

# Design of a Low Cost Single Beam UV/VIS Spectrophotometer for DNA Measurement

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**ABSTRACT:** The objective of this research is to design and build a complete simple and low cost spectrophotometer for the DNA measurement. The designed system consists of :two light sources deuterium and halogen lamp ,a monochromator based on Fastie Ebret design ,a photodiode, a chopper amplifier circuit and a data acquisition system. The system is controlled using an Atemga microcontroller .the experimental results show good wavelength accuracy.

**KEYWORDS:** Chopper amplifier, DNA, Photodiode, microcontroller, Spectrophotometer.

## I. INTRODUCTION

DNA (deoxyribonucleic acid) is a polymer found in all living cell, it contains all genetic information needed for controlling cellular growth and development. DNA contains the genetic information necessary for the production of other cell components [23].

DNA has been the subject of many physical and chemical investigations, leading to discovery of genetics code and genome sequencing, this effort lead to identification of point mutations and their correlation to pathologies such as cancer and it is a major target for drug interaction. Thus it is very important to explain the factors that determine affinity and selectivity in binding molecules to DNA [23]. From this point of view the expansions and technological to detect DNA are very important

Spectrophotometers can be used to estimate DNA and RNA concentration and to analyze the purity of the sample. Christian and Wartburg made the first measurement using spectrophotometer for nucleic acid at 260-280nm [20].

Spectrophotometry is more and often the method of choice in the analysis of biomedical substance, assessment of food quality, physical properties of various objects, classification and monitoring of optical telecommunication links [22]

Spectrophotometer is a quantitative method uses the absorbency of light by substance and takes advantage of two laws of the light absorbency [25]. Lambert law proposed that the proportional of light absorbed by a medium is independent of the intensity of incident light [26]and Beer stated that the absorption of light is directly proportional to both the concentration of the absorption medium and thickness of the medium [25] called path length and allow the measuring sample of different path lengths.

$$\text{Absorbency} = \text{concentration} \times \text{pathlength} \quad (1)$$

The major drawback of the absorbency detection in microvolumes is that the optical pathlength through the sample decreases as sample volume decrease and this affects the sensitivity [27]. The spectrophotometer is an instrument used to measure the absorbance of a sample according to beer Lambert law. It contains the light sources, spectrometer, detectors and display unit. The light sources which can be used include deuterium, halogen, xenon, and tungsten lamp, etc..

There are three type of spectrophotometer based on the optical beam: single beam, split beam and double beam [28].The spectrometer act as a wavelength dispersion element, in the spectrophotometer. The heart of the spectrometer is the monochromator, whose function is to separate the light according to their wavelengths.

The monochromator contains collimation and focusing mirrors, in addition to gratings and slits. The monochromator forms an image of the entrance slit in the exit slit plane at the wavelength present in the light source [2]. There are two configurations for the monochromator; Fastie ebret and Czerny –turner configuration [10].

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There are a lot of detectors used in spectrophotometers such as photomultiplier tube, charge - coupled devices and photodiode. The function of the detector is to convert the light (photons) to current or voltage for further processing [9]. The signal received from the detector is the low current signal which requires amplification and signal conditioning so as to reject different types of noise. This requires developing and building of a signal conditioning and amplifier circuits. The paper is organized as follows: in section II the system design is presented. This includes the design of optical and electronic circuits. Section III presents the results obtained from this design. This includes the validation of the system and its application. Finally the conclusion and future scope development were discussed in section IV.

## II. SYSTEM DESIGN

The system design of the spectrophotometer contains the design of the spectrometer and the photometer. The spectrometer includes the light source, sample cell, monochromator and slits. The grating in the monochromator is controlled using a microcontroller. The photometer includes the photodiode, preamplifier and the data acquisition system.

Figure 1 below presents the elements of the spectrophotometer. The light source is deuterium lamp L2D2 from Hamamatsu company, monochromator which contain spherical and concave mirrors, quartz as a sample cell, the detector is silicon S11227 photodiode, amplifier circuit based on chopper stabilized operation amplifier and the computer used as display.

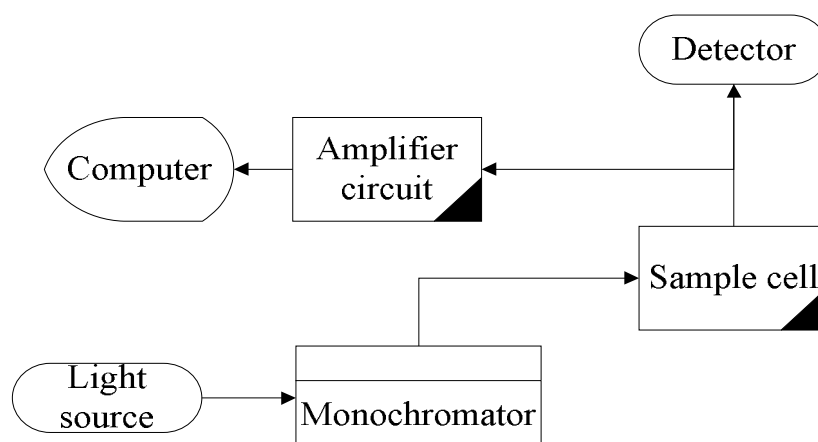


Figure 1. block a diagram of the system setup of spectrophotometer

### A. The spectrometer

The optical system uses an enhanced illumination system to increase optical throughput, sensitivity and to reduce the bandwidth. We have chosen a commercially available light source (UV or VIS), mirrors and holographic grating, as can be seen from Figure 2. The light source is focused on the entrance slits of the monochromator, then to the off-axis ellipsoidal mirror which collects light from the entrance slits and refocuses it to the large spherical concave mirror and to the grating rotated by a stepper. The off-axis ellipsoidal mirror is used to enhance the throughput of the monochromator due to under filling of the grating that translate the light away from the required position. It collects light from the entrance slits and refocused it to the large spherical concave mirror. The large concave mirror is used to let the grating remains fully illuminated and collects all of light [10]. The spherical concave mirror reflects the light to the plane holographic diffraction grating, the light is dispersed by grating into various wavelengths and the grating is rotated by a stepper motor in order to make a scan for all the wavelengths to the exit slit.

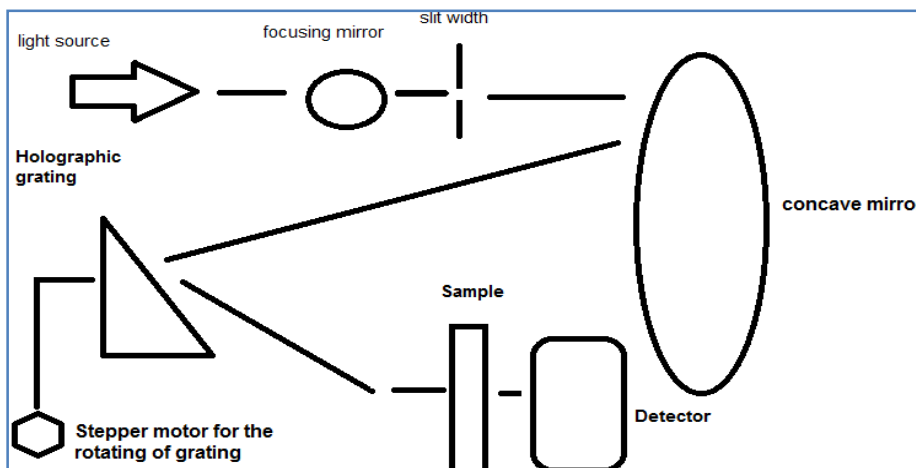


Figure 2. Fastie Ebret monochromator

The cell unit is designed to meet the challenge of the low quantity of the sample for the DNA or RNA; therefore we proposed a new type of the cell it which contains two lenses, one on the side for light coming from monochromator and the other in the opposite side.

The spectral range of the monochromator is determined by the efficiency of the grating and maximum angle of rotation of the grating. Fastie ebret configuration suffers from many problems such as spherical aberration, coma, astigmatism and curved focal field. To solve these problems, mirrors free from aberration are used besides an off-axis mirror. In order to isolate any other wavelengths, the grating is rotated by a stepper motor, the angular position of the holographic grating depends on the stepper motor rotation and when the grating rotated different wavelength are directed through exit slit and to the detector.

Table-1 summarizes the specification of the enhanced optical system which has been based on the Fastie Ebret configuration using holographic grating, the focal length of concave mirror and the angles calculated and measured from the designed setup, according to [10] the F/value in and F/value out of the system are obtained.

Table-1 the specification of the enhanced optical system design

Type	specifications
Type of optical configuration	Fastie ebret monochromator
Grating type	Plane holographic grating
Grating size	300× 300 mm
Grating grooves	2400 groove/mm
Focal length of concave mirror	500mm
Angle between incident beam and diffracted beam	12°
F/value in	0.57
F/value out	1.52
Wavelength detector	SI 1227
Spectral range	190nm-720nm

### B. Grating control

A Stepper motor has been used to rotate the holographic grating in order to scan all the wavelengths coming from the concave mirror. Figure 3.illustrate The control circuit of monochromator contains, stepper motor, an Atmega 16L 8PU microcontroller in addition to an ULN2003A driver, which is used to drive the stepper motor, it is operates on 5 volt DC and it has 6 leads with stepping angles 5.625/64 and 2 phases [2].

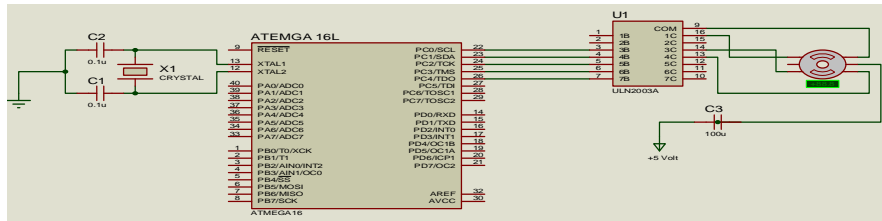


Figure 3. the circuit of the stepper motor and the microcontroller

### C. photodiode and preamplifier

We select the silicon photodiode SI 1227 from Hamamatsu photonic company as a detector for the system, table -2 summarizes photodiode general specifications required for the setup which contains effective photosensitive area which receive light from optical system, the wavelength range of the photodiode cover the range from UV to visible and the shunt resistance is slope of the current to voltage of the photodiode, the ideal photodiode have an infinite shunt resistance.

Table-2 SI1227 Photodiode specifications

Parameter	value
Effective photosensitivity	5.7mm <sup>2</sup>
Wavelength(nm)	190-1000nm
Short circuit current	3µA
Shunt resistance	20 G Ω

The function of the photodiode is to convert optical signal from spectrometer to electrical signal to be conditioned by the amplifier circuit.

In this set up the silicon photodiodes type SI 1227 33BQ has been selected ,the selection is based on the spectral wavelength from(190nm -1000nm) with peak wavelength 720nm and low dark current 5 pA and short-circuit current between 2 to 3µA[3].

The expected current from photodiode when subjected to the light source is increased directly by the power of the light source, this given by equation 2. In this experiment the three light sources have been used, deuterium lamp it is output power 30 watt, halogen lamp 20 watt and LED lamp 0.25 watt.

$$I_{MIN} = \text{power} \times \text{photodiode area(mm}^2) \quad (2)$$

Therefore to detect a small amount of the light, the large area photodiode has been selected [12]. The preamplifier circuit based on the CMOS ICL7650S Super chopper stabilized amplifiers is best choice of low frequency measurements[31] such as in our experiment ,it has been selected for many reasons first the diode shunts resistance is a function of a temperature and this increase the noise in the circuit. Second the input biases current doubles every 10° C rises in the temperature [30]. This has impact on the circuit and the offset voltage output. Therefore the Super chopper stabilized amplifiers is the right solution, a chopper stabilized amplifiers is a 14 pins IC device, having two amplifier, main amplifier and null amplifier in one chip, the input signal from the photodiode connected to the main amplifier thus, it is bandwidth determine the overall signal. The key specifications of the CMOS ICL7650S Super chopper stabilized amplifiers shown Table- 3 represent the input offset voltage ,which is indicator of voltage errors in the output, the low input offset voltage is required in the high performance applications, input bias current caused DC offset and exceeded photodiode current, the high bandwidth increase the noise in the photodiode circuit and the chopping frequency used to modulate the frequency in order to minimizes the noise and to prevent the errors due to aliasing.

Table-3The key specifications of the ICL7650S Super chopper stabilized an amplifier

Parameter	Value
Input offset voltage	5 µV
Input bias current	10pA
Gain Bandwidth	2MHz
Chopping frequency	200 Hz

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Figure 4. Shows CMOS ICL7650S Super chopper stabilized amplifier. In figure 4 U1 is a main amplifier, the null amplifier U2 monitors the input offset voltage of the main amplifier and drive it is output voltage to zero by applying a correcting voltage at main amplifier null pin [31], the correction voltage held by C1 and C2 holds the correcting voltage of a nulling amplifier and suppress the undesired offset related ripple without disturbing the desired input signal from the overall output.

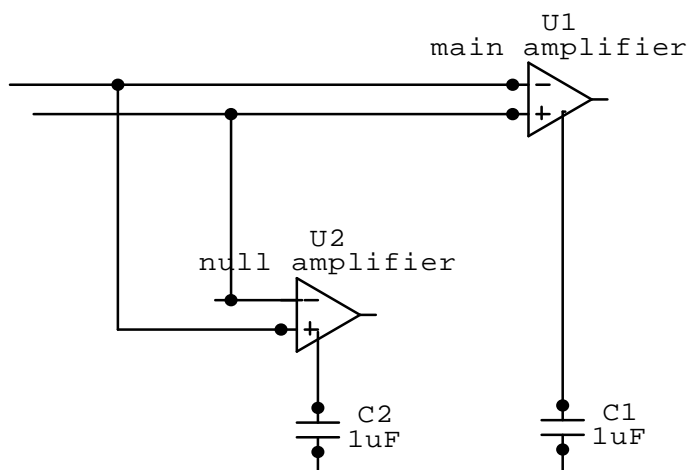


Figure 4. CMOS ICL7650S Super chopper stabilized an amplifier

The basic chopper stabilized amplifier circuit is given in Figure 5. The ICL7650S chopper amplifier is connected to two capacitors .1µF for the nulling connections and the combinations of R1, R3, R4 and R5 serve as an anti aliasing filter to prevent aliasing due to error from chopping frequency, therefore the input frequency must be much less than one half the chopping frequency of the ICL7650S amplifier. The output resistor 10k ohm and C2 is used to minimize  $V_{out}$  drop during auto zero cycle.

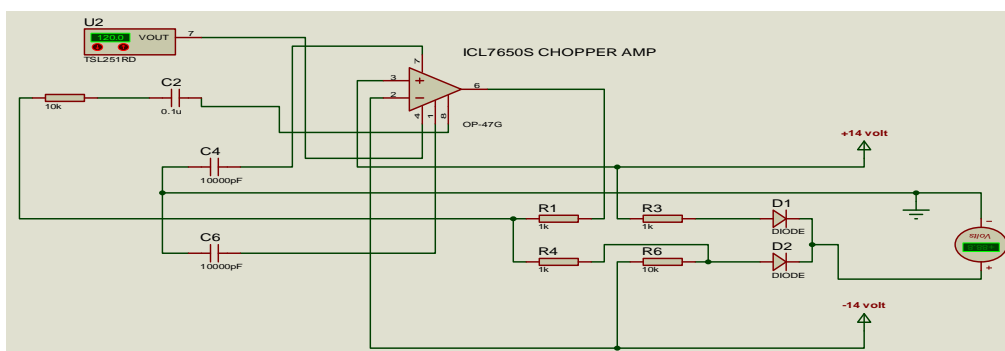


Figure 5. Chopper amplifier circuit

## D. data acquisition and serial interface to computer

To display the data onto the computer we establish interface between computer and amplifier circuit using AVR Atmega16L 8PU microcontroller, MAX232 and RS232 cable. Max232 is a 16 pin IC having dual RS232 transmitter and receivers, it requires external capacitors for its operation as shown in the figure 6. In an asynchronous RS232 communication, data sent and received as frames. Communication between amplifier circuits and computer start by sending the start bit which is logic 0, sent for the duration of the bit time, then the 8 data bit is sent, communication stop by sending the stop bit. Our baud rate is 9600 bits and the frequency of oscillator 8 KHz. The speed of a serial communication depends on the baud rate and the frequency of oscillator.

The serial data is displayed in the personal computer by using a Matlab R2009 software package to plot the data.

Figure 6. Illustrates the interface circuit to the computer, AVR Atmega16L 8PU microcontroller contains software program code for receiving data from amplifier circuit and transmits it to the computer by using MAX232. The

MAX232 serve as CMOS level converter for RS232, the function of RS232 is to convert the output voltage from the microcontroller between 0 to 5 volt to RS232 levels.

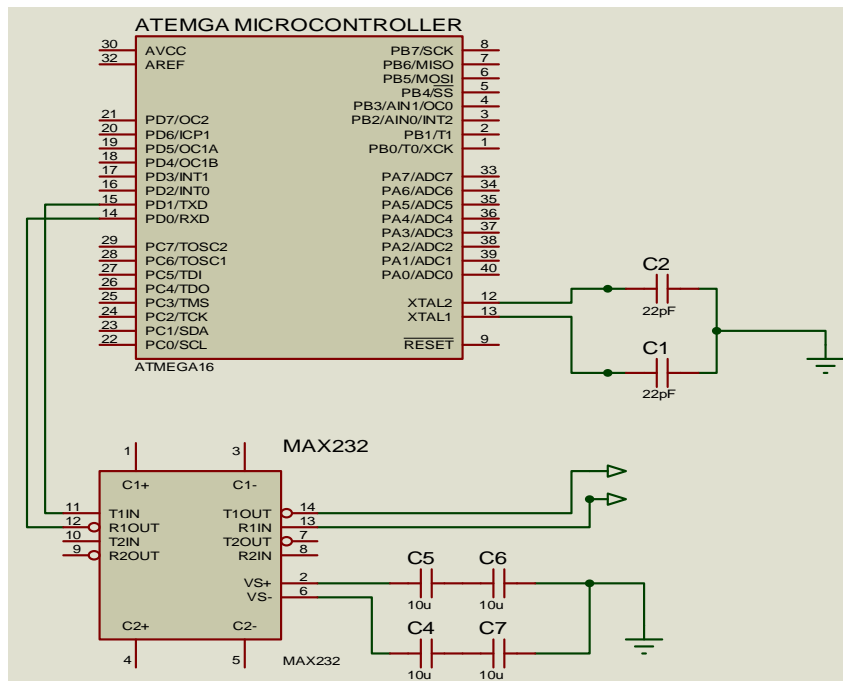


Figure 6. Serial interfaces circuits of a microcontroller and computer

### III. RESULT AND DISCUSSION

#### A. The performance of the system

To test the performance of our spectrophotometer and validate the results from it, the calibration of the wavelength accuracy test is addressed by using holmium perchlorate or holmium oxide filter [29]. The deuterium lamp (L2D2) has been used for the measurement of emission line; it showed a peak at 489nm as in the figure 7; this is the same as the emission of deuterium lamp line of the Jasco530 spectrophotometer instrument. This is a very convent and accurate way to asses that the instrument optics is aligned and working correctly. Figure 7.presents the emission lines spectrum of the Deuterium lamp L2D2 in the wavelength range between 460-510nm using scan speed 100nm/min.

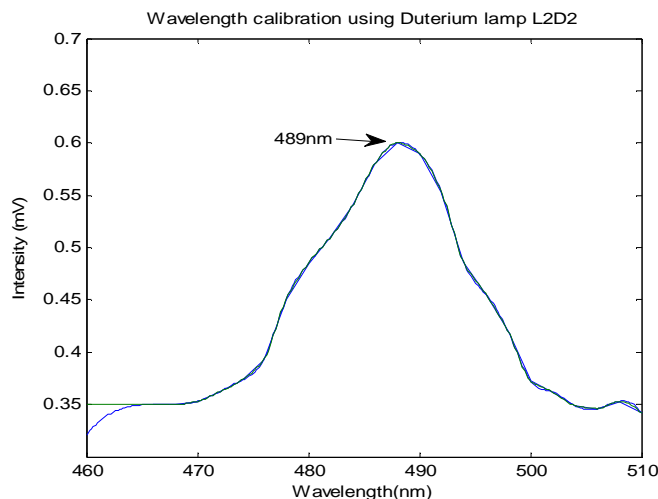


Figure 7. Emission spectrum at 489nm line of the Deuterium lamp

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The calibration testing for the spectrophotometer is performed to check the wavelength accuracy; the material used to calibrate and validate the spectrophotometer is the holmium oxide quartz wavelength standard for Unicam limited company, Cambridge, UK with reference ref/281/D and ref/281/H. The holmium oxide quartz placed in the position of the sample cell. Figure 8. Shows measurement of the absorbance spectrum at 241.3, 279.1, 288.8, 334.0 and 359.9nm of holmium oxide quartz, by using both Deuterium and halogen lamp. This test of the wavelength accuracy measurement are performed by setting the wavelength scanning speed in the 100nm/min and the measurement ranged between 200nm to 400nm.

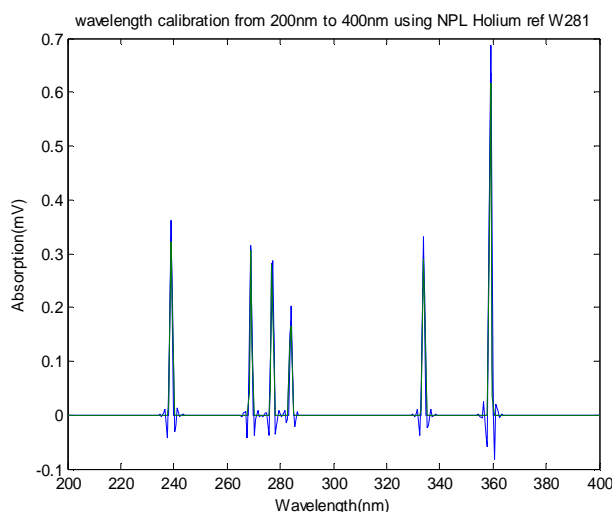


Figure 8. Wavelength calibration in the measurement range of 200 to 400nm.

The other measurement performed as shown in Figure 9. By setting the wavelength scanning speed in the 100nm/min and the measurement ranged between 500 to 700nm. The absorbance peaks in the (mV) showed at the two wavelengths 535.8 and 639.2 nm.

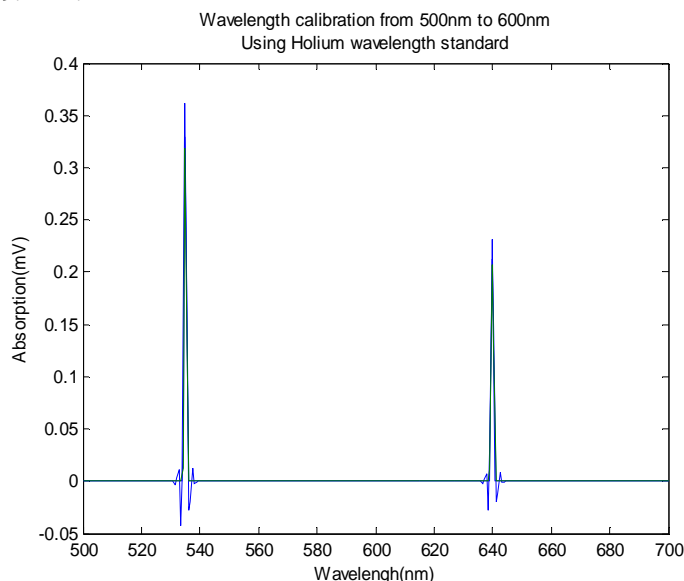


Figure 9. Wavelength calibration in the measurement range of 500nm to 700nm.

## B. Application of the spectrometer for the DNA analysis

Nucleic acid shows a strong absorbance in the region of 240-275 nm, it originates from the  $\pi$  to  $\pi^*$  transition to the pyrimidine and purine, this rising the system of the nucleic bases, e bases can be protonated and therefore the spectra of

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DNA and RNA, DNA absorbs UV light at 260 nm to 280nm due to aromatic base within their structure. The measurements of DNA made using our spectrophotometer setup as shown in Figure 10. Under these conditions, scanning speed 100nm/min, sample blanked on water using quartz cell 1 cm and the DNA sample volume concentration is 100 $\mu$ l. First the blank water has been read, then the DNA sample, the reading of the blank water subtracted from the DNA sample reading in order to obtain the absorbance result of the DNA as shown in figure 10. The peak as presents in Figure 10.Indicates the presented of the DNA in a sample, it contains Purine (Thymin, cytosinee and uracil) and pyrimidines(adenine and guanine),the absorbance peak showed at 275nm.

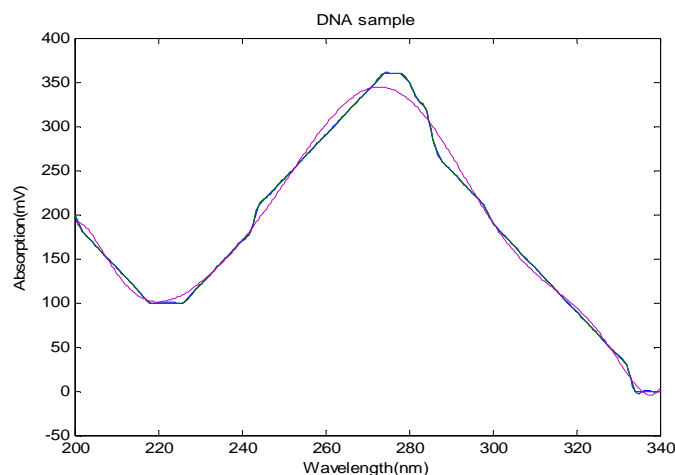


Figure 10. Absorbance profile of DNA samples from 200 to 340nm

## IV. CONCLUSION

An enhanced low cost simple spectrophotometer was designed and built. The spectrometer is based on fastie Ebert configuration and controlled using a microcontroller. The spectrometer optical part was aligned correctly, and this is validated by measuring emission lines of deuterium lamp. The wavelength accuracy testing shows good accuracy by using standard holmium quartz.

A successful prototype of the circuits of the grating control, chopper amplifier and the data acquisition system has been designed from the simple and low cost components.

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