Journal of the Optical Society of Korea, Vol. 7, No. 4, December 2003, pp. 240-244

Reagentless Determination of Human Serum Components Using Infrared Absorption Spectroscopy

Sangjoon Hahn

u-Health Project Team, Samsung Advanced Institute of Technology, Suwon 449-901, KOREA

Gilwon Yoon

Seoul National University of Technology, Seoul 139-743, KOREA

Gunshik Kim and Seung-Han Park

National Research Laboratory of Nonlinear Optics, College of Science, Yonsei University, Seoul 120-749, KOREA

(Received October 7, 2003)

Simultaneous determination of concentrations for four major components in human blood serum was investigated using a Fourier-transform mid-infrared spectroscopy. Infrared spectra of human blood serum were measured in 8.404 ~ 10.25 μm range where the highest absorption peaks of glucose are located. A partial least square (PLS) algorithm was utilized to establish a calibration model for determining total protein, albumin, globulin and glucose levels which are commonly measured metabolites. The standard error of cross validation obtained from our multivariate calibration model was 0.24 g/dL for total protein, 0.15 g/dL for albumin, 0.17 g/dL for globulin, and 6.68 mg/dL for glucose, which are comparable with or meet the criteria for clinical use. The results indicate that the infrared absorption spectroscopy can be used to predict the concentrations of clinically important metabolites without going through a chemical process with a reagent.
OCIS codes : 120.0120, 190.0190, 280.0280.

I. INTRODUCTION

Major dissolved substances in human blood and serum play an important role in metabolism and are usually sensitive indicators for the risk of various diseases as well. For example, monitoring the glucose level in serum or blood is an essential diagnosis for people with diabetes and an important element of managing the disease. Cholesterol and triglycerides in serum are known to be some of the independent risk factors for coronary heart disease. The levels of urea and creatine are closely related to kidney function. Protein or albumin in serum is commonly tested to monitor liver and renal conditions. Not surprisingly, therefore, serum and blood analyses become one of the most common tests performed and all of the major dissolved metabolites are routinely analyzed in the clinical laboratory. Most of these analyses are carried out with a photometric instrument using an enzymatic reaction. The photometric measurement method is well established and easily available. However, the chemical process such as enzymatic reaction requires expensive reagents and a relatively large volume of the sample. More than 50of the operating cost of clinical analyzers in the hospital is devoted to the expenses of reagents. Recently, there have been some reports on the optical spectroscopic method for the quantification of major metabolites in human blood, serum, tissue and other substrates [1–4]. From the clinical point of view, the optical measurement is an attractive alternative as a standard enzymatic technique because it is quite simple and rapid. Furthermore, several components can be simultaneously estimated from a single spectrum with no reagent and relatively small volume of sample. In this regard, infrared (IR) spectroscopy has emerged as an important tool to monitor the components in whole blood or serum.

Both near-IR (0.7~2.5μm) and mid-IR (2.5~10μm)

TABLE 1. Reference intervals for serum components. The concentrations are in the physiological ranges.

Component	reference interval
total protein	4.6 ~ 7.7(g/dL)
albumin	2.2 ~ 4.8(g/dL)
globulin	2 ~ 3.8(g/dL)
glucose	88 ~ 177(mg/dL)

spectroscopies are applicable for the diagnostics but each one has its own advantages. Near-IR spectroscopy has an advantage over mid-IR spectroscopy since samples with large size can be easily handled (optical pathlength can generally be millimeters, compared to micrometers in the mid-IR) [6–8]. Therefore, it has een favorably employed for the analytical tests. Meanwhile, mid-IR spectroscopy has also its potential merits. One clear advantage is its applicability for very small sample sizes (micro-liters). It also provides relatively narrow bandwidths and comparatively rich mid-IR absorption spectra, which make for more accurate analysis and better molecular fingerprints than near-IR spectroscopy [4]. Previous studies have demonstrated that the Fourier transform infrared spectrometry coupled with an ATR sampling device can be used to determine the amount of glucose in blood or plasma [5]. A transmission scheme is also employed to overcome protein adsorption on the surfaces, which is often observed in the ATR measurement. In practice, the transmission measurement has the advantage of using small cell-volume and a low priced transmission-flow-cell. However, there is hindrance to the wide spread adoption of IR spectroscopy since the IR spectrum obtained from biological samples usually consists of many overlapping bands. In addition, water, which is a strong IR absorber, is shown to conceal the characteristics of vibrational bands of the biological components and consequently the spectra are difficult to interpret. Thus, the quantitative measurement of biological metabolites using optical spectroscopy heavily depends on unscrambling the spectral data from water band. Some of the previous studies employed a drying step to eliminate one of the difficult obstacles for the quantitative analysis of an aqueous biological sample [9]. This drying process requires an extra time of about 10 minutes, for example, and adding chemicals such as thiocyanate for calibration. In this paper, we demonstrate a new approach to utilize the mid-IR absorption spectroscopic technique for determining the concentration level of human serum components such as total protein, albumin, globulin, and glucose in the physiological concentration range without using reagents or an additional step of specimen drying.

II. EXPERIMENTAL METHOD

1. Biological Samples

A total of 93 human blood serum samples were obtained from the Samsung Medical Center and analyzed for the construction of a multivariate calibration model. Reference values for total protein, albumin, globulin and glucose were obtained by the conventional method using photometric measurements based on enzymatic reactions. Table 1 lists the reference interval of the concentration range for four metabolites in human blood serum used in this work.

2. Fourier Transform Infrared(FT-IR) Spectroscopy

Absorption spectra of human blood serum were measured with a Nicolet AVATAR 360 FT-IR spectrophotometer. This spectrometer was equipped with a KBr beam splitter and DTGS detector operating at room temperature. The sample was contained in a 50 μm pathlength BaF2 transmission window cell. The spectra were ratioed against the single-beam spectrum of a clean transmission window cell and converted to an absorbance scale. However, even a minute variation of cell thickness may induce error in the spectrum. In order to perform pathlength calibration we obtained blank absorption spectra with an empty cell which contains a typical interference pattern (Fig. 1). To calculate the cell thickness we employed following equations.

$$L(\text{in min}) = \frac{nw_1w_2}{2(w_2 - w_1)(1000)} \tag{1}$$

where L = cell thickness (in mm), w_1 = starting wavelength(in μm), w_2 = ending wavelength (in μm), and n = number of fringes between w_1 and w_2.

About 100 μL of blood serum was used to fill the

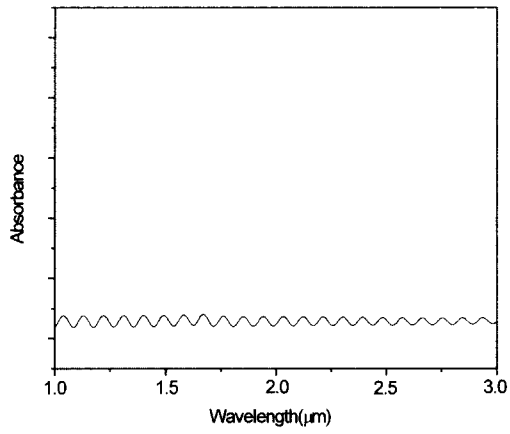

FIG. 1. Interference fringe of an empty absorption cell

window. After measurement, the cell and the tubing were rinsed with 600 μL of distilled water. The absorption spectra were acquired from 93 serum samples. We obtained FT-IR spectrum over the wavelength range from 8.404 to 10.25 μm with a spectral resolution of 4 cm^{-1} and with an 8 mm aperture. Each spectrum was obtained from 128 interferograms. Autogain was applied. Typically, it took about ten minutes to obtain the absorption spectrum of each serum sample. We employed the Happ-Genzel apodization function to convert the interferogram to an absorption spectrum.

3. Construction of a Statistical Model

For the multivariate analysis [10,11] of absorption spectra, a partial least squares(PLS) calibration method was constructed separately for each of four components. The spectra were analyzed between 8.404 $\sim$ 10.25 μm. The highest absorption peak of glucose, the most important component of our interest, is located in this range. All the spectral preprocessing and statistical computation were carried out with Pirouette 2.6 software (Informetrix Inc., Woodinville, WA). The best results were obtained when the preprocessing included one-point baseline correction and mean centering. One-point correction subtracts a constant Y value from each point of the spectrum. Four out of ninety-three spectra were considered as outliers and removed in the development of calibration models due to large spectral residuals. Table 2 lists the number of samples used in the development of the PLS calibration model.

III. RESULTS AND DISCUSSION

Fig. 2 presents the mid-IR spectra of human blood serum between 8.404 $\sim$ 10.25 μm for a total of 93 samples. Previous studies have shown that this spectral region features the characteristic absorption bands of saccharides such as sucrose, fructose, and glucose [3]. A typical mid-IR spectrum of the glucose solution is displayed in Fig. 3 as an example. It shows the aforementioned coupled absorption bands between 8.404 $\sim$ 10.25 μm (1190 $\sim$ 976 cm^{-1}) region associated with elongation of vibrations of C-C and C-O bonds. More detailed discussion on this matter was reported by Heise [12]. trictly speaking, the spectra obtained with the complex biological samples originate from the different absorption bands of many constituents in the serum such as sugar and protein. Nevertheless, the spectral variation is strongly dependent upon the glucose concentration.

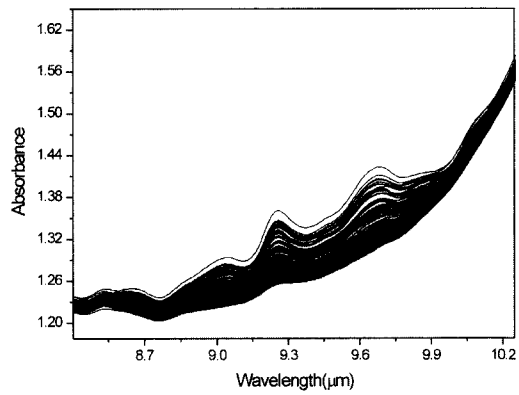

FIG. 2. Absorption spectra of blood serum in the spectral range between 8.404 and 10.25 μm.

TABLE 2. Summary of PLS calibration model for blood serum samples in terms of SECV. Spectral region under analysis is 8.404~10.25 μm. Clinically acceptable ranges are also given from the reference [11].

Component	number of samples	correlation coefficient	SECV	clinically acceptable range [11]
total protein	89	0.96	0.22 g/dL	0.7 g/dL at 7g/dL
albumin	88	0.98	0.14 g/dL	0.35g/dL at 3.5g/dL
alobulin	90	0.87	0.19 g/dL	0.44g/dL at 4.4g/dL (with assumption of 10%)
glucose	89	0.96	6.68 mg/dL	12.6 mg/dL at 126mg/dL 20 mg/dL at 200mg/dL

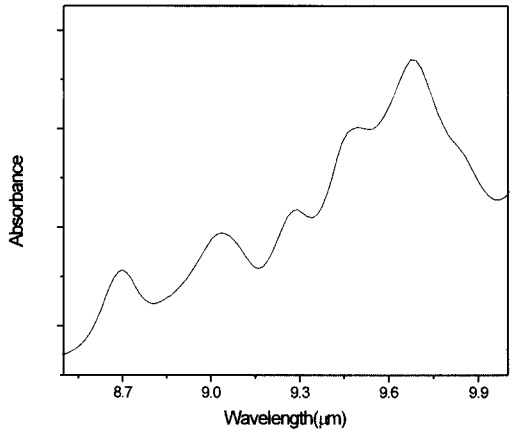

FIG. 3. Glucose spectrum over the spectral region(8.404 ~ 10.25 μm). Glucose in water whose concentration is 3.7g/dl in 50 μm cell.

Fig. 4 represents typical glucose absorption spectra for various concentrations dissolved in water, in which the absorption from the water is compensated. The enlargement of the characteristic absorption band clearly appeared in the spectrum with increasing glucose concentration. The magnitudes of the absorption peaks at 9.7μm, 9.25μm, 9.07μm, and 8.67μm linearly increases as the glucose concentration is increased, as shown in Fig. 5.

In order to determine the concentrations of all other components in the human blood serum simultaneously, the spectral region between 8.404 ~ 10.25 μm was chosen consistently. As described in section II, table 1 shows the reference concentrations for four components predetermined by chemical and enzymatic methods. Note that the concentrations of glucose in the samples lie within the ranges of 88 ~ 177 mg/dL. It is important to determine the optimal number of loading vectors to avoid under- and over-fittings for establishing the calibration model. The F-test was

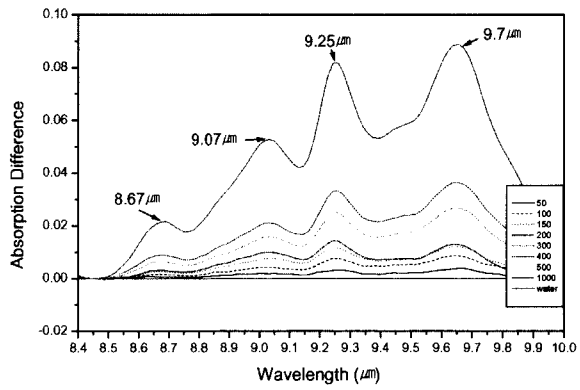

FIG. 4. Glucose absorbance for concentrations of 50, 100, 150, 200, 300, 400, 500 and 100 mg/dL. The water baseline compensation was performed.

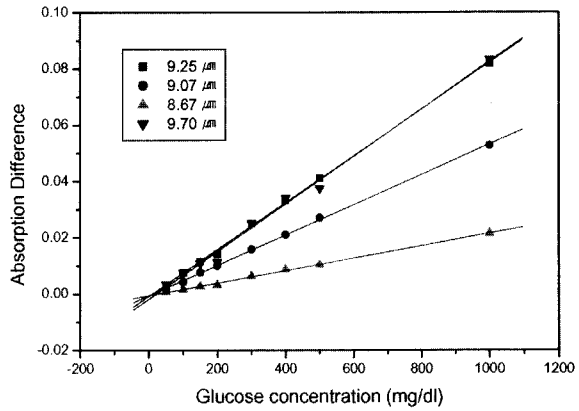

FIG. 5. Linear fitting of the absorption peaks of glucose solution.

used to determine the optimal number of factors for the calibration model. The 4 ~ 5 ranks were found to be sufficient for the establishment of a calibration model for each analysis. The standard error of cross-validation (SECV) was taken as the assessment of the calibration models. The cross-validation was performed by a leave-one-out method. The SECV values are listed along with correlation coefficients in Table 2.

Fig. 6 shows the results from the PLS, which predicts the glucose concentration versus the reference values. The SECV for the glucose was found to be 6.68 mg/dL. The result is quite comparable or superior to other results [9]. In this and all other instances, the correlation coefficient between the reference and predicted values was greater than 0.87 for all the components in our study.

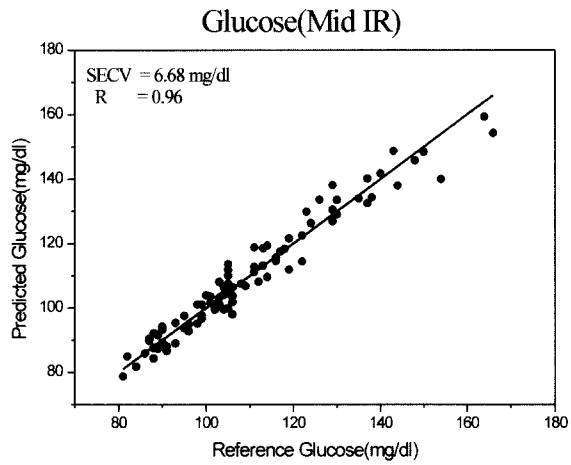

FIG. 6. The concentrations of glucose in blood serum predicted by the multivariate calibration model over the spectral region(8.404 ~ 10.25 μm) were plotted against the reference values.

IV. CONCLUSIONS

We have shown that a mid-IR spectroscopic method can be applied to serum analysis without using reagents. This technique seems to be a very attractive candidate for the development of a small analyzer with simple components [13,14]. Generally, multivariate statistical analysis in the mid-IR spectral region produces a more reliable prediction than near-IR spectroscopy because mid-IR spectrum consists of relatively well resolved band shapes due to the narrow band width. Actually, SECV obtained from the near-IR spectroscopy is $\sim$ 17.7 mg/dl, which is much better than the values measured by the conventional method. Therefore, our study successfully demonstrates the potential of mid-IR transmission spectroscopy in determining the concentrations of several major metabolites simultaneously in the clinically relevant range of concentration [15]. In addition, our results indicate that the wavelength range from 8.404 to 10.25 μm can be used to predict the concentration of total protein, albumin, globulin and glucose in human blood serum with errors being in the same or better range than those commonly stated in the literature. We want to emphasize that it is possible to obtain correlation coefficients greater than 0.87 even from aqueous biological samples, meaning that the mid-IR transmission spectroscopy of aqueous biological samples without reagent and without specimen drying is a reliable method for clinical use.

ACKNOWLEDGEMENTS

This research was supported in part by the Ministry of Science and Technology of Korea through the National Research Laboratory Program (Contract No. M1-0203-00-0082).

REFERENCES

[1] G. Budinova, J. Salva, and K. Volka, "Application of Molecular Spectroscopy in the Mid-Infrared Region to the Determination of Glucose and Cholesterol in Whole Blood and in Blood Serum," *Appl. Spectrosc.*, vol. 51, no. 5, pp. 631-639, 1997.

[2] G. Janatsch, and J. D. Kruse-Jarres, "Multivariate calibration for assays in clinical chemistry using attenuated total reflection infrared spectra of human blood plasma," *Anal. Chem.*, vol. 61, p. 2016, 1989.

[3] F. Cadet, C. Robert, and B. Offmann, "Simultaneous Determination of Sugars by Multivariate Analysis Applied to Mid-Infrared Spectra of Biological Samples," *Appl. Spectrosc.*, vol. 51, no. 3, pp. 369-375, 1997.

[4] R. Vonach, J. Buschmann, R. Falkowski, R. Schindler, B. Lendl, and R. Kellner, "Application of Mid-Infrared Transmission Spectrometry to the Direct Determination of Glucose in Whole Blood," *Appl. Spectrosc.*, vol. 51, no. 6, pp. 820-822, 1998.

[5] H. M. Heise and R.Marbach, "Human oral mucosa studies with varying blood glucose concentration by non-invasive ATR-FT-IR-Spectroscopy," *Cellular and Molecular Biology*, vol. 44, no. 6, pp. 899-912, 1998.

[6] N. A. Cingo, G. W. Small, and M. A. Arnold, "Determination of glucose in a synthetic biological matrix with decimated time-domain filtered near-infrared interferogram data," *Vib. Spec.*, vol. 23, pp. 103-117, 2000.

[7] I. Amato, "In Search of Human Touch," *Science*, vol. 258, no. 5085, pp. 892-895, 1992.

[8] M. A. Arnold, G. W. Small, "Determination of physiological levels of glucose in an aqueous matrix with digitally filtered Fourier transform near-infrared spectra," *Anal. Chem.*, vol. 62, no. 14, pp. 1457-1464 (1990).

[9] R. A. Shaw and H. H. Mantsch, "Multianalyte Serum Assays from Mid-IR Spectra of Dry Films on Glass Slides," *Appl. Spectrosc.*, vol. 54, no. 6, pp. 885-889, 2000.

[10] H. Martens and T. Naes, *Multivariate Calibration* (John Wiley and Sons, New York, 1989), pp. 1-30.

[11] R. Kramer, *Chemometric Techniques for Quantitative Analysis* (Marcel Dekker, Inc., New York, 1998) pp. 1-8.

[12] H. M. Heise, *Infrared and Raman Spectroscopy of Biological Materials*, U-H Gremlich and B. Yan Eds., (Marcel Dekker, New York, 2001), pp. 259-322.

[13] O. S. Khalil, "Spectroscopic and Clinical Aspects of Noninvasive Glucose Measurements," *Clin. Chem.*, vol. 45, no. 2, pp. 165-177, 1999.

[14] S. Sasic and Y. Ozaki, "Statistical Two-Dimensional Correlation Spectroscopy: Its Theory and Applications to Sets of Vibrational Spectra," *Anal. Chem.*, vol. 73, no. 10, pp. 2294-2301, 2001.

[15] C. A. Burtis and E. R. Ashwood, *Tietz Textbook of Clinical Chemistry* (3rd edition, W.B. Saunders Co., 1999) pp. 375-377.