APPLICATION NOTE

UV/Visible Spectroscopy

Determination of Cholesterol Level in Human Serum - Enzymatic Colorimetric Method

Introduction

Clinical chemistry uses chemical processes to measure levels of chemical components in the blood. It is very useful for the early diagnostic of disease and for monitoring organ function. The most common specimens used in clinical chemistry are blood and urine. Table 1 shows the common blood tests and measurable items using UV/Vis spectrophotometers.In this application note, the cholesterol level in human serum was determined by the enzymatic method using the LAMBDA™ 465 UV/Vis Spectrophotometer and UV Lab™ software.

Principle

The cholesterol esters of the sample are hydrolyzed by cholesterol esterase. 4-Cholesten-3-one and H_2O_2 are then formed from the released free cholesterol by cholesterol oxidase. A measurable red quinoneimine derivative, that has an absorbance at 500 nm, is formed from hydrogen peroxide (H_2O_2) and 4-amino-antipyrine in the presence of phenol and peroxidase.

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Cholesterol levels in serum are calculated using Equation 1. (Normal range : 130 - 250 mg/dl)

Equation 1

$$\text{Cholesterol Level (mg/dl)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 300 \cdots (1)$$

A schematic representation of the reaction is shown in Equation 2.

Equation 2

$$\text{Cholesterol ester} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Reagents and Apparatus

1. Cholesterol buffer solution (Cholesterol kit, 100 ml) - phenol 132 mg, NaH_2PO_4 0.78g, NaH_2PO_4 0.71 g
2. Enzyme reagent (Cholesterol kit, 100 ml dilution) - cholesterol oxidase 12 unit, cholesterol esterase 3.5 unit, peroxidase 6700 unit, 4-aminoantipyrine 17.0 mg/dl
3. Cholesterol standard solution (Cholesterol kit) - 300mg/dl
4. Human serum sample
5. D.I water
6. Water bath
7. LAMBDA 465 (UV/Vis Spectrophotometer)
8. UV Lab software
9. Cuvettes (10 mm pathlength)

Procedure

1. Prepare an enzyme solution by dissolving the enzyme reagent to 100 ml in the cholesterol buffer solution.
2. Prepare the mixture as shown in Table 2.
3. Place in a water bath at 37 °C for five minutes.
4. Measure blank solution.
5. Measure standard solution.
6. Measure sample solution. (Perform the measurement quickly, within one hour).

Table 2. Mixture preparation for measurement.

	Blank	Standard	Sample
Enzyme sol.	3	3	3
D.I Water	0.02		
Cholesterol std.		0.02	
Serum Sample			0.02

Instrument Parameters

The LAMBDA 465 instrument parameters are as follows. Figure 1 shows experimental method.

Experiment Setup

Data type: Absorbance
Sampling: Single cell
Mode: Scan no. : 30; Integration no. : 1

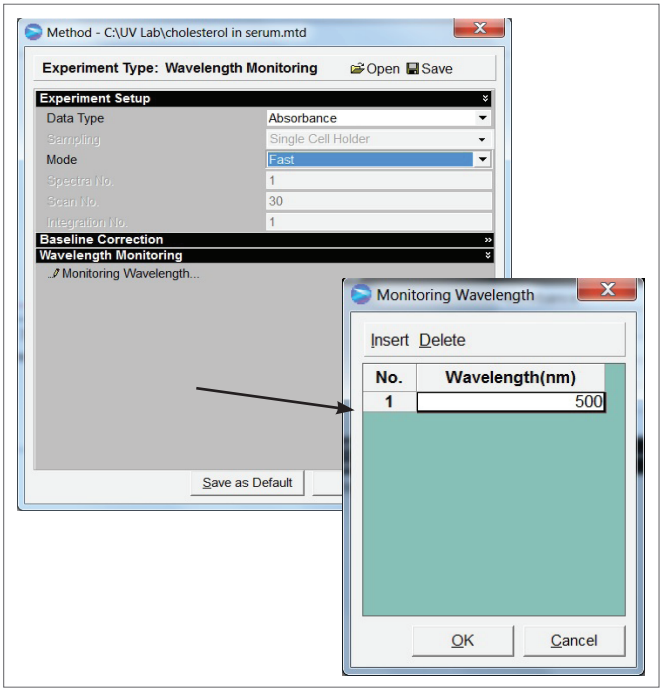

Figure 1. Experimental method

Result

Figure 2 shows the spectra of cholesterol. The absorbance values and cholesterol level of serum sample are shown in Table 3. The determined cholesterol level in serum is 253.84 mg/dl calculated using Equation 1.

Table 1. Common blood tests and measurable parameters using UV/Vis spectrophotometer.

Common Blood Tests	Tests using UV-Vis Spectrophotometer
WBC, RBC, Hb, HCT, MCV, MCH, MCHC, Platelet, GOT, SGPT, ALP, γ-GTP, Total protein, Albumin, Total bilirubin, BUN, Creatinine, Uric acid, Total cholesterol, Triglyceride, LDH, CPK, Amylase, Glucose, VDRL, anti-HIV, HBs Ag, HBs Ab, Fe, P, Ca, Mg	Hb, SGOT, SGPT, ALP, γ-GTP, Total protein, Albumin, Total bilirubin, Creatinine, Uric acid, Total cholesterol, Triglyceride, LDH, Amylase, Glucose, Fe, P, Ca, Mg

Table 3. Cholesterol level in human serum.

Name	AU(500nm)	Concentration(mg/dl)
Standard	0.2775	300
Serum Sample	0.2348	253.84

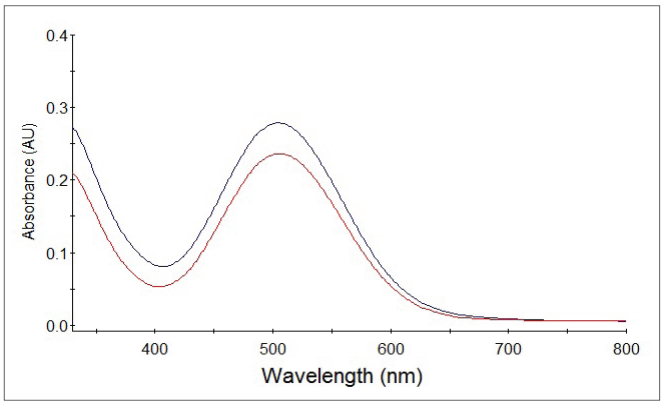

Figure 2. Absorption spectra of cholesterol by enzymatic colorimetric method.

Conclusion

The determination of cholesterol level in human serum by enzymatic colorimetric method was performed using the LAMBDA 465 UV/Vis spectrophotometer and the UV Lab software. After the reaction, the measurement time was minimized using the LAMBDA 465, enabling us to collect data quickly over the full wavelength range from 190 to 1100 nm. Data processing was performed effectively by the powerful and easy to use software. The calculated cholesterol level by Equation 1 was slightly higher than the normal expected range (130 - 250 mg/dl).

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