Quantitative analysis for detection of cholesterol using colorimetric platform

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Abstract

For blood serum analysis in the human body, a miniaturized platform was designed in order to produce a useful, affordable, and simple-to-use healthcare system. The current effort intends to construct a platform that can perform all types of clinical blood serum analysis by applying colorimetric tests and detecting the absorbance of light that passes through the tested sample. The sample's cholesterol content was examined in order to assess and confirm the platform's use. For both known and unidentified samples, cholesterol detection was carried out throughout a linearity range of 1 mg/dL to 1000 mg/dL. The created colorimetric and optical platform has the benefits of being easily accessible, useful, affordable, resilient, requiring a small amount of reagent, and not requiring highly specialized labor. Due to the stability, usage of powerful processing units, and incorporation of cutting-edge electrical mechanisms, the quantitative analysis time was slashed to thirty seconds, enabling quick inspection and great throughput. Thus, the provided framework might be a great complement to the existing resource area as a general platform for every clinical analysis by just changing the reagents and wavelength filter.

Keywords: Cholesterol, Blood; Colorimetric; Quantitative; Optical

Introduction

Our blood consists of a waxy substance called cholesterol. Cholesterol is required to build cells in our body that are healthy, there is a risk of heart disease due to the high level of cholesterol in our body (J. Moronaka, L.R. Faulkner, 1993). At a high level of cholesterol, fatty deposits form in the blood vessels. In the course of time the deposits significantly multiply, which creates difficulty for sufficient amount of blood to pass through the arteries. The deposits often break apart unexpectedly and due to which clot formation takes place which increases the chances of heart attack or stroke (E.W. Raines, R. Ross, 1995). Although high cholesterol might be a hereditary condition, it is frequently brought on by an unhealthy lifestyle that can be changed. A good diet, regular exercise, and occasionally medication can all help lower high cholesterol. Since high cholesterol has no symptoms, the only way to diagnose it is through a blood test.

Because it is bound to proteins in our bodies, cholesterol is transported in the blood (Norum KR, Berg T, 1983). A lipoprotein is made up of both protein and cholesterol. Depending on how lipoprotein is created, there are many forms of cholesterol. High-density lipoprotein (HDL), a "good" type of cholesterol that traps excess cholesterol and transfers it back to the liver, and low-density lipoprotein (LDL), a "bad" type of cholesterol that transports cholesterol particles throughout the body (Xu S, Liu Z, et.al. 2013).

Triglycerides, a kind of fat found in the blood, is another lipid profile component that is traditionally measured. Heart disease risk can also be increased by high triglyceride levels. Laziness, obesity, and a poor diet are factors that can be managed to lower dangerous cholesterol and triglyceride levels. Some outside factors that may also be important include genetic composition, which can make it more difficult for the body to eliminate LDL cholesterol from the...
blood or break it down in the liver (Zhou Y, Briand V, 2009). The cholesterol molecule contains three main parts: 1) a tetracyclic carbon ring (A–D) that functions as a steroid core, 2) a polar hydroxyl group that is attached to the A ring, and 3) a short nonpolar carbon chain attached to the ring, D (Fig. 1) (Song Y, Kenworthy, 2014). All four rings of the sterol group are in the trans conformation, making cholesterol a blueprint molecule. The double bond between C5 and C6 helps to maintain cholesterol rigidity.

Figure 1 shows the structure of cholesterol and the carbon atoms it contains

To measure cholesterol levels, various techniques have been developed. There are three types of cholesterol detection techniques: 1) Conventional chemical techniques that follow the Abell-Kendall protocol; 2) fluorometric and colorimetric enzymatic assays; and 3) analytical instrumental approaches like gas and liquid chromatography or mass spectrometry. Each of these techniques offers unique benefits (Li LH, Dutkiewicz EP, 2019). Traditional chemical processes require multiple steps to complete but are modest in size and cost. The cost of the enzymes used in enzymatic assays is high, but the limit of detection (LOD) is typically low. The most precise and sensitive techniques are chromatographic and mass spectrometric, however the equipment needed is expensive and requires substantial sample pretreatment (Cohen A, Hertz HS, et.al,1989). The modified Abell-Kendall method, which was also given the go-ahead by NIST, is the accepted reference standard for the estimation of total cholesterol for clinical application. The Liebermann-Burchard (L-B) reaction is a multi-step, classical chemical process used to generate colour (Abell LL, Levy BB, et.al., 1957). For measuring cholesterol, ultraviolet (UV) spectrophotometry (λ = 410 nm) is frequently employed. The free cholesterol and cholesterol esters are determined by the fluorometric enzyme test, which is based on an enzyme-coupled reaction. Cholesterol esterase converts esterified cholesterol to cholesterol. Cholesterol oxidase then utilises the resultant cholesterol to produce cholest-4-en-3-one, hydrogen peroxide, and the matching ketone product. Following that, a reliable and sensitive fluorescence probe is used to detect hydrogen peroxide (Allain CC, Poon LS, et al., 1974). The routine measurement of cholesterol uses chromatographic methods including gas and liquid chromatography. To separate cholesterol from other interfering molecules, chromatography is utilised. It can be used to calculate the amounts of total, free, and esterified cholesterol (based on sample pretreatment). Enzymatic and conventional chemical processes are substantially less exact than chromatographic techniques, which also involve expensive resources and highly experienced labor. The substantial sample preparation is required, including derivatization and preliminary analysis (Beggio M, Cruz-Hernandez C, et.al., 2018). For neutral-free sterol molecules, matrix-assisted laser desorption/ionization (MALDI) is not an effective ionisation method. However, there is mounting proof that cholesterol levels can be measured using MALDI-MS. In a work by Hidaka et al., the analysis of cholesterol in human serum lipoproteins was done using MALDI-TOF (time-of-flight) MS (Hidaka H, Hanyu N, et.al., 2007). For the chemical analysis of biological systems, ambient ionisation mass spectrometry (AIMS) was created (Hsu CC, Dorrestein PC, 2015). Real-time, in-situ, and quick mass spectrometric analysis using ambient ionisation techniques only requires a small amount of sample pretreatment. Desorption Electrospray Ionization (DESI) (Takats Z, Wiseman JM, et.al, 2004) and Direct Analysis in Real Time (DART) are two quick AIMS-based methods that are quicker than direct ESI methods for cholesterol ionisation and can be used directly for quantitative cholesterol measurement (Cody RB, Laramee JA, et.al. 2005).

We reviewed the significance of determining cholesterol levels in clinical samples and provided the most cutting-edge technique for cholesterol detection in related domains in this paper.
2 Materials and Methods
2.1 Materials and Chemicals
Icon Enterprises in India provided a cholesterol reagent kit that was available for acquisition. It is based on the Trinder method's fundamental tenets, and contains a phosphate buffer, cholesterol oxidase (CHOD), cholesterol esterase (CE), peroxidase (POD), stabilizers, and an activator. The components used for cholesterol detection are a peristaltic pump, a light source such as a halogen lamp, 510nm wave filter, flow cell, ADS1115 ADC 16-bit, photodiode, detector, raspberry pi 4, and LCD display.

2.2 Cholesterol detection using optical readout method
For colorimetric analysis to detect cholesterol, the reagents and sample must be combined. The resulting colour is red, which indicates the presence of cholesterol in the blood serum and is directly proportional to the concentration of cholesterol. As a result, the intensity of the colour indicates the amount of cholesterol present in the blood. For the purpose of detecting cholesterol in blood, a miniature platform using quantitative analysis has been created. The Beer-Lambert rule, which connects light absorption to the properties of the material it travels through, serves as the foundation for the basic notion of cholesterol detection. The amount of light that is transmitted when monochromatic light passes through a colorimetric solution depends on the concentration of the absorbing component. To ascertain the concentration of the target analyte, the transmitted light is quantified in terms of absorbance. Equation (1) and the ratio of incident light intensity to transmitted light intensity were used to estimate the cholesterol concentration in order to determine the related light absorption. (Baokun Liu, Junming Zhao, et al., 2021). As shown in figures 2 and 3, if the transmitted light is I0, the incident light intensity in the colour solution, then this law states that, if I0 is the incident light intensity in the color solution and it is the transmitted light, then according to this law

\[ \log_e T = -kct, \quad (3) \]

or

\[ \log_e \left( \frac{1}{T} \right) = kct, \quad (4) \]

where c is the concentration of absorbing material, t thickness of the light path, and k absorption constant. The quantity \((-\log T)\) or \(\log(1/T)\) is termed as extinction E/OD or the absorbance:

\[ A = \log \left( \frac{1}{T} \right) = \log \frac{100}{\text{%transmission}}, \quad (5) \]

\[ A = 2 - \log(\%\text{transmission}) \]

Therefore \(A = kct\).

If t is constant, then \(AaC\).

![Fig.2. Schematic diagram for Beer's Lambert law](image)

![Fig.3. Relation between percent of transmission and concentration](image)

2.2.3 Instrumentation Details
The signal processing unit is fundamentally dependent on advancements in electronics and microprocessor technology. The automated analysis for analytical data processing and data collection uses LCD panels and portable PCs. The amount of light that passes through the flow cell and the chosen wavelength filter directly affects the voltage that the photodiode and preamplifier produce as their output. The output of the detector is converted into a digital value via a 16-
2.2.4 Sample preparation for cholesterol detection

Cholesterol reagents were diluted with sample solutions to create cholesterol sample solutions with concentrations ranging from 1 mg/dL to 1000 mg/dL. Samples with varying concentrations were made using a standard cholesterol solution of 100 mg/dL for testing purposes. The standard cholesterol, blank sample, and test sample solution are pipetted into clean, dry test tubes labelled as Blank (B), Standard (S), and Test (T), respectively, as shown in Table I, and mixed. The test tubes are then incubated for 5 minutes at 37°C or for 10 minutes at room temperature, or 25 to 30°C, depending on the experiment.

<table>
<thead>
<tr>
<th>Addition Sequence</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 Cholesterol (Total) Reagent</td>
<td>1000µl</td>
<td>1000µl</td>
<td>1000µl</td>
</tr>
<tr>
<td>Standard (S)</td>
<td>-</td>
<td>10µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td></td>
<td>10µl</td>
</tr>
</tbody>
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3 Results and Discussions

The created platform was evaluated on numerous known and unknown cholesterol sample samples after system integration. As test samples, several cholesterol samples were used to assess the results. For each concentration, corresponding absorbance values were estimated and calculated using the platform that was created. As cholesterol levels rise, the absorption value rises. Following three iterations of the same set of experiments, it was discovered that the absorbance value varied linearly with respect to cholesterol samples with concentrations ranging from 1 mg/dL to 1000 mg/dL. This relationship is depicted in Fig. 5, and it can be used to estimate the concentration of cholesterol in unidentified samples. Limit of detection, or the lowest concentration that can be measured experimentally using the platform that has been designed, which has R2 = 0.99878, n = 3 for known samples and R2 = 0.99872, n = 3.
for random unknown cholesterol samples as shown in the figure 6.

Fig. 5. Plot of Concentration vs Absorbance from range 1mg/dL to 100mg/dL
Fig: 6 - Testing of cholesterol concentration for random unknown samples

**4Conclusion**

For carrying out quantitative analysis and determining the targeted analytes in blood serum, a miniature platform has been created. A platform that includes a monochromatic light source's optical assembly, a fluid dosing device for automatic sample dosing into the flow cell, a photodetector unit for recording transmitted light, a signal conditioning unit for signal manipulation, a Raspberry Pi 4 for processing, and a display unit. Experiments to identify cholesterol in blood serum were conducted to validate the platform's functionality. Due to the built platform's strong processing power, it only took a half a minute to examine the sample. The developed platform has a detection limit of 1 mg/dL and runs linearly throughout a range of 1 mg/dL to 1000 mg/dL ($R^2 = 0.9987$, $n=3$). By adjusting the reagents and according to the desired spectral wavelength, the same platform can be utilised for additional biochemical tests, offering a comprehensive solution for numerous blood biochemical investigations. The determination of cholesterol in blood samples was completed successfully using a colorimetric technique. Detection of cholesterol needs a strong and trustworthy replacement approach for the widely used chromatographic ones, which are expensive and call for highly specialised labour and serve as practical tools. Future research will concentrate on creating a platform for calculating cholesterol levels in dairy and food items and ensuring more consistency in clinical analyses.

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