression is usually evaluated with the aid of pre- and postblood samples that are analyzed for urea concentration in a clinical laboratory. An online method to continously monitor the removal of urea has strong advantages. Such a method would permit adjustment of the treatment during a single session. Methods based on urease chemistry, or ionic dialysance, exist to estimate the removal of urea. A method based on infrared absorption spectroscopy has two advantages over these methods. The infrared method is reagentless, and it monitors the urea concentration directly. No derived substance is needed. We believe it to be faster and more accurate.

We have previously demonstrated the increased day-to-day stability and improved accuracy of a dual-beam, optical null, Fourier transform near infrared (FTNIR) spectrometer compared with a single-beam configuration of the same instrument for the measurement of urea and glucose in aqueous solutions in the 5000 to 4000 cm^{-1} spectral region.[2] This spectral region has previously been shown to provide lower prediction errors than the overtone spectral region.[3,4] The long-term stability and robustness of this optical null instrument was, however, not tested. In this paper we share the experiences gained during a 1-month test of this instrument carried out in a clinical environment.

The most-studied biological aqueous solutions of clinical interest are blood, blood serum, urine, and recently, spent dialysate. In addition, amniotic fluid and oral mucosa have been studied. A comprehensive review of the clinical applications of near- and mid-infrared spectroscopy with a large section devoted to measurements on biological fluids is given by Heise.[5] The analyte of primary interest has been glucose. This interest has arisen from the potential for continuous monitoring of blood glucose concentrations in patients suffering from diabetes.[6] The concentrations of other analytes such as urea, sulphate, phosphate, creatinine, and protein have been determined by near- and midinfrared spectroscopy in a number of different applications.

Eddy and Arnold[7] demonstrated the measurement of urea and glucose in spent dialysate with near-infrared spectroscopy. The online capabilities of a standard FTNIR instrument have been demonstrated in another publication.[8] Measurement of the urea in spent dialysate in this wave number region is therefore well established and ideally suited for testing the capabilities of a dual-beam instrument. In this study we demonstrate the online ability of our dual-beam instrument to accurately predict the concentration of urea in spent dialysate over a 1-month period, using a simple calibration model based on a few samples of dialysate, which were prepared with known concentrations of urea, glucose, and creatinine. The calibration dataset was increased once, *prior* to the clinical

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measurements, and a calibration model based on second-derivative spectra was constructed. Over the following month, this calibration model was used to monitor the concentration of urea in spent dialysate online while treating eight patients (two each week) at the Nephrological Clinic at Copenhagen University Hospital (Rigshospitalet). For comparison, approximately ten samples of spent dialysate were collected during each treatment and later analyzed by the clinical chemistry department of the hospital.

2 Experimental

The dual-beam instrument has been described in detail in another publication.[2] Briefly, it consists of a Bomem MB154 with two input ports, equipped with a Peltier-cooled indium-arsenide (InAs) detector. External optics collect light from a quartz halogen light source and send it through two transmission cells and into the spectrometer through the input ports. The intensity of the light entering the two input ports may be regulated using two diaphragms placed between the source and samples. The spectrometer works in a so-called double-input-single-output configuration, where identical samples in the two transmission cells result in the measurement of an optical null signal because the two inputs take different paths through the interferometer.[9] Obtainable nulling ratios (the ratio of a single-beam spectrum of one channel to a dual-beam spectrum) were around 60. The intensity reaching the detector was reduced by means of a long-wave pass filter with a cutoff at 5000 cm^{-1} while water absorption and detector sensitivity excluded the midinfrared region from 4000 cm^{-1}. The transmission cells (Foss Electric A/S, Denmark) were flow cells with CaF_2 windows and a path length of 1.00 mm that were thermostatted to $37 \pm 0.2°C$. In our previously published work, temperature was more strictly regulated to be within 0.02°C. One cell was filled with pure water and acted as a reference, while the other cell contained the samples to be investigated. Because the primary absorber in the dialysate is water, an almost nulled signal was measured which contained the difference between the two channels.

2.1 *Calibration Samples*

Calibration samples were prepared from a Baxter 7061 concentrate that was diluted to a ratio of 1:35 through a 1:10 diluted solution with the prescribed addition of $NaHCO_3$ (Merck), yielding a sample volume of 1 liter. Table 1 shows the nominal composition of the dialysate based on the Baxter 7061 concentrate, as used in the dialysis treatment. Also shown is the nominal composition of dialysate based on a Baxter 7026 concentrate, which was used for treating some patients. Appropriate volumes of 0.01 M glucose and creatinine (BDH Lab. Supplies, Poole England; Merck) and weighed amounts of urea (Merck) were added during dilution to obtain a calibration sample set with the desired concentration of these three molecules. All three molecules are known to have signals in the spectral region of interest.[7] The calibration samples differed from the nominal composition of the dialysate based on Baxter 7061 concentrate only in their concentrations of these three molecules.

The calibration sample set was constructed as a full factorial design with two levels and three design variables, giving a total of eight samples with concentrations of glucose of 5.00 to 10.00 mM; urea, 0.00 to 10.00 mM; and creatinine, 0.00 to 1.00 mM. A calibration based on a dataset consisting of spent dialysate samples was not chosen. Such a procedure requires a large number of samples, is sensitive to errors in the clinical chemical reference method, and cannot easily separate different trace components because there is a covariance between their concentrations. The procedure of synthetically preparing the calibration samples requires comparatively fewer samples and eliminates reference error and covariance problems. Components that are present in the spent dialysate, but absent in the synthetically prepared ones, represent a source of error for the chosen method of calibration. This error is estimated to be small because interfering components are present in much smaller concentrations than urea. It has previously been shown that a calibration based on pure spectra is capable of extracting urea signals from absorbance spectra.[8]

Table 1 Nominal composition of dialysate based on Baxter 7061 and 7026 concentrate.

	Concentration (mM)			
Type	Na^+	K^+	Ca^{2+}	Mg^{2+}
7061	138	2	1.25	0.5
7026	138	2	1.75	0.5

	Concentration (mM)			
Type	Cl^-	CH_3COO^-	HCO_3^-	$C_6H_{12}O_6$
7061	108.5	3	32	5
7026	109.5	3	32	5.55

2.2 *Measurements*

Double-sided (symmetrical) interferograms containing 2048 points were measured at 32 cm^{-1} resolution with 128 coadditions. The spectrometer was operated through a personal computer (PC) using Windows NT4.0 with Grams/32AI Version 6 (Galactic Industries Corp.). Each day a single-beam sample, a single-beam reference, and a dual-beam spectrum of pure water were measured prior to the dual-beam measurements on dialysate. The single-beam spectra were measured at the lowest amplification and had an interferogram amplitude of approximately 3.6 V. The dual-beam spectra were measured at the highest amplification (about 15 times the lowest amplification).

For the online measurements, the sample cell was filled by the use of a tube connected through a peristaltic pump to the dialysate outlet of the dialyzer used for the treatment. Figure 1 is a schematic drawing of the dialyzer with tube connections. Dialysate was pumped at 35 ml/min from the outlet of the dialyzer for 1 min, thereby passing a volume approximately seven times that of the whole tubing system through the cell. The dialysate was then kept under slight overpressure in the flow cell and thermostatted to $37 \pm 0.2°C$. The preset temperature was reached within 2.5 min after sample collection. After the temperature stabilized, a dual-beam spectrum was

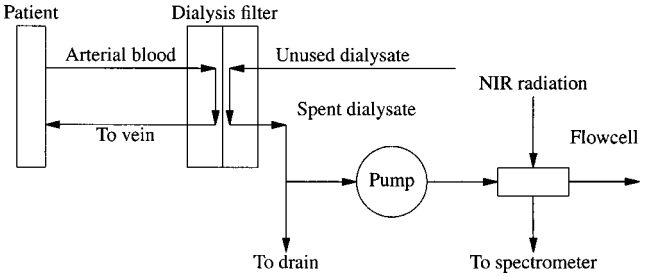

Fig. 1 Schematic drawing of dialyzer with tube connections.

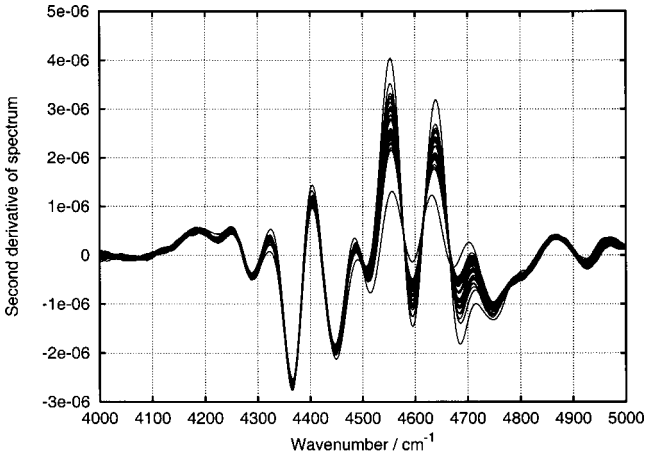

Fig. 2 Representative set of 41 measured second-derivative spectra from a single treatment session.

measured. This procedure was repeated through the measurement session. The total time required for one measurement was 5 to 6 min, which yielded between 30 and 50 points per treatment, depending on the treatment time. Approximately 10 samples were collected during each treatment session and sent to the clinical chemistry department at the hospital, where they were analyzed for urea concentration using a Modular P automated analyzer (Roche, Mannheim, Germany). The assay uses a coupled urease–glutamate dehydrogenase enzyme system. The assay has a measuring range of from 0.83 to 66.4 mM in plasma and 8.3 to 730 mM in urine, with an interserial covariance (CV) of less than 3%. Based on measurement of dialysate samples with no urea, we estimate the uncertainty to be 0.4 mM. One set of reference values contained obviously erroneous values and was discarded. For the calibration measurements, the input to the peristaltic pump was connected to a beaker filled with the calibration sample to be measured. The procedure was otherwise identical to the online measurements of the spent dialysis fluid. Two measurements were taken of each sample, resulting in a calibration dataset containing 16 measurements.

2.3 *Patients*

The patients all gave their consent to participate in this study; they were all males aged above 50 years. The patients were chosen so as to yield great variability in the measured concentrations of urea in the spent dialysate. Some patients suffered from diabetes. Different methods of vascular access were employed; some patients had a fistula and some had a Dialock. This was reflected in the urea concentration of the spent dialysate, so that maximum values lay in the 3.5 to 14-mM range and end values lay in the 1.9 to 8-mM range. The dialyzers used in the treatment were one of two types: a Gambro AK-200 or an Integra Hospal. The blood flow lay between 200 and 400 ml/min, while the dialysate flow was 500 ml/min for all patients.

2.4 *Data Analysis*

The spectra were transferred to an Intel-based Gnu/Linux workstation for further analysis. During the measurements, the transfer was carried out online as spectra were measured in order to monitor the variation in urea concentration with time. All data analysis was carried out with programs written in ANSI C based on routines from *Numerical Recipes in C*.[10]

The partial least-squares (PLS) routine used in the analysis was a translation of the Splus code by M. Denham[11] into ANSI C. For each measured interferogram, the following calculations were carried out. The mean value of the interferogram was subtracted. The interferogram was then apodized with a cosine window and zero-filled by a factor of eight. The intensity spectrum was calculated as $I(\overline{v}) = \mathrm{Re}(\overline{v})\cos\Phi(\overline{v}) + \mathrm{Im}(\overline{v})\sin\Phi(\overline{v})$; where $\mathrm{Re}(\overline{v})$ and $\mathrm{Im}(\overline{v})$ are the real and imaginary parts of the Fourier transform of the interferogram, and $\Phi(\overline{v})$ is the phase spectrum of the Fourier transform of a single-beam interferogram of pure water. The phase spectrum was calculated as $\Phi(\overline{v}) = \mathrm{atan}(\mathrm{Im}(\overline{v})/\mathrm{Re}(\overline{v}))$ from the real $\mathrm{Re}(\overline{v})$ and imaginary $\mathrm{Im}(\overline{v})$ parts of the Fourier transform of the single-beam interferogram. The use of a single-beam phase spectrum is important because a phase spectrum calculated from a dual-beam interferogram is ill-defined. A combination of low spectral resolution, high nulling ratio, and leakage between data points, which may have opposite phases, gives rise to this problem.[12,2] The spectra were restricted to the spectral range 5000 to 4000 cm^{-1} and second-derivative spectra were calculated by finite-centered differences as $d^2I_i/d\overline{v}_i^2 = (I_{i+1} - 2I_i + I_{i-1})/2$; where I_i is the intensity spectrum and the index i refers to a given spectral point. No other pretreatment was carried out. The second derivative spectra measured on the calibration dataset were used to construct PLS models with different numbers of factors. A spectral range of 5000–4000 cm^{-1} was used for all the PLS models. Based on the calibration dataset alone, the three-factor PLS model was chosen to predict urea concentrations during the treatment of patients.

3 Results and Discussion

Figure 2 shows a set of 41 second-derivative spectra measured during one representative treatment session. One can see that the major changes in the spectrum occur in the 4700 to 4500 cm^{-1} region. These signals arise from a combination of symmetric and asymmetric N—H stretching vibrations with N—H bending vibration in urea. The N—H bonding in aqueous solutions has red-shifted these signals. Details are given by Eddy and Arnold.[7] A representative online measurement of urea concentration during treatment, based on the three-factor PLS model, is shown in Fig. 3, along with the results of the clinical chemical analysis for urea in the measured samples. One observes a sharp rise as the treatment is initiated, and a gradual decline following this sharp rise, re-

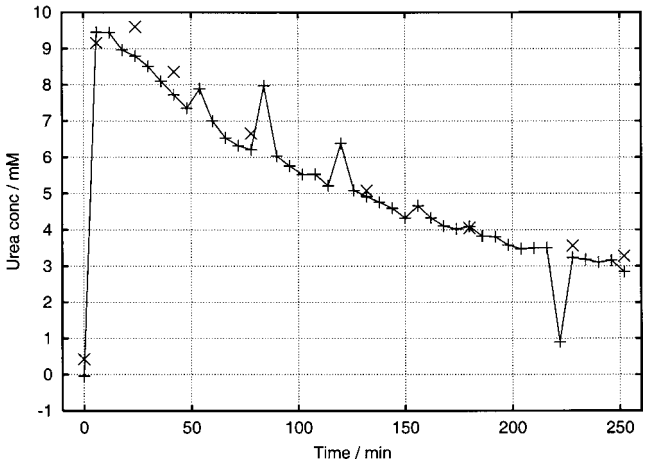

Fig. 3 Representative example of online measurement of urea concentration during treatment. Prediction of urea concentration from NIR spectra based on the three-factor PLS model, "+," compared with the results of the clinical chemical analysis, "×."

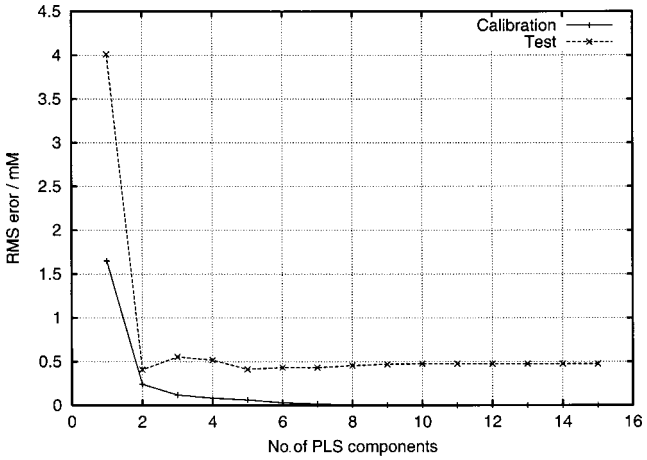

Fig. 4 Root-mean-square error of calibration and prediction as function of the number of PLS components.

sembling an exponential decay. A number of spikes are present on this concentration curve, sometimes going up, sometimes going down. At first we were troubled by this behavior, but as the nurse responsible for the treatment pointed out, this behavior coincided with recalibration and cleaning performed by the dialyzer every half hour. During this cleaning process, the dialyzer is inactive and pure dialysate is pumped into the flow cell. Therefore one may measure an almost zero value of urea when this happens. If one measures a sample taken shortly after this pause, a buildup of urea concentration manifests itself as a markedly higher concentration in the spent dialysate. The next measurement then falls to the expected value. The samples measured in the flow cell were not identical to the samples sent to the clinical chemical department. The latter were collected from the outlet of the dialyzer during the 1 min of pumping. Therefore when urea concentration is varying rapidly, as when the dialyzer recalibrates itself, or at the start of the treatment, a large discrepancy between the prediction of urea concentration from the clinical chemical analysis and the near-infrared (NIR) spectrum may occur.

Other deviations from a steady decline in urea concentrations were also observed. These deviations were caused by physiological changes in the patient's condition during the treatment. For instance, some patients began to shiver during the treatment because of cooling by the continuous blood flow through the dialyzer. When this happened, we found that the urea concentration in the spent dialysate increased, so that the efficiency of the treatment was increased. When the patient stopped trembling, the urea concentration returned to the steady decline. In one other case, the patient developed symptoms of dialysis dysequilibrium and the concentration of urea in the spent dialysate began to fluctuate. When the patient's condition improved, the usual steady behavior returned. Thus, changes in the decline in urea concentration in the spent dialysate were clearly correlated with changes in the patient's condition.

Excluding the reference measurements that were taken when the concentration was changing rapidly, the root-mean-square error of prediction compared with the clinical chemical analysis was 0.56 mM for the three-factor PLS model used during the online analysis. A two-factor PLS model provides a slightly better root-mean-square error of prediction of 0.41 mM. The root-mean-square error of calibration and prediction are shown as a function of the number of PLS components in Fig. 4. The regression vector (scaled to unit maximum amplitude) for the calibration model with two PLS factors is shown in Fig. 5. The regression vector is seen to select urea signals at 4700 to 4500 cm^{-1}. Figure 6 shows the prediction of urea concentration from the two-factor PLS model versus the results obtained from clinical chemical analysis. The discrepancy between the clinical chemical analysis and the prediction from the NIR spectra is seen mainly as a bias. Therefore a more accurate description in a measurement situation could be obtained by correcting for this bias by subtracting the urea concentration value predicted for the pure dialysate measured just prior to the start of treatment. One observes that predictions of concentrations above 10 mM are consistently lower than the reference value. We have observed the same effect in measurements with midinfrared transmission spectroscopy on the same samples.[13] This could indicate that the explanation

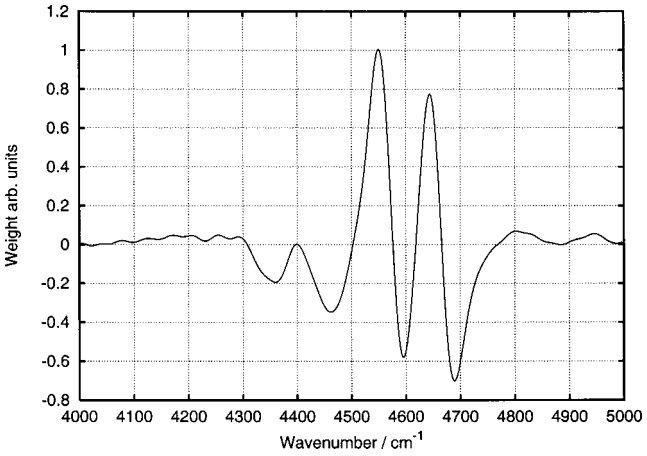

Fig. 5 Regression vector for the two-factor PLS model scaled to unit maximum amplitude.

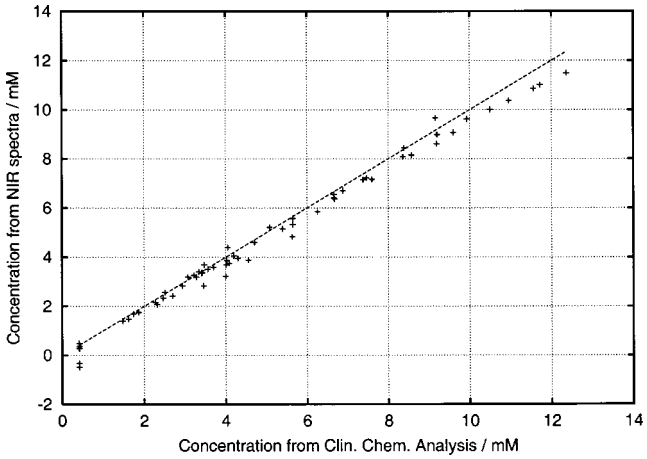

Fig. 6 Urea concentration predicted from NIR spectra with the two-factor PLS model versus urea concentration as determined by clinical chemical analysis.

of this discrepancy may be found in the reference method. These high concentration values of urea are outside the range spanned in the calibration set used to build the PLS models, and the discrepancy could arise from this extrapolation. This systematic deviation was observed only later when the reference values from clinical chemical analysis were obtained. This happened after the treatment sessions were completed and the instrument taken apart. Therefore a recalibration using a larger urea concentration range was not possible. Finally, we note that high urea concentrations are invariably found at the beginning of the dialysis treatment. Therefore some temporal effect, possibly in the composition of the dialysate, may be present. The results obtained compare well with our previous results[2] where a principal component regression model with four factors was shown to be optimal when based on raw dual-beam spectra.

During the first online measurement session, an increase in the intensity of the measured dual-beam interferogram was encountered. We believe it was the result of a change in the intensity through the reference cell caused by accidental tampering with the diaphragm placed next to it. This did not change the decay profile of the online measurement when the PLS model with three factors was employed. The prediction using the two-factor PLS model was slightly sensitive to this change, however. To correct for this change in intensity, the diaphragm was adjusted to maximize the nulling ratio with pure water present in both reference and sample cells prior to the second measurement session. The PLS calibration models with two and three factors continued to provide good results, indicating a robustness for this kind of adjustment.

During the online measurements, the phase spectrum from a pure water, single-beam spectrum, measured on the same day, was used in calculating the dual-beam intensity spectra from the interferograms. We were curious as to whether it would be possible instead to use the phase spectrum of the pure water single-beam spectrum measured for the calibration dataset. We therefore tried to substitute this one phase spectrum for the calculation of all the subsequent dual-beam intensity spectra. This made no difference in the ability to predict urea concentrations from the measured spectra. We further tried to optimize the calibration model by shrinking the wave number region to 4750 to 4450 cm^{-1}. This did not yield any improvement.

4 Conclusion

The dual-beam instrument provided online measurements of urea concentrations in a clinical environment during the treatment of patients over a span of 1 month. The instrument was sufficiently stable to allow measurements under out-of-laboratory conditions with a relaxed temperature control, compared with previous measurements made with a similar instrument. The instrument continued to provide accurate measurements after adjustment of the light intensity of the reference arm, and did not require a daily measured single-beam spectrum for the construction of a phase spectrum. The root-mean-square error of prediction was 0.4 mM.

Acknowledgments

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