Reagentless determination of hemoglobin using near infrared spectroscopy

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Introduction

There is a growing demand for rapid and cost effective new methods in the field of medical laboratory analysis. The objective of this study was to explore whether measurements in the near infrared (NIR) spectral region can be related to hemoglobin content of human whole blood.

Methods

Seventy-two whole blood samples were collected and investigated in order to determine the relationship between their NIR spectral data and hemoglobin content based on laboratory data determined by a standard method. Donors were selected randomly without respect to age, sex,

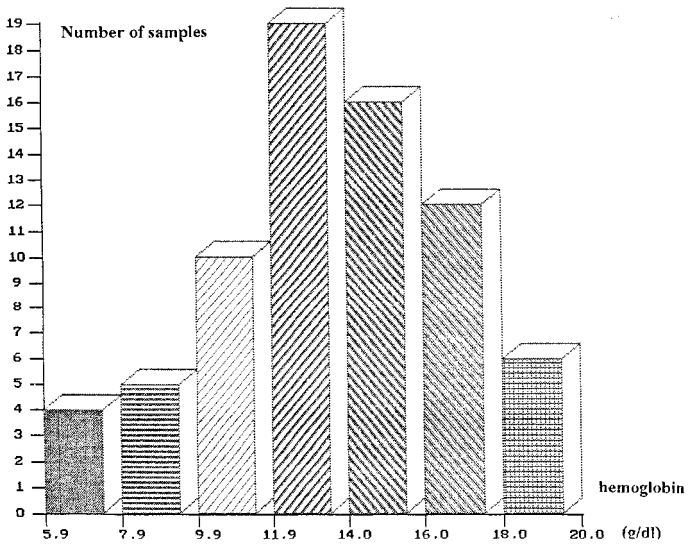

Figure 1. Hemoglobin content distribution of the 72 whole blood samples (frequency histogram).

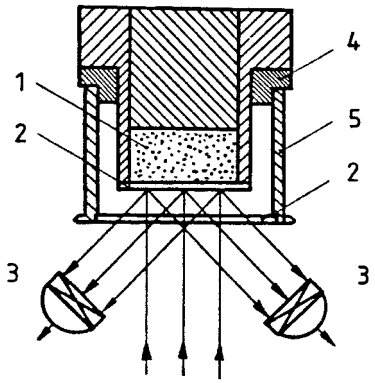

Figure 2. Schematic representation of transflectance sample holder cell. 1. "Absolute white" powder (reflector). 2. Quartz windows. 3. Lead sulfide detectors. 4. Distance ring for adjustment of sample layer thickness. 5. Sample holder house.

state of health or medical treatment. Both arterial and venous blood were used. Hemoglobin content of samples ranged from 5.9 to 20 g dL^{-1} (Figure 1).

Diffuse transflectance (TF) data ranging from 1000 to 2500 nm were measured and recorded with a Spectralyzer 1025 computerized research composition analyzer. The instrument was operated in a single beam mode with a reference spectrum measured on halon etalon as a reference

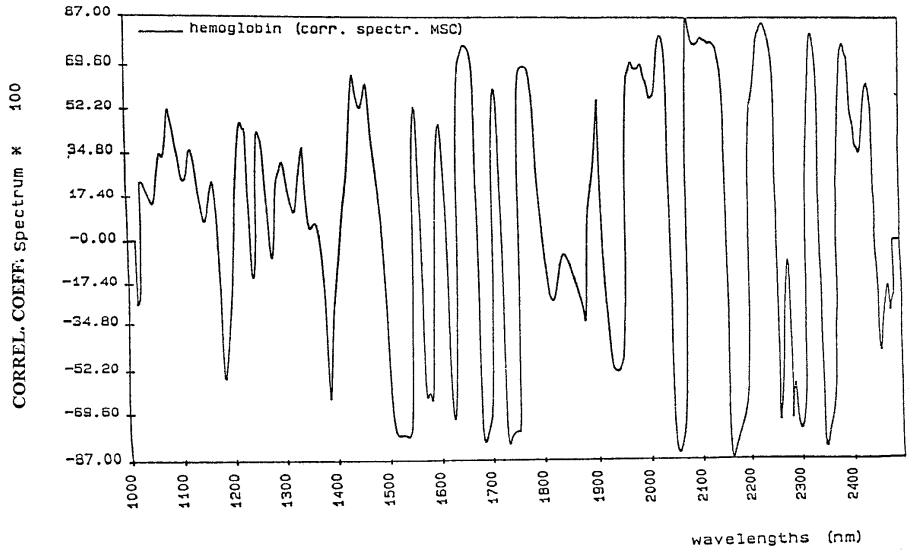

Figure 3. Correlation plot for hemoglobin determination in whole blood derived from a single term second derivative equation (correlation coefficients at each wavelength plotted against wavelength). Arrow point at peaks where correlation coefficients are high and where the iterative regression procedure was tested. See also λ_1 values in the first column of Table 1.

Table 1. Characteristic wavelengths for hemoglobin and performance data obtained with linear summation equations.

λ_1 (nm)	λ_2 (nm)	λ_3 (nm)	λ_4 (nm)	*SEC* (g dL^{-1})	*r*	*SEP* (g dL^{-1})
2164	2432	2472	—	1.347	0.905	1.348
2164	2432	2472	2266	1.250	0.920	1.251
2080	2390	1214	—	1.375	0.901	—
2080	2390	1214	2162	1.329	0.910	—
1438	1646	1200	—	1.381	0.901	—
1438	1646	1200	2456	1.305	0.913	—

standard and data were stored in a computer. Transflectance data were collected at every 2 nm with 256 digital conversions per point. As a result, 750 transflectance points (spectral values) were obtained. Transflectance spectra were transformed and stored as log(1/*TF*) spectra. Plotting log (1/*TF*) as a function of wavelength gives a special curve that is comparable to an absorption curve that has peak readings at wavelengths that correspond to absorption bands of the sample. Figure 2 shows the cuvette used in our experiments.

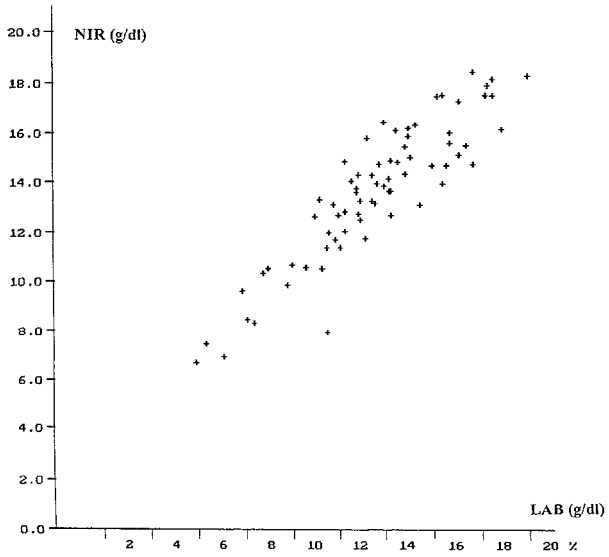

Figure 4. Scatter plot of hemoglobin content of blood determined by NIR spectroscopy versus reference laboratory data of the calibration sample set. The hemoglobin content of the 72 blood samples determined by NIR was calculated using the four term equation given in the second line of Table 1.

Results

The qualitative relationship between spectral data and hemoglobin content of whole blood was determined by multiple linear regression (MLR). The optimal three wavelengths were determined with an iterative procedure while the fourth wavelength was determined with normal stepwise MLR. The form of the multi-term linear equation was:

$$Q_h = k_0 + \sum_{n=1}^{4} k_n \, V'' \, \lambda n$$

Where Q_h stands for the hemoglobin content, k_o is constant (intercept), k_n are coefficients (slope terms), λ_n are characteristic wavelengths, and $V''\lambda_n$ are the values of the second derivative spectra that belong to the characteristic wavelengths, n is the number of terms. The first characteristic wavelength λ_1, is at or near to one of the absorption peaks of the constituent to be determined. λ_{2-4} are used for correction for disturbing effects at λ_1 elicited by changes in the concentration of other constituents or alterations in physical parameters. The correlation plot for selecting the primary characteristic wavelengths of hemoglobin is shown in Figure 3. Table 1 summarizes the parameters used and obtained for determining hemoglobin content. Figure 4 shows the scatter plot for hemoglobin.

Conclusions

For determination of hemoglobin content of human whole blood NIR spectroscopy represents a rapid, accurate, and inexpensive analytical method that uses no chemicals and reagents. By using fundamental laboratory methods for calibration the accuracy of NIR spectroscopy can be improved and will be comparable to that of the most sophisticated analytical techniques.

Acknowledgment

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