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Abstract. Bacterial contamination of blood products is one of the most frequent infectious complications of transfusion. Since glucose levels in blood supplies decrease as bacteria proliferate, it should be possible to detect the presence of bacterial contamination by measuring the glucose concentrations in the blood components. Hence this study is aimed to serve as a preliminary study for the nondestructive measurement of glucose level in transfusion blood. The glucose concentrations in red blood cell (RBC) samples were predicted using near-infrared diffuse-reflectance spectroscopy in the 1350 to 1850 nm wavelength region. Furthermore, the effects of donor, hematocrit level, and temperature variations among the RBC samples were observed. Results showed that the prediction performance of a dataset which contained samples that differed in all three parameters had a standard error of 29.3 mg/dL. Multiplicative scatter correction (MSC) preprocessing method was also found to be effective in minimizing the variations in scattering patterns created by various sample properties. The results suggest that the diffuse-reflectance spectroscopy may provide another avenue for the detection of bacterial contamination in red cell concentrations (RCC) products. © 2012 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.JBO.17.1.017004]

Keywords: glucose; red cell concentrations; near-infrared; diffuse-reflectance spectroscopy; bacterial contamination; nondestructive measurement.

Paper 11467 received Aug. 30, 2011; revised manuscript received Nov. 21, 2011; accepted for publication Nov. 28, 2011; published online Feb. 7, 2012.

1 Introduction

Septic reactions from bacterial contamination of blood products are considered to be one of the most frequent complications of blood transfusion. The U.S. Food and Drug Administration reported bacterial contamination as the third most frequent cause of death related to transfusion and data compiled from 11 prospective studies showed that, on average, there is about one case of sepsis per 2695 units transfused. Such bacterial contamination may be caused by bacteremia (i.e., the presence of bacteria in donor’s blood), the introduction of contaminants such as skin commensals during donor venipuncture, or the incomplete disinfection of processing equipment, etc.

One of the measures taken against this problem is to limit the shelf life of the blood supply. For example, in the U.S. the storage time of platelet (PLT) concentrates was reduced from seven to five days in 1986 due to a higher risk of bacterial sepsis with older units, and in Japan the retention period for red cell concentrations (RCC) was reduced from 42 days to 21 days in 1995. This reduction of storage life, though, may cause shortages in blood supply in countries such as Japan, where the population is aging. Therefore, a prolongation of the shelf life of blood components would become useful in maintaining a stable supply of transfusion blood.

In some European countries bacterial screening tests are employed before transfusion to reduce the occurrence of bacterial sepsis. For instance, in one such bacteria-detecting system, a small amount of blood is drawn from the transfusion bag into a smaller pouch and incubated. Inside the pouch oxygen (O2) consumption is monitored and used as a marker for bacterial contamination. This method requires the use of an additional sample pouch as a surrogate indicator for bacteria. This is an extra and inessential step as there is a method that enables the detection of bacteria directly from the transfusion bag.

Besides O2 level, glucose consumption is also found to increase in bacterially contaminated blood samples. For example, one study showed that the glucose level of RCC contaminated with Yersinia enterocolitica decreased by nearly 500 mg/dL (from 700 mg/dL to 200 mg/dL) whereas that of a sterile sample decreased by around 100 mg/dL (from 700 mg/dL to 600 mg/dL). This result suggests that the detection of glucose levels could serve as a useful indicator for bacterial contaminations in blood components.

One of the most promising methods for the nondestructive measurement of blood glucose levels is near-infrared (NIR) spectroscopy because the NIR region contains the overtone and combination bands for glucose absorption. One important contributor in a successful component analysis using NIR spectroscopy is the selection of a suitable measurement system (e.g., transmittance or reflectance spectroscopy) and an optimal...
wavelength region. Previous studies that took measurements in biological components have utilized both shorter (800–1300 nm) and longer (1300–2500 nm) wavelengths in the NIR region. In general, the longer wavelength regions produced larger absorbance signals but resulted in shorter optical path lengths. In a study conducted by Heise, transmittance spectroscopy was used to measure glucose levels in human blood plasma. The results showed that measurements taken in the longer wavelength regions produced more accurate glucose quantification results than those taken in the shorter wavelength regions. Similar results were also obtained by Amerov et al. in a study that utilized diffuse-transmittance spectroscopy to detect glucose levels in whole blood samples. They tested wavelength regions in the NIR range between 1111 and 2500 nm and showed that the longer wavelength region from 2062 to 2381 nm generated a higher correlation between predicted and reference glucose levels than the shorter wavelength region from 1117 to 1370 nm (correlation coefficient $r = 0.960$; standard error of prediction $[\text{SEP}] = 17.3$ mg/dL). Furthermore, in a study by Kim and Yoon, which utilized the diffuse-transmittance method in the wavelength range of 1110 to 2400 nm, results also indicated that spectra taken in the long wavelength region between 1390 and 1888 nm and 2044 to 2392 nm produced the best prediction of glucose levels in whole blood ($r = 0.976$; $\text{SEP} = 26.1$ mg/dL).

In both Amerov et al. and Kim and Yoon’s studies of whole blood, the SEP of glucose was lower than 30 mg/dL, which suggests that NIR spectroscopy can be utilized to measure blood glucose levels. Moreover, since glucose concentrations in transfusion products are known to decrease from the normal level of around 600 mg/dL to less than 200 mg/dL when bacteria proliferate, NIR spectroscopy is a promising method to detect the presence of bacterial contamination in blood components.

In contrast to studies that utilized diffuse-transmittance method, Maruo et al. used NIR diffuse-reflectance spectroscopy for the noninvasive measurement of blood glucose level in vivo. They suggested the diffuse-reflectance method yields larger absorbance signals than the diffuse-transmittance method because it can take changes in both glucose concentration and the scattering property of the body into account when forming the absorbance signals. Therefore, for materials with high scattering properties, such as the RBC components used in the present study, diffuse-reflectance may produce larger absorbance signals. Furthermore, unlike the diffuse-transmittance method, diffuse-reflectance measurements do not require the optical path length to be fixed, which would enable the measuring machine to have a simpler design.

Since there has not been any published research on the measurement of glucose concentrations in whole-blood or RCC utilizing diffuse-reflectance spectroscopy, this paper attempts to serve as a preliminary study. In this study glucose concentrations in blood samples were predicted using NIR diffuse-reflectance spectroscopy in the 1350 to 1850 nm range. The effect of variations in hematocrit (Hct) level, temperature, and donor properties among the samples was also observed to test whether the present method could be used in real-life settings. From the results of the present study, the possibility of optical detection of glucose in RCC by NIR diffuse-reflectance spectroscopy can be evaluated.

2 Methods

2.1 Device

We developed a diffuse-reflectance spectrometer to measure the NIR spectra of RBC samples. Figure 1 is a schematic diagram of the instrument. The instrument consisted of a 150-watt halogen lamp light source (Philips, TYPE 6550), an optical fiber bundle that included both detector and illuminator fibers (Fujikura, cladding diameter 200 μm, core diameter 175 μm, NA = 0.2), and mini spectrometer containing a grating and a 256-channel linear array photo detector (Hamamatsu Photonics K. K., C9914GB). The optical signal from the spectrometer was transferred to a computer (Panasonic, Let’s Note), where the optical signal was processed to predict the glucose concentrations.

The detector and illuminator optical fibers were fixed at the measuring tip (see Fig. 1) of the optical fiber bundle. Figure 2 shows the geometry of the optical fibers at the measuring tip. The tip of the optical fiber was designed such that twelve illuminator fibers surrounded one central detector fiber in a circle. The distance between the detector fiber and each of the illuminator fibers was 650 μm. This design maintained a sufficient average NIR radiation path length and allowed the illuminator to emit high-intensity radiation.

![Fig. 1 The schematic diagram of the diffuse-reflectance spectrometer used in the present study.](image1)

![Fig. 2 The geometry of detector and illuminator fibers at the measurement tip of the sample.](image2)
For spectral measurement, the measuring tip was directly submerged into the RCC. Inside the spectrometer, radiation from the halogen lamp was gathered through a concave mirror, transmitted through the illuminator fibers, and irradiated into the samples. Then, in the RCC the diffuse-reflected radiation was scattered many times in the turbid media, and only the radiation that reached the detector fiber was transmitted back to the spectrometer. Finally, the transmitted signal was sent to the computer, where the absorbance spectra were calculated. The reference spectra needed to form the absorbance spectra were taken by irradiating a standard 10% reflectance target (Labsphere) at the beginning of the experiment. Although this spectrometer is able to record measurements in wavelength regions between 1100 and 2200 nm, only spectral signals taken in the 1430 to 1850 nm range were used for glucose prediction. The accumulation time of each linear array photoreceptor was 50 ms, and 200 spectral data were averaged per measurement.

The quality of the spectra was also assessed by taking a spectral measurement every 5 min for 120 min and then conducting a root-mean-square (rms) noise analysis of 100% lines on the collected data. Results showed that the average rms of 100% line of the wavelength region between 1430 and 1850 nm was 87.4 μAU, which we predicted to be sufficient for the purpose of the study.

### 2.2 Red Blood Cell Samples

The RBC samples used for this experiment were prepared by the Japanese Red Cross Society (JRCS) using two packages of Red Cells Concentrates-Leukocytes Reduced “Nisseki” (RCC-LR) that did not qualify for transfusion after a blood test. The blood from the two packages was not mixed together in this experiment.

To make the RBC sample, the disqualified RCC-LR was centrifuged to separate the RBC portion from the rest of the blood. A typical RCC-LR contains glucose concentrations up to 600 mg/dL and Hct values around 50 to 60%. The collected RBC portion was first adjusted into samples with Hct levels of 50 or 60% by diluting it with physiologic saline solution. Then, within each Hct group, the samples were further adjusted into samples with glucose concentrations of 0, 100, 200, 300, 400, 500, or 600 mg/dL. Because the Hct and glucose levels were hand-adjusted, it was likely for the actual Hct and glucose values to deviate from the ideal values. Therefore, the actual Hct and glucose levels were measured using an automated hematocrit analyzer (Sysmex, K-4500) and Glucose CII-test Wako (Wako Pure Chemicals, Osaka), respectively, and used in the data analysis. Since the measurements were taken from the supernatant of the samples, the effect of haemolysis on glucose measurement was considered negligible.

After the RBC samples were separated into their respective Hct and glucose levels, 25 mL of each sample were transferred to a glass container, inside which the measurement took place. To create different temperature conditions, these containers were first placed in a 25 °C water bath and taken out at the time of measurement. Then, following the first round of measurements, they were transferred to a 27 °C water bath for the second round of measurements. All experimental steps, from preparation of the RCC samples to measurement of the spectra, were completed within one hour; hence, the properties of each sample were assumed to be constant throughout the experiment. Furthermore, the Hct levels and glucose concentrations were not correlated to each other (r = −0.08).

Table 1 shows a summary of the experimental conditions and the measured Hct, temperature, and glucose values for each sample. RBC from Donor A was separated into four measurement conditions: 1. Hct 50% at 25 °C; 2. Hct 50% at 27 °C; 3. Hct 60% at 25 °C; and 4. Hct 60% at 27 °C, as shown in Table 1. RBC from Donor B was adjusted to two measurement conditions: 1. Hct 50% at 25 °C and 2. Hct 50% at 27 °C. Each measurement condition had RBC samples with seven glucose levels ranging from 0 to 600 mg/dL, making 42 independent blood samples in total. Three spectral measurements were taken for each sample, producing a total of 126 spectral readings available for analysis.

### 2.3 Prediction Method

Partial least squares (PLS) regression analysis was used to form calibration models for glucose quantification based on the measured data. Because the number of data points for each condition was limited, the cross validation method was utilized. Furthermore, the effect of multiplicative scatter correction (MSC) preprocessing method, which is known to reduce the scattering effect for quantitative prediction, was observed using the spectral data from each condition.

### 3 Results and Discussion

Figure shows all 126 spectra taken from samples in conditions 1 to 6. The unprocessed spectra showed multiplicative and
additive variations due to different sample parameters in Hct level, temperature, and donor properties.

### 3.1 Glucose Sensing in Samples from the Same Condition

The glucose concentration in each sample was estimated from all the spectral data taken from its respective condition. Table 2 summarizes the cross validation and calibration regression results of samples from conditions 1 to 6. Fairly robust correlations were obtained between predicted and measured glucose levels for all groups. The average correlation coefficient of cross validation (rCVal) was 0.994, and the standard error of cross validation (SECV) was 17.1 mg/dL. Moreover, the average correlation coefficient of calibration (rCal) was 0.998, and the standard error of calibration (SEC) was 9.9 mg/dL. Our results showed improvement in prediction performances compared to previous studies on optical glucose sensing in blood. For example, Amerov et al. obtained rCVal of 0.980 and SECV of 21.6 mg/dL in the wavelength region between 1550 and 1754 nm in their research utilizing diffuse-transmittance spectroscopy. Compared to their study, the SECV was improved by around 4.5 mg/dL in the present study. Although different types of measurement systems were utilized, their study could serve as a basis of comparison for results from our study because similar experimental setups and a similar wavelength region were used.

A closer look at the results from a single condition also shows that correlations obtained are fairly high. For example, Fig. 4(a) is a regression graph that illustrates the results obtained from samples in condition 4. The correlation was fairly high, with rCVal of 0.997 and SECV of 13.3 mg/dL. Furthermore, Fig. 4(b) is a regression vector obtained from the same set of samples. The vector had a positive peak around 1580 nm, which is near the peak absorbance spectra of glucose. In addition, Kim and Yoon also observed two positive peaks around 1580 nm and 1750 nm in their regression vector, which are similar to those seen in Fig. 4(b). These evidences support the validity of this calibration model.

### 3.2 Glucose Sensing in Samples from Different Conditions

Several datasets were constructed by pooling together spectral data from various conditions, as shown in Table 3. These calibration sets were then used to predict glucose levels to test whether a good calibration model could be constructed from data taken from samples with various conditions. The effects of donor, Hct, and temperature differences on glucose prediction were observed.

In the first set, data that varied only in the original donor were combined (see Table 3 “donor difference”). The rCVal was 0.971, and the SECV was 36.1 mg/dL. For the second dataset, which includes samples with different Hct content (see Table 3 “hematocrit difference”), rCVal was 0.985 and SECV was 29.9 mg/dL. For the third dataset, which included spectral

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**Table 2** Prediction of glucose concentrations in spectral region between 1430 and 1850 nm for RBC samples from conditions 1 to 6.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of spectra</th>
<th>Number of factors</th>
<th>SECV (mg/dL)</th>
<th>rCVal</th>
<th>SEC (mg/dL)</th>
<th>rCal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>7</td>
<td>27.4</td>
<td>0.988</td>
<td>9.9</td>
<td>0.998</td>
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<td>2</td>
<td>21</td>
<td>5</td>
<td>14.2</td>
<td>0.997</td>
<td>7.8</td>
<td>0.999</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>4</td>
<td>21.6</td>
<td>0.993</td>
<td>13.8</td>
<td>0.997</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>4</td>
<td>13.3</td>
<td>0.997</td>
<td>10.1</td>
<td>0.998</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>4</td>
<td>10.1</td>
<td>0.996</td>
<td>6.8</td>
<td>0.998</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>5</td>
<td>16.0</td>
<td>0.990</td>
<td>10.7</td>
<td>0.996</td>
</tr>
</tbody>
</table>
data taken at various temperatures (see Table 3 “temperature difference”), rCVal of 0.990 and SECV of 16.5 mg/dL were obtained. The last dataset consists of samples that differed in all parameters, including donor, Hct, and temperature (see Table 3 “donor, hematocrit, temperature difference”). Its rCVal was 0.969, and its SECV was 36.0 mg/dL.

The dataset for “temperature difference” exhibited the highest correlation between the reference and predicted glucose concentrations, which is likely due to the fact that samples from this set had the same donor and Hct values and differed only in their measured temperatures. Because the scattering properties of samples with different temperatures were relatively constant, a better calibration model was achieved. In contrast, for datasets that contained samples with varying scattering properties due to Hct or donor differences, the accuracy of glucose prediction decreased.

### 3.3 Effects of MSC Preprocessing Method

Spectral data from samples in Tables 2 and 3 were preprocessed by MSC method. Tables 4 and 5 summarize the calibration models created from the MSC preprocessed data. The effect of MSC preprocessing can be seen by comparing Fig. 4(a), which displays all 126 raw spectra, with Fig. 4(b), which illustrates MSC preprocessed spectra from all 126 samples. It is evident that the multiplicative and additive variations in the raw spectra were compensated by the MSC preprocessing method.

**Table 3** Calibration models for glucose prediction in wavelength regions between 1430 and 1850 nm using datasets that consist of spectral data from different conditions.

<table>
<thead>
<tr>
<th>Calibration set</th>
<th>Samples used</th>
<th>Number of spectra</th>
<th>Number of factors</th>
<th>SECV (mg/dL)</th>
<th>rCVal</th>
<th>SEC (mg/dL)</th>
<th>rCal</th>
</tr>
</thead>
<tbody>
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<td>Donor difference</td>
<td>Condition 1 &amp; 5</td>
<td>42</td>
<td>10</td>
<td>36.1</td>
<td>0.971</td>
<td>14.2</td>
<td>0.996</td>
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<td>Condition 1 &amp; 3</td>
<td>42</td>
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<td>0.985</td>
<td>18.6</td>
<td>0.994</td>
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<tr>
<td>Temperature difference</td>
<td>Condition 5 &amp; 6</td>
<td>42</td>
<td>8</td>
<td>16.5</td>
<td>0.990</td>
<td>9.7</td>
<td>0.996</td>
</tr>
<tr>
<td>Donor, hematocrit, temperature difference</td>
<td>Condition 3, 4, 5 &amp; 6</td>
<td>84</td>
<td>12</td>
<td>36.0</td>
<td>0.969</td>
<td>18.6</td>
<td>0.992</td>
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</tbody>
</table>

**Table 4** The effects of MSC preprocessing on the prediction of glucose concentration in a spectral region between 1430 and 1850 nm for RBC samples from conditions 1 to 6.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of spectra</th>
<th>Number of factors [MSC]</th>
<th>SECV [mg/dL] [MSC]</th>
<th>rCVal [MSC]</th>
<th>SEC [mg/dL] [MSC]</th>
<th>rCal [MSC]</th>
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</thead>
<tbody>
<tr>
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<td>18.5</td>
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<td>0.999</td>
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<td>26.0</td>
<td>0.989</td>
<td>12.6</td>
<td>0.998</td>
</tr>
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<td>4</td>
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<td>0.994</td>
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<tr>
<td>6</td>
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<td>17.8</td>
<td>0.988</td>
<td>8.12</td>
<td>0.998</td>
</tr>
</tbody>
</table>
were 0.992 and 19.7 mg/dL, respectively. Results show that the MSC preprocess did not improve the quantitative determination accuracy of the raw data (average raw rCVaL = 0.994; raw SECV = 17.1 mg/dL). These results are most likely due to the small differences in scattering properties between each raw sample, which minimize the effect of the MSC preprocess.

The MSC preprocess was also performed on datasets that combined samples from various conditions (see Table 3). Glucose concentrations were then predicted using the processed data. The rCVaL and SECV for the “donor difference” dataset was 0.982 and 28.4 mg/dL, respectively, an improvement compared to those of nonpreprocessed dataset, which had an SECV of 36.1 mg/dL. This improvement was probably due to the MSC preprocess’s effect, which diminishes the variation in scattering properties of the blood from different donors.

The same effect was seen in the dataset for “hematocrit difference.” The MSC preprocess rCVaL of 0.993 and SECV of 20.9 mg/dL were better than the raw SECV of 29.9 mg/dL. In addition, the dataset for “donor, hematocrit, temp difference,” the MSC preprocess rCVaL of 0.980 and SECV of 29.3 mg/dL were better than the raw rCVaL of 0.969 and SECV of 36.0 mg/dL. Again, this effect was probably due to the decrease in scattering properties caused by Hct and donor differences following MSC preprocessing.

Interestingly, the dataset for “temperature difference” did not seem to be affected by MSC preprocessing. The rCVaL and SECV after MSC preprocessing were 0.989 and 16.6 mg/dL, respectively, whereas the raw rCVaL and SECV for the unpreprocessed were 0.990 and 16.5 mg/dL, respectively. This is probably because this dataset contained samples that had the same donor and Hct level. The difference in measuring temperature alone did not cause variance in scattering property, which was significant enough for MSC preprocess to pose an effect.

In the present study, the MSC-preprocess method was able to effectively reduce the scattering properties created by different blood properties. The same effect, though, could not be observed in Kim and Yoon’s study. One possible explanation for this discrepancy is that different optical measurement systems were used (i.e., diffuse-reflectance as opposed to diffuse-transmission spectroscopy).

4 Summary

In the present study NIR diffuse-reflectance spectroscopy was used to measure the glucose concentrations in RBC samples. As we evaluated above, the glucose concentrations in RCC decrease from around 600 mg/dL down to less than 200 mg/dL when it is bacterially contaminated. Considering that the SECV obtained from even the most complex calibration set—which included samples that differed in donors, Hct levels, and temperatures—was 29.3 mg/dL, we can suggest that our method has the potential to detect bacterial contamination in RCC with sufficient quantitative determination accuracy.

The present study was a preliminary study for the evaluation of the optical measurement of glucose in blood supplies as a method of bacterial detection. We showed that the diffuse-reflectance method has several advantages over alternative methods, including simpler instrumental structure and higher glucose-sensing accuracy. In addition, we determined that the MSC preprocessing method was effective in minimizing the variation in scattering properties created when samples with different properties are combined, thereby improving the prediction performance. In the future we plan to examine methods by which glucose concentrations can be measured directly through the RCC transfusion bags using NIR diffuse-reflectance spectroscopy.

Table 5 The effects of MSC data preprocessing on glucose prediction in wavelength regions between 1430 and 1830 nm.

<table>
<thead>
<tr>
<th>Calibration set</th>
<th>Samples used</th>
<th>Number of spectra</th>
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<td>0.997</td>
</tr>
<tr>
<td>Temperature difference</td>
<td>Condition 5 &amp; 6</td>
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<td>7</td>
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<td>Condition 3, 4, 5 &amp; 6</td>
<td>84</td>
<td>9</td>
<td>29.3</td>
<td>0.980</td>
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</table>

References


