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# Fourier Transform Infrared Spectroscopy of Aqueous Solutions using Optical Subtraction

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## ABSTRACT

For the analysis of small concentrations of organics in aqueous solutions, a novel add-on accessory for dual-beam / optical subtraction spectroscopy has been built for a commercial Fourier transform infrared spectrometer. A standard FT-IR instrument requires a sample measurement and a separate reference measurement, whereas the optical subtraction instrument directly measures the difference between sample and reference. This has a number of advantages. The time delay between the two measurements is eliminated, and the effective measurement time is improved by a factor of two. Moreover, the optical subtraction provides a large reduction in dynamic range of the measured signal, which prevents detector saturation, and enables effective use of dynamic range of the analog to digital converter in the FT-IR spectrometer. This results in an increased signal to noise ratio, compared to the standard FT-IR instrument. By changing detector and light source the instrument may be used for both near- and mid-infrared spectroscopy. The increased sensitivity and stability of the optical subtraction instrument compared to the standard instrument is demonstrated by transmission measurements of aqueous urea solutions in the combination band region 4000 to 5000 cm<sup>-1</sup> (2000 to 2500 nm).

**Keywords:** Fourier Transform Infrared Spectroscopy, Biological Fluids, Optical Subtraction.

## 1. INTRODUCTION

The accurate determination of small concentrations of solvents in aqueous solutions is important in applications found in such diverse areas as dairy industry, environmental monitoring, and biomedical diagnostics. For many such applications, Fourier transform near- and mid-infrared (FT-(N)IR) spectroscopy is being used as an instrument in combination with chemometric data analysis.<sup>1-9</sup> The Fourier transform spectrometer is an instrument with remarkable stability and accuracy. Even so, it is the performance of the FT-IR spectrometer which determines the detection limit of trace organics in aqueous solutions, and it is therefore desirable to improve the FT-IR spectrometer performance.

Water is the dominating infrared absorber in aqueous solutions and small concentrations of solvents give rise to comparatively weak signals that are superposed on the water absorbance spectrum. Measuring small concentrations of solvents therefore requires a separation of the large unwanted background caused by water and the small wanted signal caused by the solvent. The usual procedure involves the measurement of a pure water reference which is then subtracted from the sample. Several problems arise from this procedure. The measured signal contains the unwanted large background caused by the absorption of water. This is true even when the background is subsequently removed by spectral subtraction of a water reference. Two measurements, on sample and reference, respectively, are required and are necessarily separated in time. This means that varying instrumental characteristics affects the accuracy of the measurement. These temporal changes may arise at a number of places, e.g., at the source, the detector, or the purge of sample compartment.

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In standard FT-IR instruments, the measured signal has a large dynamic range caused by the broad spectrum of the source and the basic working principle of the instrument (which is the measurement of intensity as a function of pathlength difference in the two arms of a Michelson interferometer). As the movable mirror is scanned across the zero pathlength difference (ZPD) point all wavenumbers interfere constructively causing a large signal known as the center burst. At large pathlength differences, the signal is small because few wavenumbers interfere constructively and many interfere destructively. Firstly, this means that one must be able to measure the large center burst signal which limits the usable intensity of the light source. Secondly, digitization of this large dynamic range signal by the analog to digital converter (ADC) is problematic. Digitization noise may occur if the dynamic range of the signal is large and the detector has a low noise level.

Reduction of signal dynamic range and prevention of detector saturation may be obtained by filtering out light in spectral regions that contain no usable signal. For aqueous transmission spectroscopy, the choice of pathlength effectively selects a narrow spectral region with a high SNR such that other regions contribute little to the detection of small signals and may be filtered out. Even so, this approach is not always sufficient and the limitations of the single-beam measurement still pertain.

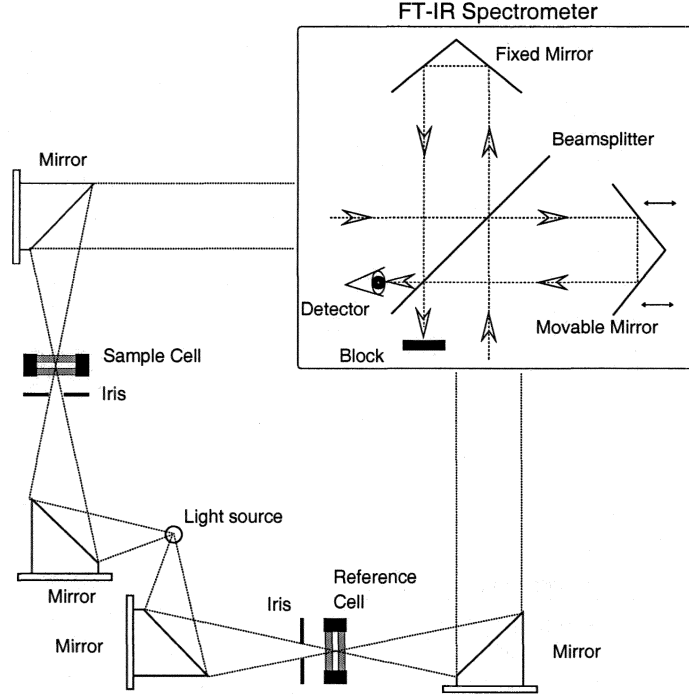
Dual-beam spectroscopy is an elegant solution to the above mentioned problems. The technique provides simultaneous measurement of the difference between sample and reference such that the small wanted signal may be measured directly without the large background signal. Dual-beam spectroscopy reduces the dynamic range of the signal. This prevents digitization noise and allows the use of more intense sources of light. The dual-beam technique requires an FT-IR spectrometer with two inputs or with two outputs, and works by adding two signals either optically on the detector or electronically on two separate detectors prior to digitization. In the mid-infrared region, beam splitter absorption and detector saturation reduces the advantages of the dual-beam technique and thermal radiation from the samples is a problem for some dual-beam configurations.<sup>22, 23</sup> These effects are not important in the near-infrared region.

Since no commercial dual-beam FT-IR instrument is available and only few companies<sup>10</sup> offer an instrument with more than one input or output, application of this technique requires custom built interferometers or modified commercial ones. The dual-beam technique has found earlier uses in such mid-infrared applications as detection of anisole in GC-FT-IR,<sup>11-14</sup> detection of polyester fibers,<sup>15</sup> detection of adsorbed polymer monolayers on mica,<sup>16, 17</sup> emission spectroscopy,<sup>10, 18, 19</sup> gas analysis,<sup>20</sup> and studies of Bacteriorhodopsin in membranes.<sup>21</sup> A review of the subject prior to 1986 is given by Griffiths and deHaseth.<sup>22</sup> These applications all measure a very small signal on a weak background with high accuracy.

We present a simple dual-beam accessory for a commercial FT-(N)IR spectrometer with two input ports designed to accurately determine small differences between an aqueous sample and reference. This device may be used for both traditional single-beam measurements and for dual-beam measurements thereby facilitating a comparison of the two modes of operation. We demonstrate the ability of the dual-beam mode of operation for near-infrared transmission measurements of urea in aqueous solutions in the combination band region 4000 to 5000  $\text{cm}^{-1}$  (2000 to 2500 nm). It is demonstrated that the noise level is reduced and the reproducibility increased in the dual-beam mode of operation. In addition, the potential of the dual-beam mode of operation for improving the detection limit is demonstrated.

## 2. THEORETICAL CONSIDERATIONS

The instrument described herein is operated in the so-called double-input-single-output mode. A schematic drawing of the FT-IR spectrometer and the add-on accessory is shown in figure 1. The sample and reference are placed before the interferometer at the two input ports and the output from one of the output ports is sent to the detector. One beam is emerging from the beam splitter as a sum of light from one arm having experienced two reflections and light from the other arm having experienced two transmissions. The other beam is emerging from the beam splitter as a sum of light from the two arms, each having experienced one reflection and one transmission. The net result is a phase difference of  $\pi$  between the two signals meaning that identical sample and reference result in a zero signal at the detector where the two signals are added. The double-input-single-output requires that the light sources for the two inputs have identical characteristics. This is conveniently achieved by collecting the light for both inputs from the same area of one source.



**Figure 1:** Schematic drawing of dual-beam instrumentation.

A general expression for the output from a dual-beam instrument has been given by Genzel, Chandrasekhar, and Kuhl.<sup>23</sup> Assume two monochromatic sources, at wavenumber  $\bar{\nu}$ , with intensities  $p_1$  and  $p_2$ . The intensity at the detector,  $P_1$ , as a function of the phase shift  $\varphi = 2\pi\bar{\nu}\gamma$ , introduced by the optical path difference  $\gamma$ , between the two arms of the interferometer, is then given by

$$P_1 = p_1 2RT(1 + \cos \varphi) + p_2 ((R + T)^2 - 2RT(1 + \cos \varphi) + 4RT \cos \Phi \cos(\varphi - \Phi)) + 4(p_1 p_2 RT)^{1/2} \cos(\varphi/2) \times (R \cos(\Phi - \delta - \varphi/2) + T \cos(\Phi + \delta - \varphi/2)); \quad (1)$$

where  $R$ ,  $T$  are the beam splitter reflectance and transmittance, respectively,  $\Phi = \Phi_r - \Phi_t$  is the phase difference between the reflected  $\Phi_r$  and transmitted beams  $\Phi_t$  on the beam splitter and  $\delta = \delta_1 - \delta_2$  is the phase difference between the two input channels with phases  $\delta_1$  and  $\delta_2$ . A similar expression for the second output,  $P_2$ , which is not used in our case, may be obtained by exchanging  $p_1$  and  $p_2$  and by changing the signs of  $\delta$  and  $\varphi$ .

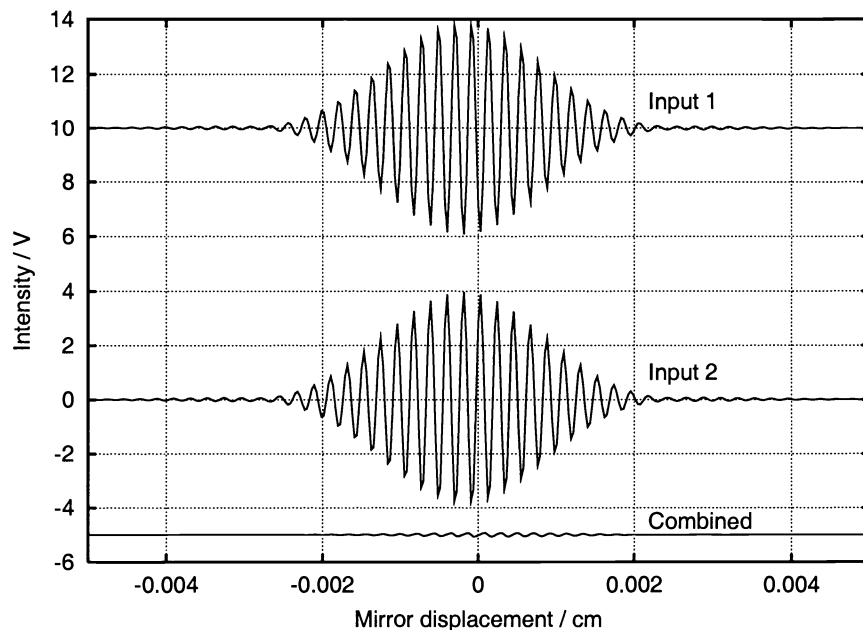
Assume that the two inputs are uncorrelated, then the last term is rapidly varying and averages to zero on the detector. Furthermore, assume an ideal lossless beam splitter such that  $R = T = 0.5$  and  $\Phi = 0$ . Then eq. 1 reduces to the following expression

$$P_1 = 0.5 (p_1 + p_2 + (p_1 - p_2) \cos \varphi). \quad (2)$$

The AC component of this signal is the measured dual-beam interferogram, which contains the difference between the two input sources. If the second input is zero, we have the usual expression for the single-beam intensity at the detector<sup>24</sup>

$$P_1 = 0.5 p_1 (1 + \cos \varphi); \quad (3)$$

where, again, the AC component is the measured interferogram. Note, that the DC component in the single-beam expression, eq. 3, is of the same order of magnitude as the AC component. In the dual-beam expression, eq. 2, with  $p_1$  and  $p_2$  chosen to be nearly equal, the AC component is nearly zero but the DC component is twice as large as in the single-beam mode.

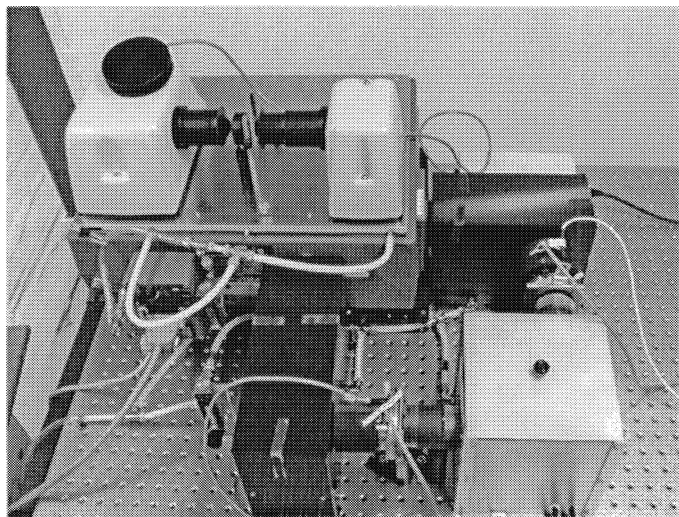


**Figure 2.** Interferograms from input 1 (offset by 10 V), input 2, and the combined dual-beam interferogram (offset by  $-5$  V).

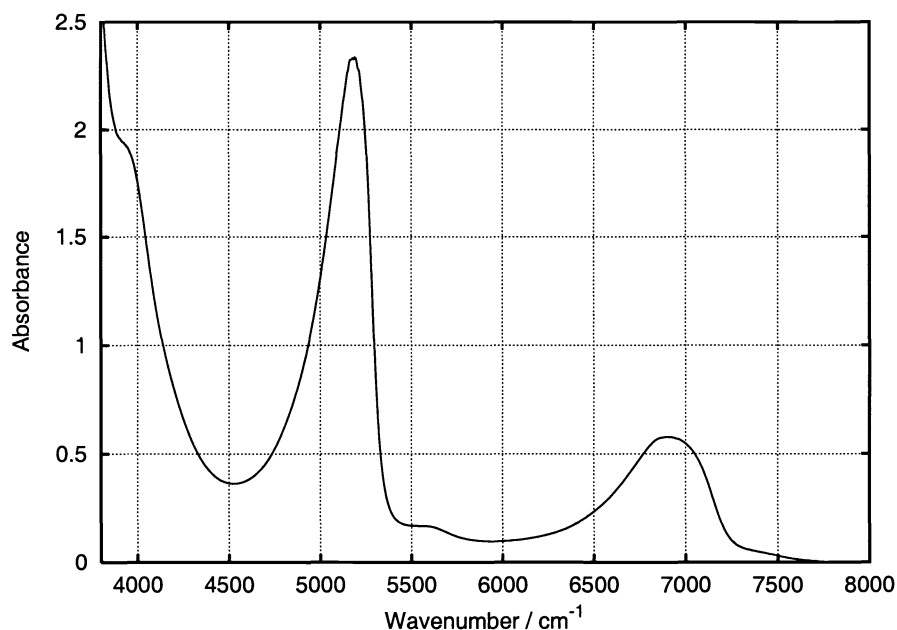
In our case, we collect the inputs  $p_1$  and  $p_2$  from one source after having passed the two beams through a sample or a reference cell, respectively. In this way, the dual-beam mode of operation provides a direct measurement of the difference between the sample and reference, where the single-beam mode of operation requires separate measurement of sample and reference. Figure 2 shows the region around the center burst for typical inputs through two identical cells containing pure water, to demonstrate that the two inputs are  $\pi$  out of phase and result in a nulled signal when combined. Achievable nulling ratios are in the range 30 to 50, with the residual signal arising from a constant difference between the two input channels. The advantages of this approach are, that the measurement is simultaneous, the common background is eliminated, and the dynamic range of the measured signal is reduced. This permits a more effective use of the ADC in the spectrometer and the use of more intense sources of light.

### 3. EXPERIMENTAL

An FT-IR spectrometer equipped with an extra input port (Bomem MB155) and a Peltier cooled InAs detector was connected to an add-on accessory consisting of two pairs of off-axis paraboloidal gold mirrors with  $90^\circ$  deflection, and one quartz halogen lamp. A photograph of the FT-IR spectrometer and the add-on accessory is shown in figure 3. The construction is similar to the one used by Tripp and Hair<sup>16,17</sup> for measurements of adsorbed polymers on mica. The main difference is, that the instrumentation described herein use only one source and has source and detector optimized for near-infrared spectroscopy. As shown in the schematic drawing, figure 1, the add-on accessory is symmetrical around the beam splitter plane of the interferometer with the light source placed in the beam splitter plane. The light from the source is collected by two mirrors. Each mirror focuses the light through a liquid transmission cell, one containing pure water, acting as reference, the other containing the sample solution. The light emerging from the liquid transmission cells are then collected and collimated by each their mirror and sent into the FT-IR spectrometer through the two input ports, where the light reaches the detector. Diaphragms, placed between the transmission cells and the light source, are used to fine tune the nulling ratio of the optical subtraction by adjusting the intensity of the two inputs. By blocking one of the inputs, the spectrometer may be used in single-beam mode using only one input port. An optical



**Figure 3.** Photograph of dual-beam instrumentation. The FT-IR spectrometer is seen at the top left with the input ports facing right and down. The light source and two mirrors are hidden in the aluminum box at the lower right. The two transmission cells are seen to the left of and above the aluminum box.



**Figure 4:** Water absorbance spectrum at a pathlength of 0.4 mm and a temperature of 32 °C.

bandpass filter (Spectrogon AB, Sweden) was placed at the standard sample compartment of the spectrometer to select the combination band region  $4000$  to  $5000\text{ cm}^{-1}$  where water has an absorption window. For easy reference, figure 4 shows the water absorbance spectrum for a pathlength of 0.4 mm.

The pathlength of the liquid transmission cells (Specac Inc.) were adjusted by rotation of one window mounted on a screw thread to a pathlength of 1 mm. The two transmission cells were adjusted to equal pathlength by adjusting one cell to give a transmittance of one relative to the other. Only one input channel of the spectrometer were used to avoid effects of asymmetry between the two input channels. The temperature of

each transmission cell was 32 °C, controlled to within  $\pm 0.02$  °C by a temperature control unit (Eurotherm 4208), which measured sample temperature through the cell filling port with a PT100 thermo element and heated the cell by a nichrome wire wound around the cell body.

The source intensity was adjusted to provide single-beam center burst intensities of approximately 3.5 V amplitude to keep within the ADC range of 4.0 V amplitude. The potential problem with detector saturation, caused by the high DC component of the intensity reaching the detector, were not encountered. Double-sided interferograms were collected at  $32\text{ cm}^{-1}$  resolution (2048 points) with 128 co-additions. Single-beam interferograms were collected with an instrument gain of one whereas dual-beam interferograms were collected with an instrument gain of approximately 16. In dual-beam mode the second input to the spectrometer contained a pure water reference.

Aqueous urea solutions were prepared by weighing reagent grade urea using an analytical balance and dissolving it in 0.5 l water. For each sample three spectra were collected, a dual-beam spectrum, a single-beam spectrum of the reference arm and a single-beam spectrum of the sample arm. Three replica of each were collected. Two data sets were collected, one containing urea in concentrations from 0 to 1 g/dl and one containing concentrations from 0 to 40 mg/dl.

Interferograms were apodized by a cosine window, zero filled by a factor of eight and Fourier transformed. The intensity spectrum was calculated using full phase information<sup>25</sup> as

$$I(\bar{\nu}) = C(\bar{\nu}) \cos \Phi(\bar{\nu}) + S(\bar{\nu}) \sin \Phi(\bar{\nu}) \quad (4)$$

where  $C(\bar{\nu})$  is the cosine and  $S(\bar{\nu})$  is the sine transform of the interferogram and  $\Phi(\bar{\nu})$  is the phase defined by  $\tan \Phi(\bar{\nu}) = S(\bar{\nu})/C(\bar{\nu})$ .

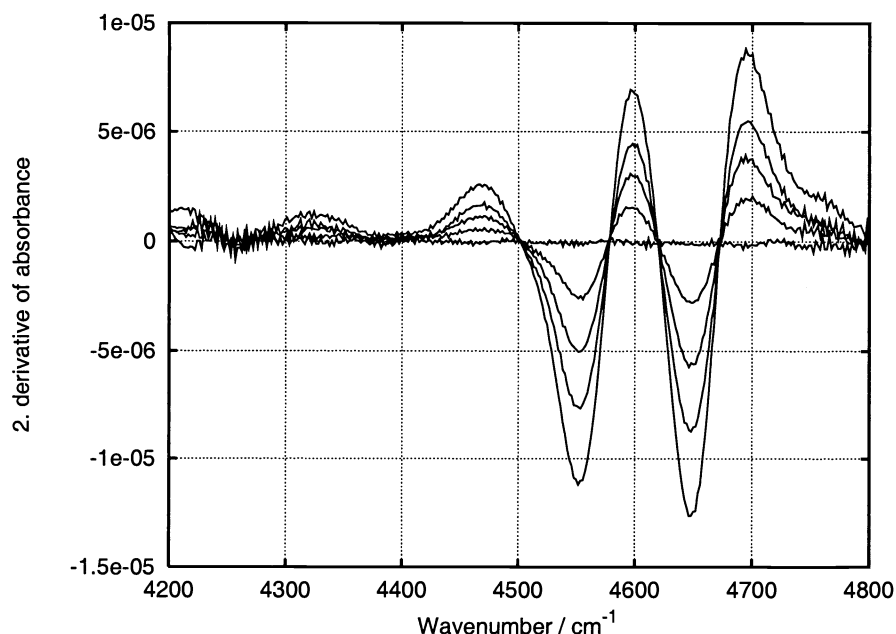
For dual-beam spectra, the phase is poorly determined because of the high nulling ratio. The measured intensity, which is the difference between the two input intensities, may be positive or negative. The assumption of positive intensity and the existing ambiguity,  $-Ae^{i\phi} = Ae^{i(\phi+\pi)}$ , between negative intensity and a phase shift of  $\pi$  means that negative intensities will be represented by a  $\pi$  phase shift. This is further complicated at low resolution where the instrument response function will be broadened so each calculated phase point will contain information from neighbouring points. Therefore, the phase of a single point will be shifted by an amount which is not merely  $\pi$ .<sup>26</sup> The real phase is a characteristic of the instrument, including detector and electronics. In single-beam measurements there is no sign ambiguity and the phase is slowly varying. This means that leakage from neighbouring points does not create any error. For that reason, a stored phase from a single-beam measurement was used for the calculation of dual-beam spectra from the dual-beam interferograms.

The absorbance spectra in single-beam mode was calculated as  $A = -\log_{10} I/I_0$ ; where  $I$  and  $I_0$  are the sample and reference spectra. In the dual-beam mode of operation, one measures the difference between sample and reference  $D = I - I_0$ . Therefore,  $I/I_0 = D/I_0 + 1$  and

$$A = -\log_{10} \frac{I}{I_0} = -\log_{10} \left( \frac{D}{I_0} + 1 \right) \approx -\frac{1}{\ln 10} \frac{D}{I_0}, \quad (5)$$

using the first term of the series expansion  $\ln(1+x) = x - x^2/2 + \dots$  since  $D$  is much smaller than  $I_0$ . The measured dual-beam signal is seen to be proportional to the absorbance with a factor which depends on the reference intensity at a given wavenumber.

The raw dual-beam spectra, with no division by a reference spectrum, were used for further data analysis and compared with equivalent analysis of the traditional absorbance measurements. The division of a dual-beam spectrum with a single-beam reference would make the curve shape of the traditional absorbance spectrum agree with the dual-beam measurement at the cost of introducing the noise present in the single-beam reference measurement. Second derivatives of the data set containing high concentrations of urea were constructed. Principal component analysis were employed to investigate the data sets containing low concentrations of urea.

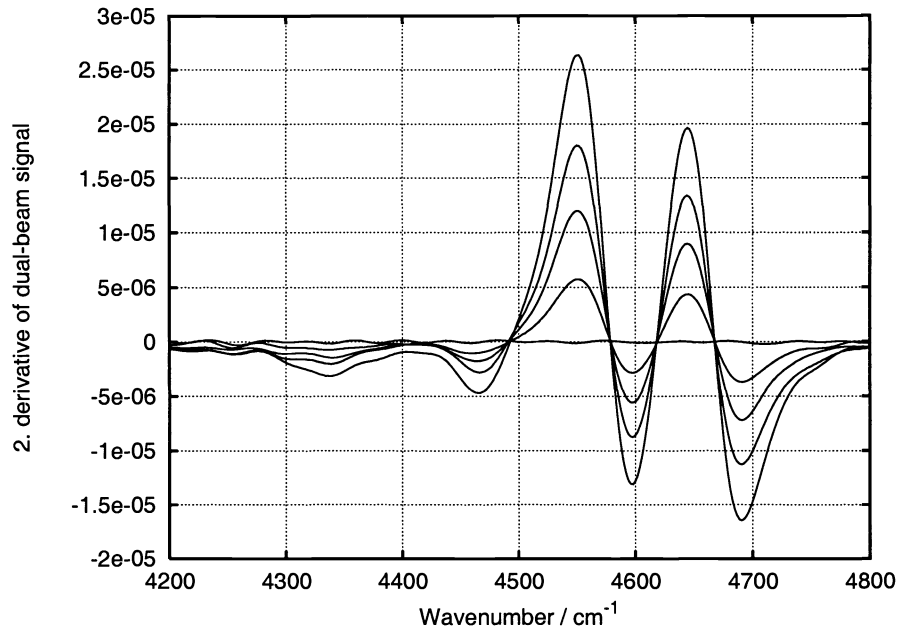


**Figure 5:** Second derivative of urea single-beam absorbance at conc. 0.1, 0.2, 0.3, 0.45, 0.675, and 1.0 g/dl.

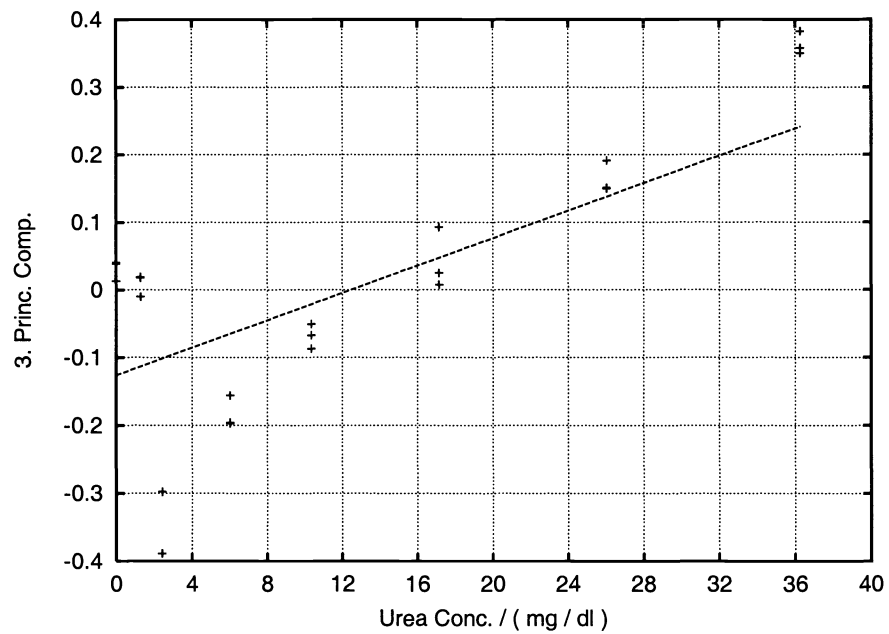
#### 4. RESULTS AND DISCUSSION

Figure 5 shows the second derivative of the traditional urea absorption spectra at concentrations 0.1, 0.2, 0.3, 0.45, 0.675, and 1.0 g/dl. Figure 6 shows the second derivative of the corresponding dual-beam measurements. The shape and magnitude of the urea signals differ between the traditional absorbance measurement and the dual-beam measurement. This is because the direct dual-beam measurement has not been divided with a single beam reference. Even so, one clearly notes that the noise present in the traditional absorption spectra has disappeared in the dual-beam measurements. At these high concentrations, it is evident that the noise of the traditional absorbance measurements does not prevent detection of urea. We do, however, expect that the absence of noise will be of importance at low concentrations. This is confirmed by the measurements of urea at concentrations in the range 0 to 40 mg/dl. Figure 7 shows the third principal component of the traditional absorbance measurements for these low concentrations of urea. The first and second component describe source intensity variations. The corresponding plot for the direct dual-beam measurements of the same samples is shown in figure 8. The single-beam data has a larger spread between replica measurements and a poorer linear relation between the third component and concentration. We estimate an improvement by a factor of at least six in reproducibility from the spread of replica measurements in the two modes of operation. A similar improvement in detection limit is estimated from the deviation from linearity at small concentrations in the two cases. The detection limit, estimated from figure 7, for the single-beam mode of operation is roughly 12 mg/dl and the detection limit for the dual-beam mode of operation, estimated from figure 8, is roughly 2 mg/dl.

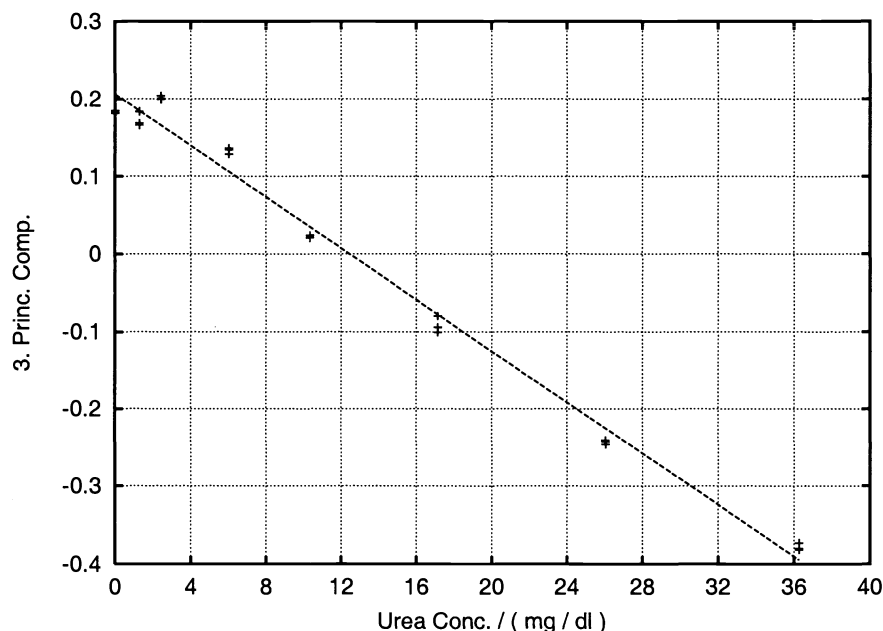
Eddy and Arnold<sup>6</sup> has measured urea from single-beam spectra of hemodialysis fluids using an optimized five factor partial least squares (PLS) model and obtained detection limits of 1.3 mg/dl. The rough analysis presented herein does not reach that limit of detection for the absorbance spectra. Even so, it is notable that a comparable detection limit is reached with the dual-beam mode of operation in combination with the same rough analysis. We expect that the combination of chemometric calibration models, such as PLS, with the superior data provided by the dual-beam mode of operation will improve the current detection limits and thereby make the technique interesting for other groups of trace organics that are present in low concentrations in biological fluids.



**Figure 6:** Second derivative of urea dual-beam signal at conc. 0.1, 0.2, 0.3, 0.45, 0.675, and 1.0 g/dl.



**Figure 7:** Third PC of single-beam absorbance data.



**Figure 8:** Third PC of direct dual-beam data.

## 5. CONCLUSION

The dual-beam technique seems highly feasible for near-infrared transmission spectroscopy of liquids. The problems usually found in mid-infrared applications with detector non-linearity and beam splitter absorption does not exist in the near-infrared. The problems with the double-input-single-output mode of operation caused by the modulation of thermal radiation from the sample and reference are of no concern either. The natural restriction of the wavenumber range by the absorption of water and the low required resolution of the broad signals found in the combination region facilitates the optical subtraction process. One disadvantage of the dual-beam mode of operation is the necessity of storing the phase of a single-beam measurement for calculating dual-beam intensity spectra. Even so, the dual-beam mode of operation provides increased stability and higher signal-to-noise ratio by removing the background absorption of water and digitizing only the difference between sample and reference, which has a much reduced dynamic range. Work in progress concentrates on quantification of the presented advantages of the dual-beam mode of operation and pursuit of lower detection limits with this instrumentation in combination with chemometric methods.

## ACKNOWLEDGMENTS

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