Rapid analysis of β-lipoprotein in human blood serum using near infrared spectroscopy

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Introduction

Two hundred and twenty-eight human blood serum samples were analysed with a Spectralyzer 1025 near infrared (NIR) spectrophotometer in the 1000–2500 nm wavelength range in order to determine the relationship between NIR spectral data and the β-lipoprotein (β-LP) content determined by routine turbidimetry.

Methods

Blood was drawn by venipuncture after an overnight fast from apparently healthy volunteers as well as from unselected ambulatory and hospitalized patients. Following collection, serum was separated from the cellular components and divided into two aliquots. Serum concentration of β-LP was determined turbidimetrically using an LKB automatic photometer at 660 nm. For NIR spectroscopy 1 mL of sample was put into the sample holder cell (cuvette) (Figure 1). Diffuse transflectance from 1000 to 2500 nm was measured and recorded with a Spectralyzer 1025 NIR

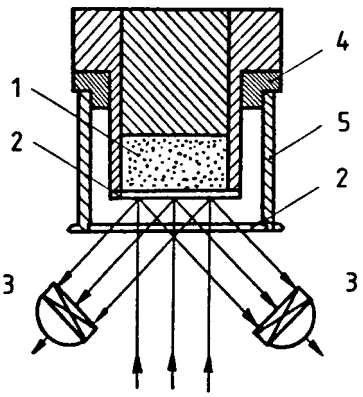

Figure 1. Schematic representation of transflectance sample holder cell. 1, "absolute white" powder (reflector). 2, quartz windows. 3, lead sulphide detectors. 4, distance ring for adjustment of sample layer thickness. 5, sample holder house. Reproduced with permission from *J. Near Infrared Spectrosc.* 2(2), 67 (1994). © NIR Publications 1994.

spectrophotometer. The instrument was operated in single beam mode with a reference spectrum measured on halon ethalon as a reference standard and stored in a computer. Transflectance (*TF*) was transformed to log(1/*TF*). This way log(1/*TF*) gives a nearly linear correlation with the concentration of the measured component. Transflectance data were collected at every 2 nm with 256 digital conversions per point. This resulted in 750 transflectance points (spectral values) for the whole wavelength region. Plotting log(1/*TF*) as a function of wavelength gives a spectral curve that is comparable to an absorption curve having peak readings at wavelengths that correspond to absorption bands in the sample.

Results

The quantitative relationship between spectral data and composition of blood serum was determined by multiple linear regression (MLR) analysis. The optimal three wavelengths (terms) 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:

$$Q = K_0 + \sum_{n=1}^{4} K_n V'' \lambda_n$$

where Q represents the concentration of β-LP, K_0 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 transflectance 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 major absorption peaks of the constituent to be determined. Additional terms at λ_2, λ_3 and λ_4 are used for correction for disturbing effects at λ_1 elicited by changes in composition or physical parameters (e.g. temperature, pH, particle size etc.). Therefore λ_{2-4} represent wavelengths where absorption is due mostly to interfering substances and effects.

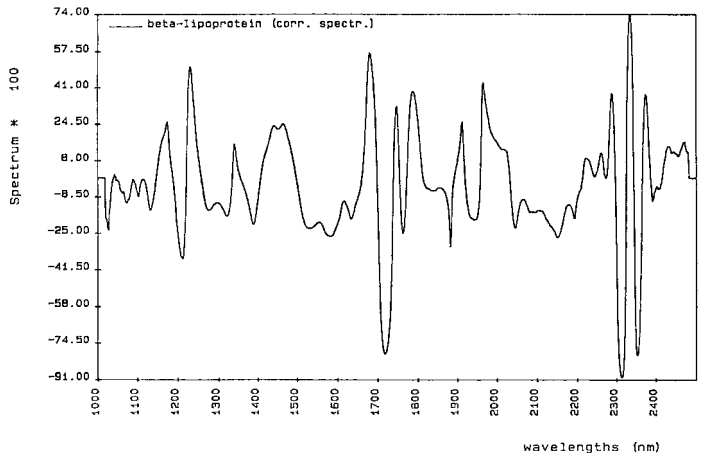

Figure 2. Correlation plot for β-lipoprotein determination in human blood serum derived from a single term equation. (Correlation coefficients at each wavelength plotted against wavelength). Reproduced with permission from *J. Near Infrared Spectrosc.* 2(2), 67 (1994). © NIR Publications 1994.

Table 1. Characteristic wavelengths for β-lipoprotein and performance data obtained with linear summation equations. Reproduced with permission from *J. Near Infrared Spectrosc.* 2(2), 67 (1995). © NIR Publications 1995.

λ_1 (nm)	λ_2 (nm)	λ_3 (nm)	λ_4 (nm)	*SEC* (TU)	*r*	*SEP* (TU)
1720	1438	2216	—	0.871	0.938	—
1720	1438	2216	2316	0.742	0.956	—
2314	2216	1978	—	0.746	0.955	—
2314	2216	1978	2356	0.735	0.957	—
2314	2216	1720	—	0.740	0.956	0.859
2314	2216	1720	1694	0.729	0.957	0.796

The correlation plot for selecting the primary characteristic wavelengths of β-LP is shown in Figure 2.

Table 1 summarizes the parameters used and obtained for determining β-LP content of human sera. The total number of samples in the calibration set was 138. β-LP content ranged from 2.0 to 20.0 TU (turbidity unit). The test set contained 90 samples with similar concentration range.

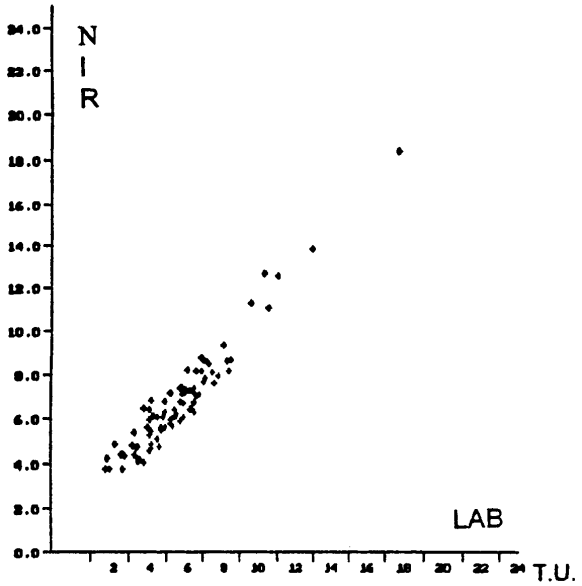

Figure 3. Scatter plots of the NIR spectroscopy-calculated β-lipoprotein (concentration in blood sera vs the laboratory reference concentration for the validation (test) data set. Reproduced with permission from *J. Near Infrared Spectrosc.* 2(2), 67 (1994). © NIR Publications 1994.

Figure 3 shows the scatter plot for β-LP. After omitting eight samples with large error from the set, standard error of calibration *(SEC)* = 0.632 and correlation coefficient *(r)* = 0.959 were obtained.[1]

Conclusions

The NIR measurement technique is a correlative method as it needs calibration. Calibration is based on an independent reference system, therefore, all errors of the reference methods will influence the *SEC* values. As our standard clinical chemistry methods do not represent fundamental reference methods, calibration and prediction results were limited to the accuracy of our routine laboratory evaluation.

This preliminary study indicates that the standard error of calibration and prediction of NIR spectroscopy is comparable to that of clinical chemistry methods (the reference methods). Omitting eight samples with large deviation from the expected value improved our results. This indicates that errors made by routine laboratory evaluation have been primarily responsible for the limited accuracy of NIR spectroscopic analysis. Therefore, for calibration of an analytical NIR spectrophotometer more fundamental reference methods will be needed.

Acknowledgements

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References

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