Determination of Cholesterol by a Diode Laser/Fiber Optic Colorimetric Spectrometer

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A simple and inexpensive colorimetric spectrometer for determining total cholesterol has been developed, comprising a diode laser as the light source, optical fibers for the light guide and a photodiode as the detector. The stability and performance of the new system was investigated by obtaining the calibration curve for standard cholesterol solutions. The total cholesterol in human serum was also measured by the analyzer and compared with the value obtained by a conventional spectrometer. The results showed that the developed spectrometer was useful for the determination of cholesterol levels. The visible diode laser used in the study exhibited good spectroscopic and operational properties for colorimetric absorption spectrometry and could be a key component for the development of a simple and economical analyzer.

Introduction

Hypercholesterolemia is one of many independent risk factors for coronary heart disease. Various detection techniques have been developed for determining cholesterol in human serum, such as fluorescence detection,[1,2] electrophoresis,[3] Raman spectroscopy,[4] etc. However, most of them do not assure on-site monitoring of cholesterol. To prevent coronary heart disease, total cholesterol should be measured on a random basis. Therefore, there is an increasing interest in and demand for a portable, inexpensive but powerful spectrometer providing easy operation for individual use. The construction of such a spectrometer depends upon the availability of a low-cost and low-power source and a simple compact optical system with effective background correction. From the clinical point of view, a colorimetric absorption spectrometer with a laser diode seems to be very attractive in the development of a small analyzer with simple components. Its advantages over conventional spectrometers with an incandescent bulb lamp as the light source are easy operation, portability and simplicity of electronics and optical alignments. The conventional stationary systems are relatively large and heavy, demanding great expenditures in optics, electronics and power supply. Laser colorimetry is based on the measurement of the typical blue color formation developed when cholesterol reacts with reagents.[5]

Diode lasers are used primarily in compact systems. As the fabrication technologies for laser diodes mature, they exhibit excellent spectroscopic and operational properties, and offer a variety of shapes and applications for signal processing. The radiation emitted by laser diodes has narrow line width and high intensity. They also exhibit high efficiency and small degradation over their long service life. During the last 10 years, new analytic instruments using such solid lasers have revolutionized absorption spectroscopy. Laser diodes suitable for spectroscopy are commercially available in the spectral range of 630-1600 nm. This is the main limitation of the application of laser diodes to absorption spectrometry such as for atoms or molecules.[6-10] Raman,[11,12] fluorometry,[13-15] etc.

Several spectrometers using a visible-light emitting diode (LED) similar to a laser diode have been introduced for colorimetric measurements.[16,17] The fundamental problem with LEDs, however, is the divergent light and relatively broad band. Advantages of diode lasers over LEDs are high intensity, monochromaticity, coherence and convergence of the light. A few papers related to diode laser colorimetry are found for the analysis of inorganic species. Soylok *et al.*[18] measured concentrations of aluminum in water samples with a visible diode laser. A diode laser at 635 nm radiation was used in their simple system. F. Son and T. Korenaga[19] determined total phosphorous in water, using a FIA system with a semiconductor laser emitting at 780 nm. They used the diode laser in order to increase the sensitivity of phosphorous detection. Our lab has introduced a colorimetric method for analysis, using a visible diode laser.[20-22] We published a thermal lensing spectroscopy system in 1996.[23] Diode lasers coupled with optical fibers are especially useful when samples are far from the on-site field monitoring of a spectrometer.

In the present paper, we describe the structure of an inexpensive and simplified spectrometer with a diode laser at 648 nm combined with optical fibers and a photodiode detector. We have named this spectrometer Diode Laser-Fiber Optic Colorimetric Spectrometer (DL-FOCS). This spectrometer represents a forward step for the construction of a miniature clinical analyzer for the individual who needs on-sight tests of cholesterol. For performance and clinical applications, the DL-FOCS was calibrated with standard solutions of cholesterol.

Experimental Section

Instrumentation. A schematic of the system is shown in Figure 1. The light source is a laser diode (LDM 145, Ima-

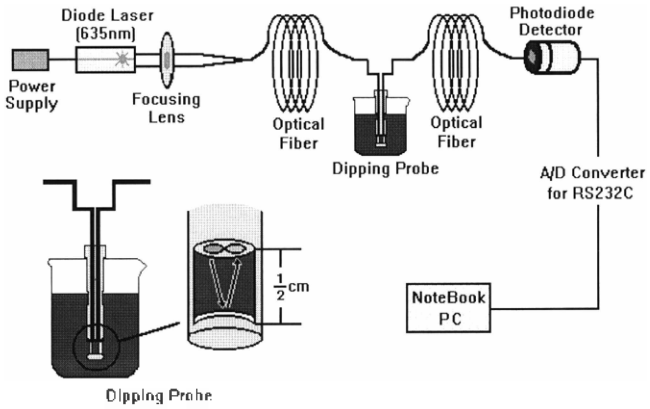

Figure 1. Schematic diagram of the Diode Laser-Fiber Optic Colorimetric System (DL-FOCS).

tronic Limited, UK) with a continuous-wave type InGaAlP semiconductor. The emission character of the diode laser was investigated with a conventional grating and a CCD detector (SD-1000, Ocean Optics, USA). For transmission of the laser, two optical fiber (ϕ 200 μm) sections were used, each of 2-m length. A commercially available dipping probe (T-200, Ocean Optics, USA) was used as the sample cell. The beam focused at a convex lens (F.L.–6 cm) was transmitted into the solution through an optical fiber whose exit was placed at a 6-mm circular bore of the dipping probe. The volume of the dipping probe unit used for the sample solution was 0.14 mL. A reflection mirror was placed at the bottom 5 mm from the bore. The beam reflected on the mirror was transmitted again to a photodiode (Pin-6DP, UDT Sensors Inc., USA) through the other optical fiber, whose entrance was also situated at the bore of the dipping probe. The optical path length in the solution for the system was 10 mm. An A/D converter (ADAM-4520, Adventech, USA) was employed to convert the electrical analog signal from the detector into a digital signal for the RS232C port of a notebook PC. A conventional spectrometer (UV-2101PC, Shimadzu, Japan) was used to verify the results obtained by the DL-FOCS.

Reagents. The blue color formation of cholesterol was achieved by the Lieberman-Burchard method.[24] Standard solutions of cholesterol to obtain a calibration curve for the spectrometer were prepared by the following procedures. Acetic anhydride, acetic acid, ethanol, sulfosalicylic acid and sulfuric acid were purchased from Aldrich Chemical Company (St.Louis, Mo, USA) and used without further purification. A 500 mg/dL stock solution was prepared by dissolving 0.5 g cholesterol in 100 mL ethanol. Cholesterol standard solutions were prepared by diluting the stock solution with ethanol. A sulfosalicylic acid solution was prepared by dissolving 5 g sulfosalicylic acid in 100 mL acetic acid. A color reagent solution of 110 mL was prepared by mixing 35 mL of the sulfosalicylic acid solution, 65 mL of acetic anhydride and 10 mL of sulfuric acid. The standard solutions were colored by adding 5mL of the color reagent solution to 0.1 mL of each standard solution. The blue color formation was completed after 10 min in cold water.

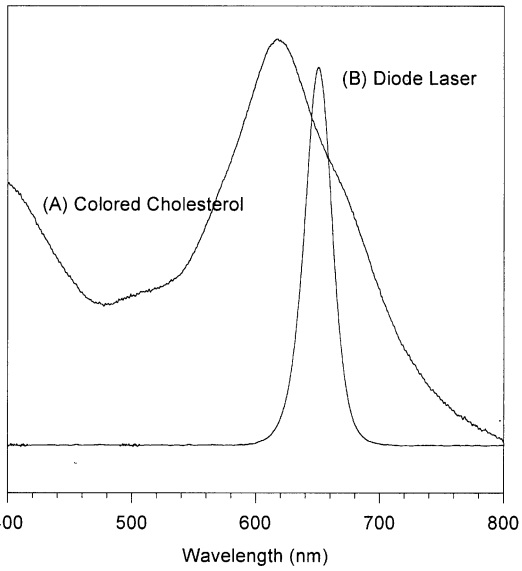

Figure 2. Radiation band of the visible diode laser and absorption spectra of colored cholesterol.

Results and Discussion

Absorption spectra of cholesterol and emission band of the diode laser. Since this experiment is based on detection of the blue complex formed between cholesterol and reagents, the relationship between the absorption of the colored cholesterol and an emission band of the diode laser is projected in Figure 2. The blue complex shows a strong absorption band near 620 nm. The emission wavelength of the diode laser has a Gaussian shape. The spectral range of the diode laser is from 620 nm to 676 nm with a peak at 648 nm. The full width at half maximum (FWHM) is 28 nm. The emission wavelength at the peak of the diode laser is a little longer than the maximum absorption wavelength of the cholesterol. However, the narrow bandwidth of the laser is well included in the broad absorption spectra. Cholesterol concentrations in this experiment were calibrated with the intensity of the full band. The simple structure, without a filter or grating followed by a photomultiplier tube and complex alignments in the optics, comes mainly from the use of the full band centered at 648 nm.

Stability of the DL-FOCS. To investigate the stability of absorption detection, a base absorption line with a blank solution in the dipping probe was examined. The DL-FOCS noise and the conventional system noise for the base absorbance are shown in Figure 3. The average trace of the conventional system is stable and localized near zero. On the other hand, the base line of the DL-FOCS was slightly unstable. The base absorbance of the system is not flat, and the floor fluctuates between $+0.00035$ and -0.00029 A.U. Laser noise arises in a great variety of ways, so an exhaustive list is difficult to give. However, in most measurements, photocurrent fluctuations resulting from laser amplitude noise are the primary nuisance.[25] The fluctuations probably reflect a change of hole-electron pairs due to temperature changes in the diode laser. The base noise influences sensitivity at low concentra-

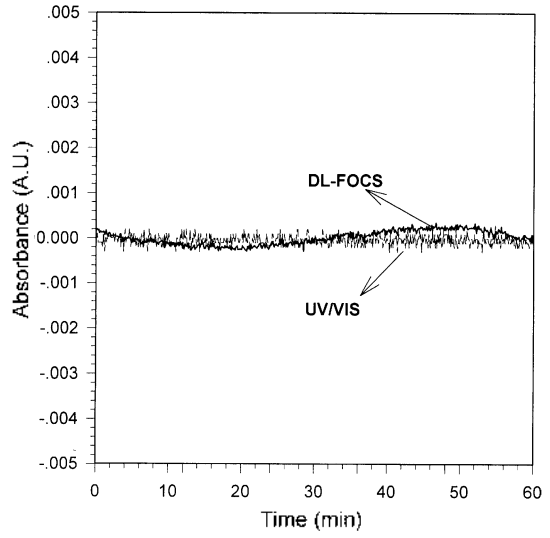

Figure 3. Comparisons of noise and stability between the DL-FOCS and the conventional spectrometer.

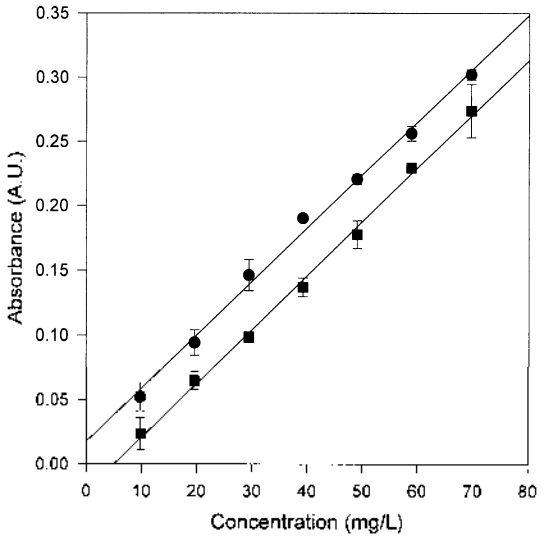

Figure 4. Calibration curves for the standard solutions of cholesterol: DL-FOCS (●), conventional spectrometer (■).

tion detection. However, the variations of the trace for day-to-day operations at room temperature were always within the noise levels of the conventional system. Modulation noise can be reduced by the use of a feedback controller. In a recent advanced experiment currently in progress, the base absorbance has been stabilized by the feedback stabilizing circuit.

Calibration and evaluation of the instrument. In most clinical tests, samples are diluted and adjusted to a proper concentration for application to an analyzer. The DL-FOCS and the conventional spectrometer were, therefore, calibrated with the standard solutions ranging between 10 mg/L and 70 mg/L, which means that the samples ranged from 50 mg/dL to 350 mg/dL. Relative to the real concentration of cholesterol in human serum, they were diluted 50 times. Figure 4 shows the calibration curves of both systems for five consecutive tests at each concentration. Absorbance *vs.* concentration is linear with a correlation coefficient of 0.9948 for the DL-FOCS and 0.9969 for the conventional spectrometer. The tendency of the curve for DL-FOCS is similar to that of the conventional spectrometer except for the higher intercept, which is believe to be residual absorbance from remainder deposited on the bottom of the dipping probe during consecutive tests.

An unknown cholesterol level in human serum from the Soonchunhyang University Hospital was analyzed using both the calibrated analyzer and the classical spectrometer. This sample was diluted in ethanol (dilution of 50 times). The diluted concentration of the sample (n=4) was 40.18 ± 3.13 (7.8% in RSD) for the DL-FOCS and 40.21 ± 2.93 (7.3%) for the conventional spectrometer. The result measured with the DL-FOCS was well agreed with the value obtained with the conventional spectrometer.

Conclusions

This work shows the great possibility of the DL-FOCS for the determination of cholesterol. The system exhibited good spectroscopic and operational properties for the colorimetric absorption spectrometry. The system has a simple structure, comprising a visible laser diode, optic fibers and a photodiode detector. The advantages of this system are that it is compact and inexpensive for the detection of total cholesterol in serum. For the tested on-site analyzer, the P.M. tube, monochromator and complicated optical alignments are not needed.

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