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Study and realization of a system for measuring hemoglobin: hemoglobinometer

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Examination Board jury

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Dedication

This project is dedicated to My parents, who have always provided me with moral and financial support, met all of my needs while I built my system, and taught me that even the most difficult tasks can be completed if they are carried out step by step.

I dedicate this project to my dearest sister, brothers and my beloved ones who has put in a lot of effort to assist us in finishing it.

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LIST OF ACRONYMS

Hb, HGB: Hemoglobin
HCl: hydrochloric acid
K3 [Fe(CN)6]:Potassium ferricyanide
KCN: Potassium cyanide
KH2PO4: Potassium dihydrogen phosphate
HiCN: hemoglobincyanide
ICSH: International Committee for Standardization in Hematology
Fe2+: oxidizing hemoglobin

CoHb: carboxyhemeglobin
O2Hb: oxyhemoglobin
MetHb:methemoglobin
SHb:sulfhemoglobin
Fe3+: methaemoglobin
EDTA: Ethylenediaminetetraacetic acid
VCS: volumetric conduction light scattering
MAPSS: multi-angle polarized light scattering
HHb: deoxyhemoglobin
CBC: Cell Blood Counter
RBC: Red Blood Cells
WBC: White Blood Cells
MCV: Mean corpuscular volume
TCMH: Mean corpuscular hemoglobin content
SLS: Sodium lauryl reagent sulphate
LED: Light emitting diode
LCD: Liquid crystal display
PTFE :Polytetrafluoroethylene
FKM: Fluorine kautschuk material
NBR: Nitrile butadiene rubber
EPDM: Ethylene propylene diene monomer

Abstract

Hemoglobin (Hb or Hgb) is an essential element within the human body since it is a protein molecule in the red blood cells which attribute their color and transport oxygen from lungs to all the tissues and carbon dioxide from tissues to lungs. It is important to measure the rate of Hb since if it is low, the person may suffer from anemia, a pathological situation which may result from multiple causes and reveal many symptoms such as tiredness, paleness, dizziness etc: and have to be treated rapidly.

The Health professionals will be able to diagnose anemia using the hemoglobinometer, a medical device which is able to measure precisely and rapidly the rate of Hemoglobin within blood. This device uses the spectrophotometry method to determine the concentration of Hemoglobin within a sample of blood.

It is with this device as far as this project is concerned. In fact, we are concerned in this project with the study and the realization of a “hemoglobinometer” based on the spectrophotometry method. The proposed device is aimed to be inexpensive; easy to manipulate and reliable in measuring and displaying the rate of Hb.

Key-words: hemoglobin, sahli’s method, drabkin solution, cbc analyzer, co- oximetry, RBC’s, photometry, Syringe, needle, sampling, reflectance

ملخص

الهيموجلوبين (Hb أو Hgb) هو عنصر أساسي داخل جسم الإنسان لأنه جزيء بروتيني في خلايا الدم الحمراء الذي ينسب لونها وينقل الأكسجين من الرئتين إلى جميع الأنسجة وثاني أكسيد الكربون من الأنسجة إلى الرئتين. من المهم قياس معدل الهيموغلوبين لأنه إذا كان منخفضًا فقد يعاني الشخص من فقر الدم ، وهي حالة مرضية قد تنجم عن أسباب متعددة وتكشف عن العديد من الأعراض مثل التعب والشحوب والدوخة وما إلى ذلك: ويجب معالجتها بسرعة .

سيتمكن المتخصصون في مجال الصحة من تشخيص فقر الدم باستخدام مقياس الهيموجلوبين ، وهو جهاز طبي قادر على قياس معدل الهيموجلوبين في الدم بدقة وبسرعة. يستخدم هذا الجهاز طريقة القياس الطيفي لتحديد تركيز الهيموجلوبين في عينة من الدم.

إنه مع هذا الجهاز بقدر ما يتعلق الأمر بهذا المشروع. في الواقع ، نحن مهتمون في هذا المشروع بدراسة وتحقيق "مقياس الهيموغلوبين" بناءً على طريقة القياس الطيفي. يهدف الجهاز المقترح إلى أن يكون غير مكلف ؛ سهل التلاعب وموثوق في قياس وعرض معدل الهيموغلوبين.

Introduction

Anemia is widely prevalent in developing world and continues to be a major public health challenge worldwide, it's affecting over 1.6 billion people globally which is almost one quarter of the world's population and is associated with impaired functional capacity, reduced quality of life, and poorer outcome in diverse disease states [1]. Defined as a limited or insufficient functional red blood cell supply in peripheral blood, anemia causes a reduced oxygen supply to tissues and can have serious health consequences that vary among populations based on multiple factors such as age, sex and altitude level [2].

Hemoglobin "Hb" assessment is a reliable indicator for anemia [3]. It occupies a very special place in the annals of chemistry. Work on the protein began in the early 1800s [4]. The compound was accidentally discovered in 1840 by "Friedrich Ludwig Hünefeld", by pressing the samples of earthworm blood held under two glass slides and allowing them to dry. He occasionally found small plate-like crystals in desiccated swine or human blood samples [5]. Until the 1864 the crystals would have been referred to as haematoglobulin by "Hoppe-Seyler", a name that combines the Greek for blood "haima", and the idea of a globule "it's a little blood blob"[6], while its role as a carrier of oxygen was discovered by the French physiologist Claude Bernard around 1870 [7]. It was one of the first proteins to be studied by X-ray crystallography, and earned Max Perutz the Nobel Prize in Chemistry in 1962 [8].

Hemoglobin (Hb) is an iron-containing metalloprotein present essentially in the blood of vertebrates within their red blood cells, as well as in tissues of some invertebrates, accounting for approximately 90% of the dry weight of the mature cell. It is comprised of heme and globin. It essentially plays the role of transporting oxygen from lungs to body tissues and carbon dioxide from the tissues to the lungs. It is measured in grams per deciliter (g/dL) of blood or grams per liter (g/L) of blood.

Hemoglobin levels vary from individual to one another based on sex, age and physiological situations. As an example; these levels are between 13 and 18 g/dl for men, between 12 and 16 g/dl for women, between 11 and 14 g/dl for pregnant women beginning of the2nd quarter; between 11 and 16 g/dl for babies from 6 to 59 months; between 11.5 and 16 g/dl for children from 5 to 11 years and between 12 and 16 g/dl for children from 12 to 14 years [9].

Today Hb measurement is the most frequently requested blood test and is performed not only in the hospital laboratory but in a variety of healthcare settings, by a range of healthcare personnel (HCP), using technology of diverse sophistication. These rely on many different techniques, most of them based on measuring the color of hemoglobin or a derivative of hemoglobin, including reagent based and "reagent-less" methods, or a variety of non-invasive methods.

In this context, this study was conducted in order to develop and realize a device called " hemoglobinometer" which allows to assess accurately and immediately the concentration of the hemoglobin. The hemoglobinometer is an automated system used to provide an easy and convenient measurement of the concentration of the circulating hemoglobin. It is based on the principle of spectrophotometry, also using less reagents and requiring only a small sample of blood taken from a vein in the arm, using a small needle.

This work is divided into three chapters:

- The first chapter: we will cite and describe the different methods of hemoglobin level measurement, its advantages and its disadvantages.
- The second chapter: will be devoted to the theoretical study of the different stages that make up the system.
- The third chapter: will be devoted to the practical realization of the system and discussion of some results obtained from testing the realized system.

Chapter I

Hemoglobin level measurement methods

ChapterI:

I.1 Introduction

Methods to measure hemoglobin (Hb) were developed more than a century ago, making hemoglobin one of the first diagnostic blood tests available to physicians in the early 20th century, when laboratory medicine was still in its infancy.

A hemoglobin test may rely on many different techniques, the majority based on measuring the color of hemoglobin or a derivative of hemoglobin, including reagent based and “reagent-less” methods, or a variety of non-invasive methods.

In this chapter we will describe several techniques used in the experimental field as well as in hospital and school laboratories where routine hemoglobin readings are so frequent. For each technique its basic principle is described along with its advantages; limitations and factors to be considered before its use.

I.2 Sahli’s method

Sahli's method is a rapid process used to test the amount of hemoglobin in blood. It was inventedin 1875by the British neurologist William Gowers (1845-1915). However, the first Hemoglobinometer using this method was designed by Hermann Sahli (1856-1933) and developed and commercialized by Hawksley and Sons Limited in London [10] in 1890-1910.

I.2.1 The principle of Sahli’s method

Sahli's method, also known as the acid heme method, is based on comparing the color of the blood sample being measured. The principle is to add blood to N/10 hydrochloric acid (HCl). Hemoglobin (Hb) present in red blood cells is converted to acid heme, a dark brown compound. The color of the acidic hemoglobin complex formed corresponds to the concentration of hemoglobin in the blood and meets the standard. This is a brown reference glass that is diluted with N/10 hydrochloric acid or distilled water in a Sahli apparatus until the color of the acidic heme complex matches that of the standard.

Based on the level reached by the final solution, we can estimate the hemoglobin value by reading directly from the scale on the Sahli’s tube. This method must be read under natural light, and it only takes 2-3 minutes. Hbis read to one decimal place in grams per deciliter. In order to be able to perform concentration measurements, a lot of equipment is required. They are: Comparator, Hemoglobin Tube, Hemoglobin Pipette and Stirrer(figure I.1).

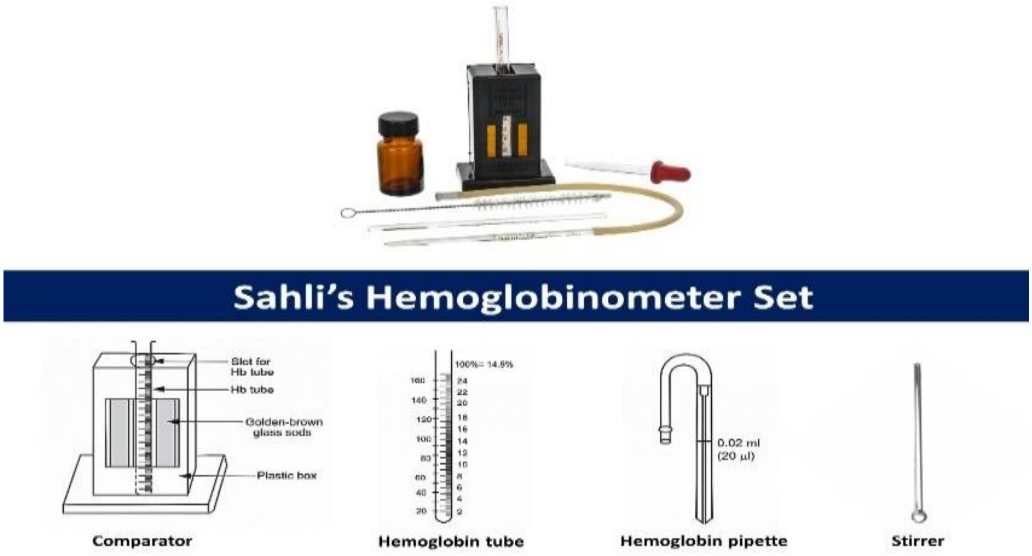

Figure I.1: Sahli’shemoglobinometer[11]

I.2.2 Required Equipment

Comparator: It is a rectangular, plastic container with a slit in the center for a hemoglobin tube. For color matching, there are brown standard glasses on either side of the slot. To produce even lighting, the back is made of white opaque glass.

Hemoglobin tube: Sahli's graduated hemoglobin tube is graduated on one side from 2 to 24 grams per percentage (g%) and on the other side from 20 to 140 percentage (%).

Hemoglobin pipette: also known as Sahli'spipette, has just one mark at 20 l, or 0.02 ml. It doesn't have a bulb like WBC and RBC diluting pipettes do.

Stirrer: The mixture inside the hemoglobin tube is stirred with the use of a thin glass rod called a stirrer.

.I.2.3 Required Reagents

- N/10 hydrochloric acid
- Distilled water

I.2.4 Advantages of Sahli’s method

The sahli’s technique has some advantages

- No time is needed for color development
- a simple bedside approach
- inexpensive reagents and equipment
- affordable and simple to carry outQuick and affordable
- doesn't require technical knowledge [12]

I.2.5 Disadvantages of Sahli's method

This technique has many disadvantages due to the three factors mentioned below:

Time factor

- because: Acid hematin's color fades quickly with passage of time
- the color of the standard comparator also does
- the acid hematin solution is unstable.

Color matching:

• Individual differences in matching the color

Technical errors include

- improper blood mixing,
- capillary blood in tissue fluid
- incorrect pipette calibration
- fetal hemoglobin that cannot be converted to acid hematin.
- Non-hemoglobin components like protein and lipids in the plasma also affect the color of the blood that is diluted with acid.

This method is a visual method that can't measure all hemoglobins. It estimates only oxyhemoglobin and reduced hemoglobins. But carboxyhemoglobin, methemoglobin and sulfhemoglobin (which all constitute about 2-12% of total hemoglobin) are not converted to acid hematin [11, 12].

I.3Drabkin colorimetric method (the HiCN method)

Nearly 40 years after drabkin colorimetric methodwas first adopted as the reference method for measuring hemoglobin by the International Committee for Standardization in Hematology (ICSH) [13], the hemoglobincyanide (HiCN) test remains the recommended method of the ICSH [14] against which all new concentration of hemoglobin methods are judged and standardized.

The detailed consideration that follows reflects its continued significance both as a reference and routine laboratory method.

I.3.1 The principle of Drabkin colorimetric method

With Drabkin's solution, which hemolyzes red blood cells and releases hemoglobin, a single whole blood sample is divided into 200 dilutions. The potassium ferricyanide K3 [Fe(CN)6] component of the drabkin solution has a pH of 7.0–7.4 and it is composed of:

- Potassium ferricyanide K3 [Fe(CN)6]
- Potassium cyanide (KCN)
- Potassium dihydrogen phosphate (KH2PO4)
- Nonionic detergent
- Distilled water

The final product should be transparent and light yellow in hue.

The method relies on oxidizing hemoglobin Fe2+ and its derivatives to methaemoglobin Fe3+ in the presence of potassium ferricyanide "K3 [Fe(CN)6]" (figure I.2). Methaemoglobin and potassium cyanide (KCN) combine to generate cyanmethemoglobin, an extremely stable molecule with a maximum absorption wavelength of 540 nm. At 540 nm, the color intensity is proportional to the hemoglobin concentration[15].

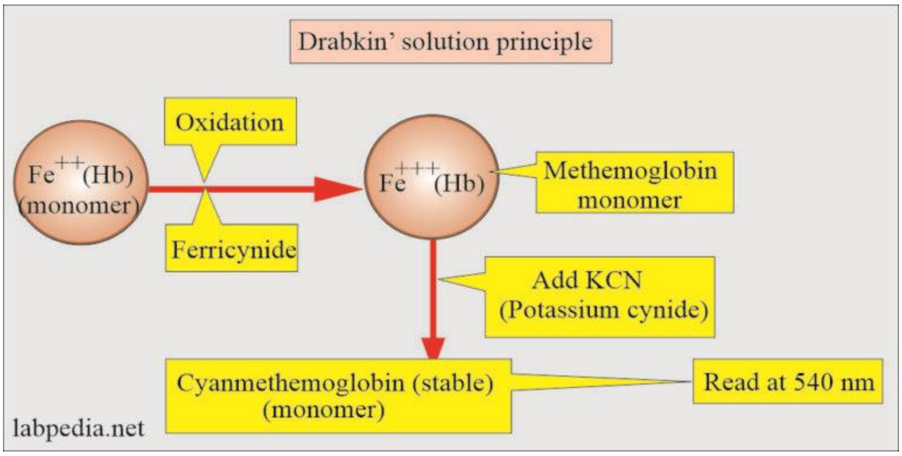

Figure I.2: Drabkin's solution principle **[15]**

After dilution of the sample with Drabkin's reagent, the absorbance of the solution is measured with a spectrophotometer.

The reading of the absorbance of the solution is affected, based on a standard curve of calibration ofabsorbance values versus the concentrations (g/L) (figure I.3). [16]

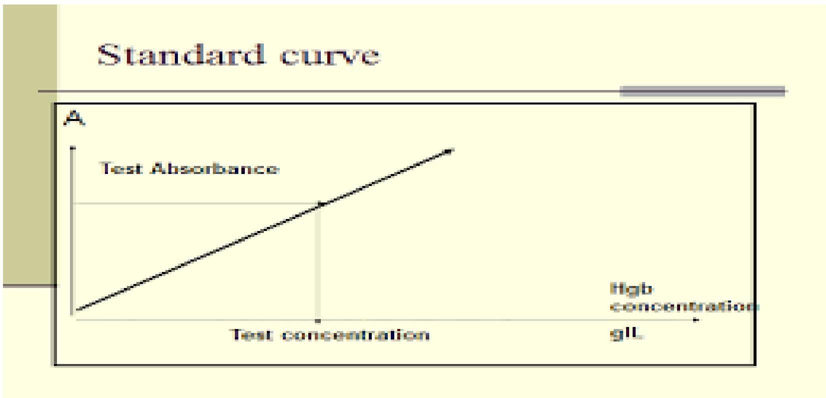

Figure I.3: Standard Curve of hemoglobin measurement [16]

The Hb concentration of the sample is determined by using the formula given below:

Concentration of unknown (g/l) = (Absorbance of unknown * concentration of standard)/ absorbance of a standard.

I.3.2 Required Equipment

- Hb pipette
- Spectrophotometer

- Reagents

I.3.3 Advantages of HiCN technique

The major advantages of the hemoglobincyanide (HiCN) techniques are:

- ❖ The availability of a reliable, globally recognized reference standard/calibrator.
- ❖ ICSH recommends this approach since it is:

• Easily adaptable to automated hematology analyzers
• Well established and fully explored
• Does not require color matching, as in Sahli's method
• Uses inexpensive reagent [17]

I.3.4 Disadvantages of HiCN technique

The HiCN method has been the golden technique for Hb assessment, but it also has some disadvantages that include:

- Manual method requires accurate pipetting and spectrophotometer
- Reagent (cyanide) hazardous substance and that is why Drabkin's solution must never be pipetted by mouth.
- The above limit its use outside the laboratory
- Abnormal plasma proteins cause turbidity when blood is diluted with Drabkin's solution.
- Turbidity results from abnormal plasma proteins when blood is diluted with Drabkin's solution.
- Does not distinguish those hemoglobin derivatives which have no oxygen-carrying capacity (MetHb, COHb, SHb). Thus may overestimate the oxygen-carrying capacity of blood if these are present in abnormal (more than trace) amounts. [17]

I.4 Portable hemoglobinometers

One of the numerous methods have been used and developed to measure hemoglobin concentration in human blood is portable hemoglobinometers that allow accurate determination of hemoglobin at the bedside. They are essentially photometers which allow measurement of color intensity of solutions.

I.4.1 The test principle of portable hemoglobinometers

The test principle of portable hemoblobinometersis based on the principle of reflectance photometry, requiring a small sample of capillary, venous or arterial blood introduced into a disposable microcuvette. This acts as a reaction vessel. The reagents necessary for both the release of Hb from erythrocytes and conversion of Hb to a stable colored product are present in dried form on the walls of the microcuvette.

In reflectance photometry (see figure 1.4), diffused light illuminates a reaction mixture in a carrier, and the reflected light is measured (signal detector). Alternatively, the carrier is illuminated, and the reaction mixture generates a diffuse reflected light (at around 535 nm) that is measured. Since the intensity of reflected light is nonlinear in response to the concentration of the analyte, the intensity of light reflected from the reagent carrier is contrasted with the intensity of light reflected from a reference surface. [18].

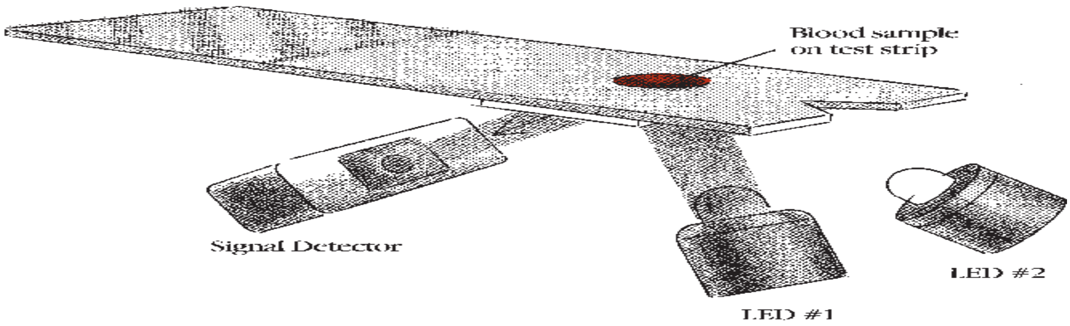

Figure I.4: the principle of reflectance photometry [18]

The instrumentis calibrated at the factory using the HiCN standard, and the test solution's absorbance is immediately translated into Hb concentration. In less than a minute, the result is shown.

I.4.2. Advantages of portable hemoglobinometers

Modern hemoglobinometers have the following benefits:

- Portability
- Battery or mains driven
- Small sample volume (10 L) collected by finger prick
- Fast (result in 60 seconds)
- No pipetting
- Ease of use
- Little training needed for non-laboratory workers
- Standardization against HiCN for findings that are comparable to those produced in a lab, and turbidity correction.

The vast majority of investigations have proven that this technology offers acceptable accuracy and precision when compared to laboratory procedures after rigorous evaluation in a variety of contexts. [19, 20].

I.4.3 Disadvantages of portable hemoglobinometers

Some studies, however, have raised concern that in the hands of non-laboratory staff results may be less satisfactory. Despite the simplicity of operation these instruments are not immune from operator error, and effective training is essential [21, 22].

There is evidence to suggest that results derived from capillary (finger prick) samples are less precise than those derived from well-mixed capillary or venous samples collected into Ethylenediaminetetraacetic acid(EDTA) bottles [23].

I.5 Co-oximetry

Co-oximetry provides the means for automated spectrophotometric measurement of the concentration of total hemoglobin in blood and the percentages of its four hemoglobin derivatives: oxyhemoglobin (O_2Hb); deoxyhemoglobin (HHb); carboxyhemoglobin (COHb); and methemoglobin (MetHb).

Many modern blood gas analyzers have an incorporated Co-oximeter; alternatively stand-alone Co-oximeters are also available.

I.5.1 The test principle of Co-oximetry

Spectrophotometric analysis of blood is the basis of Co-oximetry..Spectrophotometric analysis allows quantitative measurement of a substance in a solution by measuring the difference in intensity of incident and transmitted light when the solution is placed in the path of a light source of defined wavelength; the difference is called the absorbed light or absorbance. The principle is based on Beer-Lambert's law which dictates that absorbance of a single compound is proportional to the concentration of that compound. Spectrophotometric analysis of blood is the basis of Co-oximetry.

If the spectral characteristic of each absorbing substance in a solution is known, absorbance readings of the solution at multiple wavelengths can be used to calculate the concentration of each absorbing substance [24].

Application of this technique in measurement of hemoglobin derivatives (O_2Hb, HHb, COHb, MetHb and SHb) by Co-oximetry is based on the fact that each of the hemoglobin derivatives has a unique absorbance spectrum as shown in figure (I.5).

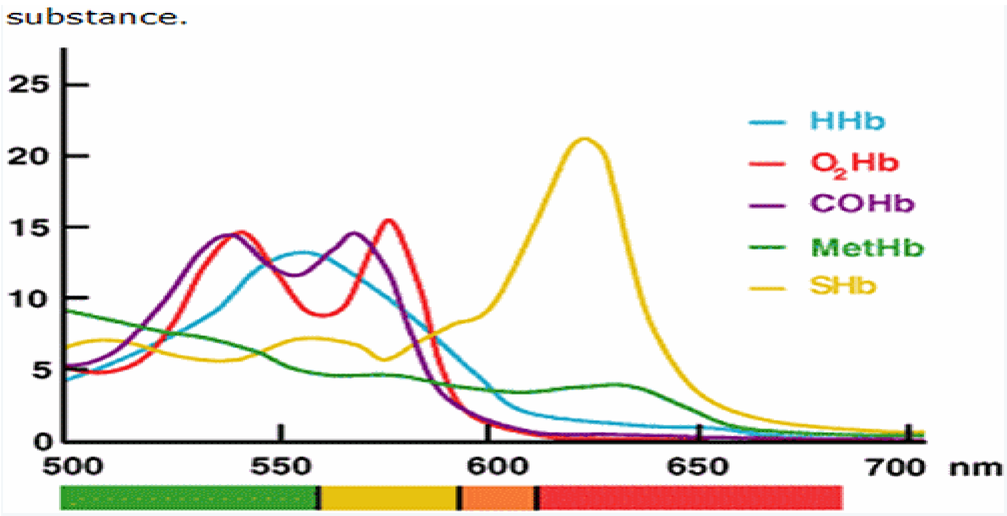

Figure I.5: Absorbance spectrum of hemoglobin derivatives [24]

I.5.2 Advantages of co-oximetry:

The following features of co-oximetry are particularly advantageous:

- Speed of analysis
- Ease of analysis
- Small sample volume
- No capital or consumable cost beyond that required for blood gas analysis
- Additional parameters (MetHb, COHb, O2Hb) measured
- Not influenced by high white-cell count [25]

I.6Automated Hb measurement method

Automated blood cell analysis has developed since the Automated Hematology Analyzer was first introduced in the 1950s, going from a single electrical impedance technique to a fusion of several techniques. These include information processing techniques, physics, chemistry, immunology, flow cytometry, volumetric conduction light scattering (VCS), and multi-angle polarized light scattering (MAPSS). The study of diverse blood cells has produced more precise and trustworthy results thanks to this blood technology.

I.6.1 The Automated Hb test principle

An automated hematology or Cell Blood Counter (CBC) analyzer measures several hematological parameters including white blood cell counts (WBCs), erythrocyte indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin content (TCMH), platelet rate, it is also as reliable in terms of both accuracy and precision for hemoglobin measurement.

The CBC analyzer uses various lysing agents to lyse the red blood cells in the blood sample. One of the most used method is the one using the sodium lauryl reagent sulphate (SLS). The chemical reaction begins by altering the globin and then oxidizing the haeme group. Now the SLS' hydrophilic groups can bind to the haeme group and form a stable, colored complex (SLS-Hb), which is analyzed using a photometric method [26]. In this case, as shown in figure I.6, an LED emits monochromatic light, and as the mixed light passes through, it is absorbed by the SLS-Hb complexes.

The absorbance is measured by a photodetector. This is proportional to the Hb concentration of the sample. The Hb concentration is a logarithmic function of this frequency measuredby the microcontroller on the MAIN board. The frequency output signal of the photodetector is counted by FPGA counters.. The counter counts up while the LED is on and counts down while the LED is off. The LED and direction of counting are switched with a 100 Hz signal. This method provides "real time backlight correction", which makes the Hb measurement more precise in changing backlight environment situation as well.

There are two kinds of Hb measurements: Diluent measurement and a sample measurement. [27]

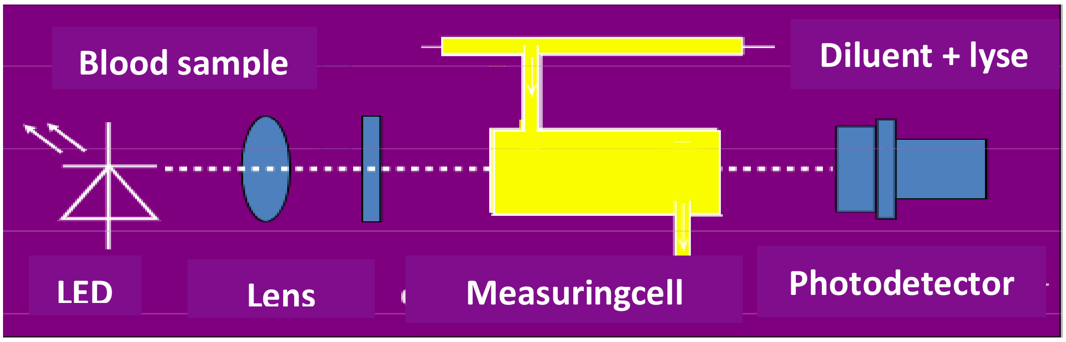

Figure I.6: principle of automated Hb measurement [28]

I.6.2 Advantages of Automated Hematology Analyzer

- Quickness when handling a lot of samples effectively.
- The precision and accuracy of quantitative blood testing.
- The capacity to run numerous tests on a single platform.
- A significant decrease in the amount of labor needed.
- Essential for accurately calculating red cell indices

I.6.3 Disadvantages of Automated Hematology Analyzer

- **Flags**: The manual evaluation of a blood smear is labor-intensive and required when flagging a laboratory test result.
- Red cell morphological comments cannot be produced. Red cells with abnormal forms (like broken cells) cannot be identified.
- Results that have been artificially boosted or lowered as a result of interference.
- Expensive and having significant operating costs.
- regular calibration to guarantee accurate and exact results [29]

I.7 Conclusion:

Through this chapter, description of the different methods available for estimating hemoglobin has been made, with their own advantages and disadvantages. Thus, through the description of the basic principle of each technique, and to solve the problem related to the hematology labs,we are focusing onthe automated hematology analyzer method as basic principle for hemoglobin estimation to realize our hemoglobinometer.

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[25]; Scharnhorst V, van der Laar PJ, Vader HL. Hemoglobin in samples with leukocytososcan be measured on the ABL 700 Series blood gas analyzers. ClinChem 2003; 49: 2107-08.

[26]: https://www.sysmex-europe.com/academy/knowledge-centre/technologies/sls-detection-method.html
[27]: Hematology Analyzer Service Manual https://www.manualslib.com/manual/1566704/Diatron-Abacus-Plus.html#manual

[28]: https://docplayer.fr/3726184-Objectifs-pedagogiques.html

[29]: https://dripmotion.com/advantages-and-disadvantages-of-autoanalyzer/

Chapter II

Theoretical study of the proposed system

II.1 Introduction

After studying the different basic principle of each hemoglobin level measurement method, we will, in this chapter, illustrate and analyze the different stages that make up our device. The proposed device allows the measurement of the hemoglobin concentration using the photometry method.

As is illustrated in the figure II.1 (bloc diagram of the device) below the proposed device is made up of four main parts:

The mechanical part: which consists on a stepper motor responsible for vertical and horizontal movement of the needle and limit switches responsible for stopping stepper motor movement.

The pneumatic part: which consists respectively on a three-way solenoid valves needed to fill the counting chamber with diluent, lyse and blood, a two-way solenoid valve and a pump to drain the counting chamber after measuring.

The Analog part: which is concerned with the photometry environment allowing the measurement of the hemoglobin concentration. This consists mainly on a light source and a sensor which allows the conversion of the transmitted light through the blood sample into frequency which in turn is converted into an electrical signal using a frequency voltage converter. The variation of this electrical signal is proportional to the hemoglobin concentration in the blood sample.

The interface and processing part: which mainly consists on a microcontroller (ArduinoMega 2560 Board) responsible for digitizing the electrical signal obtained in the Analog part, the calculation and the execution of commands to control the stepper motor, the limit switches, the pump, the solenoid valves, the led, the buttons, the LCD displayer...

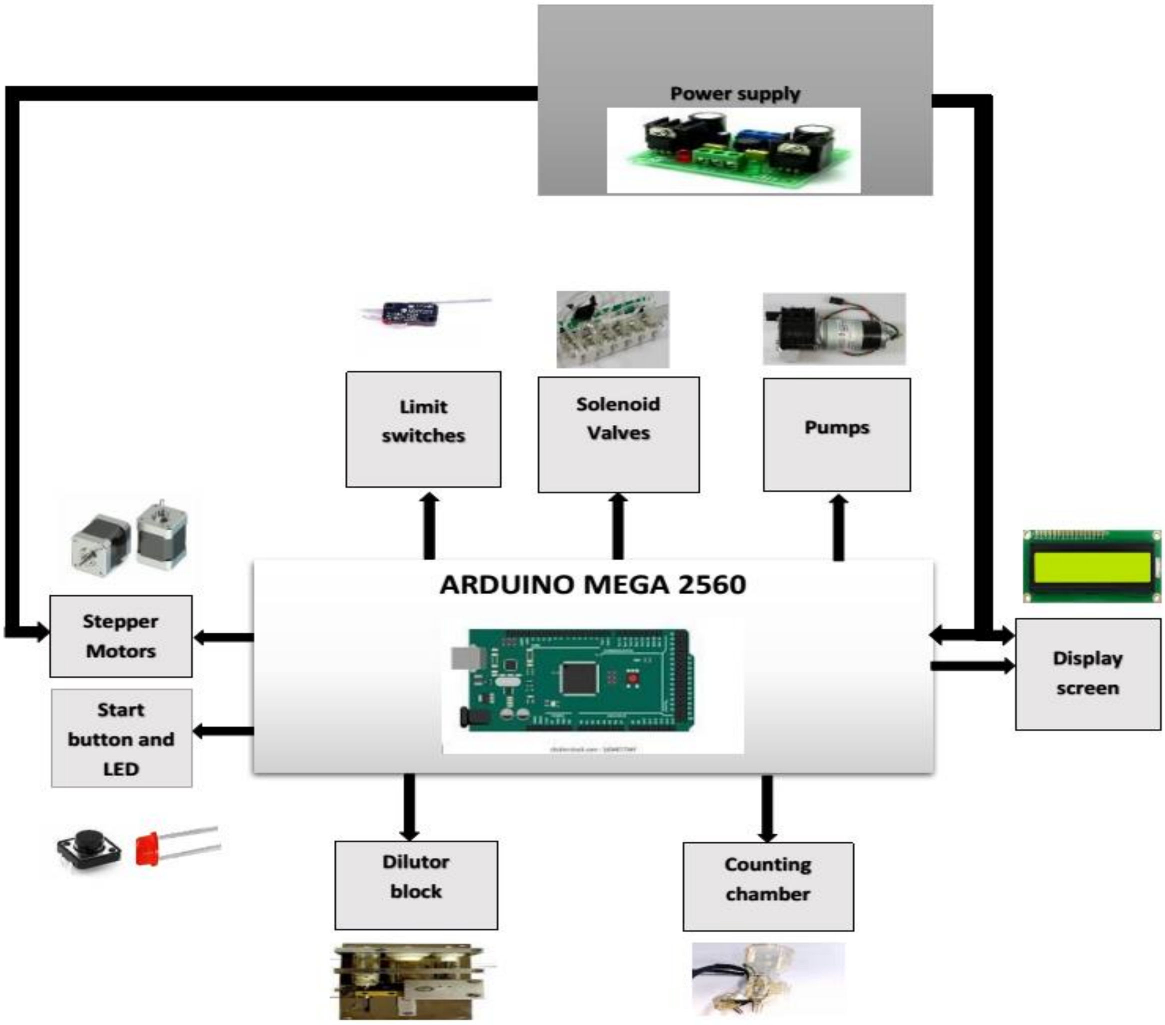


Figure II.1: The system bloc diagram

II.2 The mechanical part

As stated above the mechanical part consists on a stepper motor responsible for vertical and horizontal movement of the needle and limit switches responsible for stopping stepper motor movement. These devices are required to ensure the adequate displacement of the blood filling needle (syringe) or in other words its positioning. One should know that the stepper motor will be controlled by the Arduino Board as it will be discussed in the paragraph below.

II.2.1 Stepper motor

An electromechanical device called a stepper motor transforms electrical power into mechanical power. Additionally, it is a synchronous, brushless electric motor that has a large number of steps per entire rotation. As long as the motor is properly sized for the application, the movement of each step is accurate and repeatable, allowing the motor's position to be precisely regulated without the use of a feedback device.

With this kind of control, optical encoders and other pricy sensor and feedback components are not required. Simply keeping track of the input step pulses allows one to determine the position. It is one of the positioning systems with the widest range of applications. They are simpler and more durable than closed loop servo systems, and are often digitally operated as a component of an open loop system. [30].

II.2.1.1 Stepper motor construction

Stepper motor is made up of the following parts:

Stator: The motor's stationary component. The stator is made up of a group of electromagnets in a stepper motor.

Rotor: The motor's non-stationary component. A stepper motor's rotor is a permanent magnet.

The diagram below depicts how a basic stepper motor's electromagnets and permanent magnets are arranged [31].

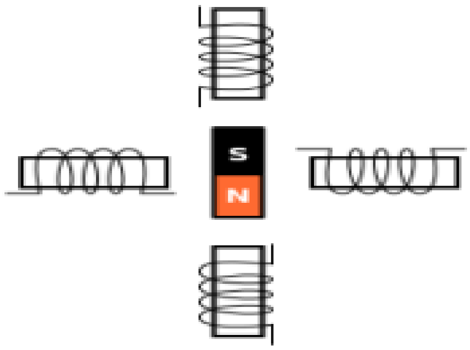


Figure II.2: stepper motor construction [31]

II.2.1.2 Stepper motor working principle

The stepper motor uses the theory of electromagnetism. The electromagnetic stators are positioned all around the magnetic rotor shaft. Depending on the nature of the stator, the poles of the rotor and stator may or may not be toothed. The rotor moves to align with the stator once the stators have powered it up. In this way, the stators are energized in the sequence at different poles to rotate the stepper motor. "Stepper motor working principle" Figure II.3 below illustrates this. [31].

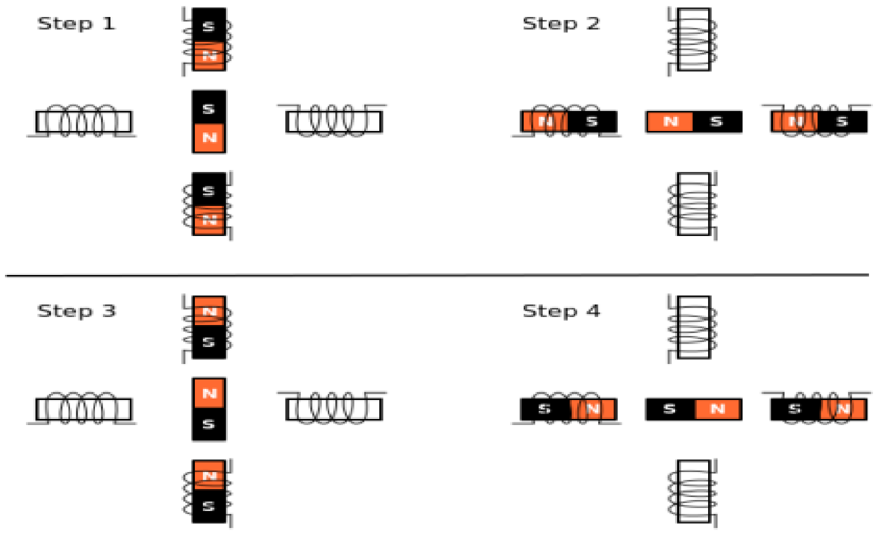


Figure II.3: stepper motor working principle [31]

II.2.1.3 Stepper motor winding:

Stepper motors are available in two basic winding types: bipolar and unipolar.

II.2.1.3.1 Bipolar wound stepper motor

A form of stepper motor with only one winding per phase is known as a bipolar stepper motor. Bipolar stepper motors are two-winding indicated as A and B as shown in "bipolar wound stepper motor" figure II.4, four-wire stepper motors. For the stepper motor, to function, a mechanism should be available to switch the direction of current flow in the coils. This is required so that during Step 1, as indicated in "stepper motor working principle" figure II.3 , the current in Coil A flows in one direction, and during Step 3, the current in Coil A flows in the opposite direction [31].

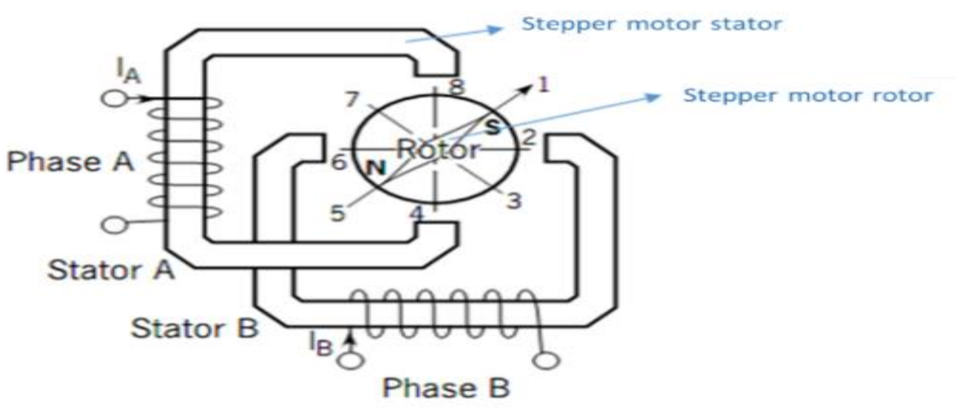


Figure II.4: bipolar wound stepper motor [32]

II.2.1.3.2 Unipolar wound stepper motor

Bipolar Wound Stepper Motors, require additional circuits to reverse the polarity of the coils. To simplify this, another class of stepper motors called “unipolar wound stepper motor” as shown in the figure II.5 below, provide an additional center tap in the windings. The center tap is always connected to the power supply.

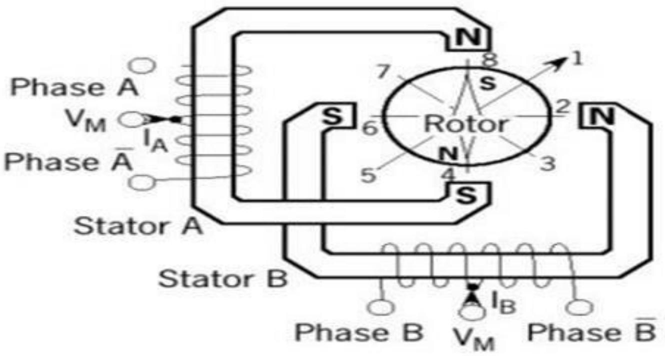


Figure II.5: unipolar wound stepper motor [33]

II.3.1.5 Stepper Motor Advantages and Disadvantages

Advantages:

- The motor's rotational angle is inversely proportional to the input pulse.
- If the windings are powered, the motor has its maximum torque when it is stationary. Accurate placement and repeatability of movement due to the accuracy of good stepper motors, which ranges from 3 to 5% of a step and is non-cumulative from one step to the next.
- Excellent starting, stopping, and reversing response.
- Exceptionally dependable due to the absence of contact brushes in the motor. • The motor's responsiveness to digital input pulses enables open-loop control, making the motor simpler and less expensive to manage. As a result, the motor's lifespan solely depends on the lifespan of the bearing.
- A load that is directly linked to the shaft can produce synchronous rotation at very low speeds.
- Because the speed is inversely related to the frequency of the input pulses, a wide variety of rotating speeds can be achieved.

Disadvantages

- If resonances are not effectively managed, they may happen.
- Extremely fast operation is difficult[30].

II.3.2 limit switch

A limit switch is an electromechanical device that responds to the physical pressure that an object exerts on it. It is employed to determine if an object is present or not. When there is nothing touching the switch actuator mechanism, a limit switch will be in its "normal" status, as shown in the figure II.7 below [34].

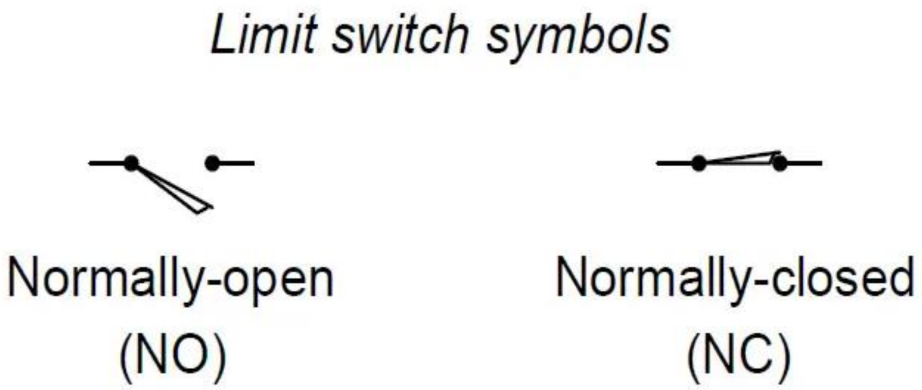


Figure II.7: limit switch symbols [34]

II.3.2.1 limit switch working principle

A roller-tipped lever is typically used in limit switch designs to establish contact with the moving component. The NC and NO contacts inside the switch are connected to the screw terminals on the switch body.Most limit switches of this design share a “common” terminal between the NC and NO contacts as illustrated in the following figure II.8 [34].

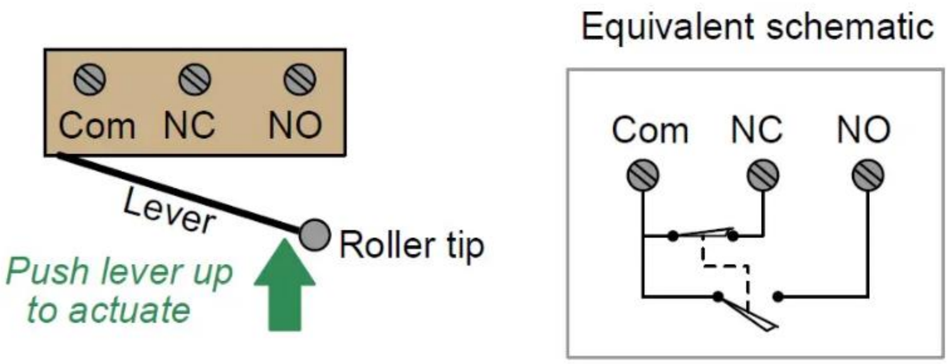


Figure II.8: limit switch working principle [34]

II.3 The pneumatic part

Itconsists respectively on a three-way solenoid valves needed to fill the counting chamber with diluent, lyse and blood, a two-way solenoid valve and a pump to drain the counting chamber after measuring.

II.3.1 the solenoid valves

A solenoid valve is a crucial component of any fluid control system. It is an electro-mechanical valve that is commonly employed to control the flow of liquid or gas which as a result, eradicates the need for an engineer to manually control the valve, saving time and money. Usually, solenoid valves are used whenever the flow of media has to be controlled automatically.

II.3.1.1 Solenoid valve construction

A solenoid valve is a crucial component of any fluid control system. It is an electro-mechanical valve that is frequently used to regulate the flow of liquid or gas. As a result, the engineer is not required to physically manage the valve, saving time and money. Usually, solenoid valves are used whenever the flow of media has to be controlled automatically.

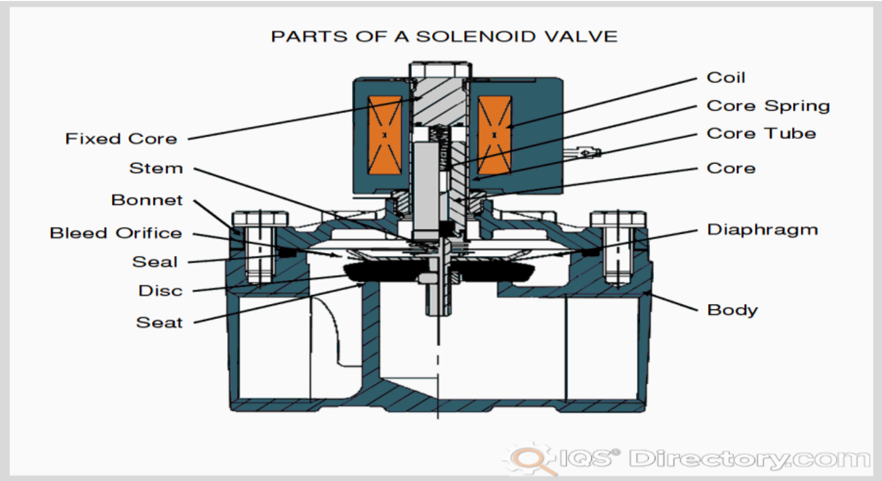


Figure II.9: solenoid valve construction [35]

The solenoids have varying parts depending on the type of action required. The valve body components are the same for every valve but with different designs and materials. Listed below are the general solenoid and valve body components [35].

- **Coil:** the coil is one of the main parts of the solenoid which consists of an insulated copper wire wound tightly around a core tube. As described earlier, a magnetic field is generated when current is applied.

- **Core:** a solenoid's core, also known as the armature or plunger, is its moving component. This metal can be easily magnetized and demagnetized at low magnetic fields since it is a soft ferromagnetic metal. The core is drawn to the magnetic field created when the coil is energized, which opens or closes the valve.

- **Core Spring:** When the magnetic field is turned off, the core spring places the core back in its original position. Depending on how the valve operates, different core spring designs and configurations are used in the solenoid assembly. Some designs do not employ springs to produce a return motion, such as latching solenoid valves.

- **Core Tube**: The coil is coiled around the core tube. Additionally, it serves as a soft magnetic core, enhancing the magnetic flux produced by the coil.

- **Fixed core**: installed at the core tube's closed end, this enhances the magnetic flux. Additionally, the substance is a soft magnetic metal.

- **Diaphragm**: a pliable material that separates the solenoid assembly from the fluid is the diaphragm. The fluid pressure is intended to be contained by the diaphragm.

- **Stem:** the valve's stem, to which the core or plunger is attached, is a valve component. The stem moves along with the coil as it draws the core, triggering the valve.

- **Disc:** when the valve is closed, the disc prevents fluid from flowing. Instead of a disc to restrict fluid flow, some solenoid valve designs utilize bellows, diaphragms, or pinch devices. The disc is often made of corrosion- and erosion-resistant materials, such as stainless steel or polytetrafluoroethylene (PTFE), depending on the purpose.

- **Seat:** The seat is the orifice that contacts the disc as the valve is closed. Depending on the valve design, the seat can not be present like the disc. Additionally, the seat is built of a material that resists erosion and corrosion. The valve will cease working and become passing if the seat or disc is destroyed.

- **Seal:** the seal separates the solenoid assembly and the outside environment from the fluid, much like the diaphragm does. Numerous seal materials, including polytetrafluoroethylene (PTFE), fluorine kautschuk material (FKM), nitrile butadiene rubber (NBR), and ethylene propylene diene monomer (EPDM), are available, depending on the application and the process fluid.

- **Bonnet:** located at the top of the valve body, the valve bonnet. Through the bonnet and into the valve, the core tube and stem protrude.

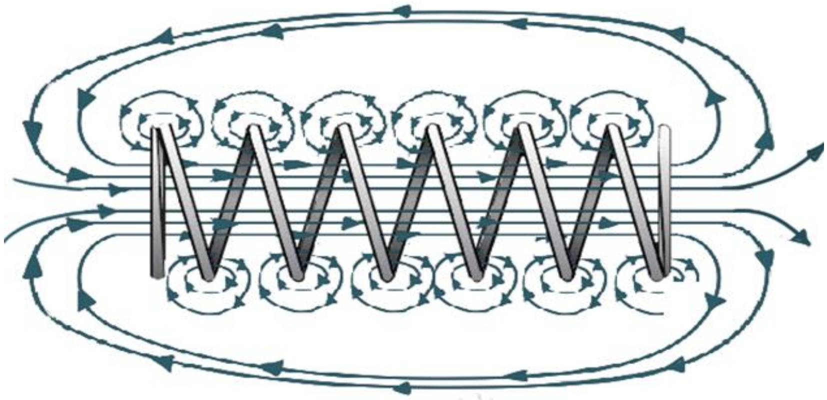
- **Body:** the valve's main component, the body is where the diaphragm, disc, seat, and inlet and output ports are located.

- **Bleed Orifice:** a bleed orifice is put on the diaphragm for solenoid valves with indirect or semi-direct action. An equalizing hole is used in some valve designs. The bleed orifice enables the valve to open or close using line pressure.

- **Pilot Channel:** a pilot channel is built into the valve body for indirect acting solenoid valves. This is where fluid enters the downstream side of the valve from the top of the diaphragm.

II.3.1.2 solenoid valve working principle

When electrically charged or de-powered, solenoid valves are control devices that either shut off or permit fluid flow. An electromagnet serves as the actuator. A plunger or pivoted armature is pulled by an electrified magnetic field in opposition to the force of a spring (Figure II.10). When de-energized, the spring action forces the plunger or pivoted armature to return to its initial position. [36].



FigureII.10: Representation of solenoid magnetic field [35]

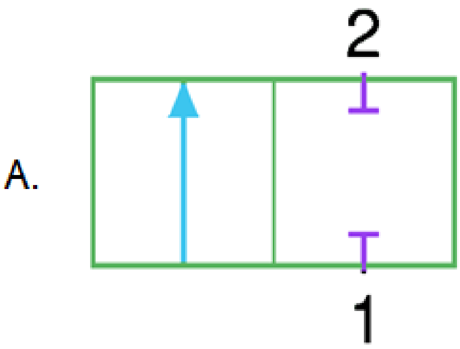
The number of loops will increase the flux or lines in the magnetic field. As a result, the solenoid's electromagnetic force increases, increasing the force required to actuate the valve.

Increasing the supply voltage to the solenoid will increase the force of attraction. Both DC and AC can be used to power solenoid valves. 6, 12, 24, and 240 volts are typical DC voltages, while 24, 120, 240, and 480 volts are typical AC voltages at 60 Hz.

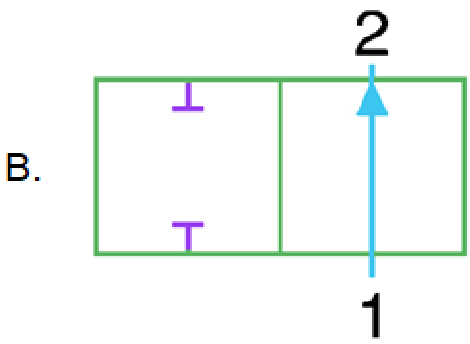
II.3.1.3 Types of solenoid valves

II.3.1.3.1 Two- way valves

Shut-off valves with one inlet port and one outlet port are known as two-way valves (Figure II.11). As shown in figures II.11 and II.12, the solenoid valve can be designed to be either normally open or normally closed. In the normal state, the flow is stopped by the core spring holding the valve seal on the valve seat with the help of fluid pressure. When powered, the valve opens as the core and seal are drawn into the solenoid coil. The total spring force, static and dynamic pressure forces of the medium, and the electro-magnetic force are all less than the electromagnetic force [36].



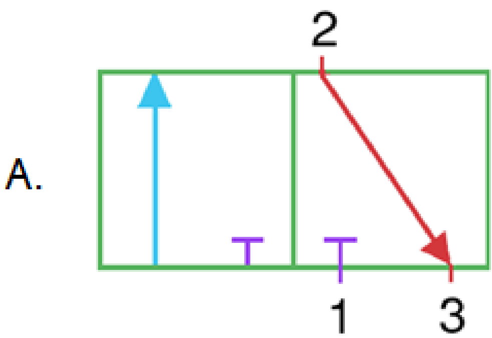
FigureII.11: Two-way normally closed solenoid valve [35]



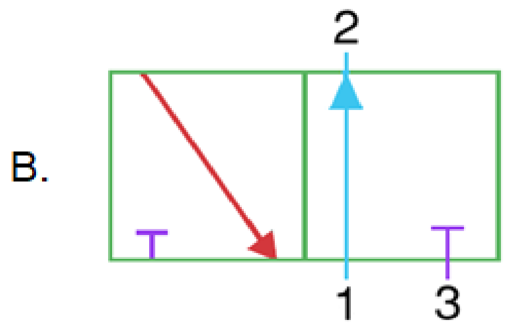
FigureII.12: Two way normally opened solenoid valve [35]

II.3.1.3.2 Three-way solenoid valve

Three-way valves have three port connections: inlet (pressure port), exhaust and outlet (actuator port). It can also be configured as normally open and normally closed in the de-energized state shown in figure II.13 and figure II.14. When the coil is energized, the mode reverses.



FigureII.13: Three-way normally closed valve [35]



FigureII.14: Three-way normally opened valve [35]

A plunger-style core was used in the design of the 3-way valve. Under the valve seat, fluid pressure increases. For instance, in a three-way valve that is ordinarily open, fluid flows from the inlet port to the outlet port while the exhaust port is closed when the valve is de-energized. The outlet port is connected to the exhaust port when the device is powered up, and the inlet port is shut off. In contrast, typically closed valves work the other way. On the other hand, the universal function is utilized to choose the direction of flow from one port to another [35].

These solenoid valves can also be controlled by Arduino, the specifics are presented in the following chapter.

II.3.2 The drainage pump

The liquid accumulates energy by the action of inertial centrifugal force to increase the pressure, according to the drainage pump's operating principle, which relies on the impeller revolving with high resistance.

In order to avoid cavitation, the pump body and inlet pipe must be filled with water prior to operation. Centrifugal force causes the rotating water to flow away from the impeller when it turns rapidly, which causes the blades to also spin rapidly. The center of the impeller creates a vacuum area once the water in the pump is discharged. Under the influence of atmospheric pressure or water pressure, the water from the water source is forced into the water input pipe through the pipe network. This makes it possible to establish continuous water pumping[37].

FigureII.15: automated pump [38]

In order to regulate the pump, it must be wired with a relay module and connected to Arduino as well.

II.4 The analog part

Itis concerned with the photometry environment allowing the measurement of the hemoglobin concentration. This consists mainly on a light source and a sensor which allows the conversion of the transmitted light through the blood sample into frequency which in turn is converted into an electrical signal using a frequency voltage converter. The variation of this electrical signal is proportional to the hemoglobin concentration in the blood sample.

Photometry is the common principle used for hemoglobin assessment. This hemoglobin assessment is simply optioned from the transmittance of monochromatic light at 540nm wavelength through the blood sample, light is absorbed by the mixture. The absorbance is measured by a photo sensor.

The Photo sensor to be used depends on several factors: a wide spectral response, high sensitivity, high speed of operation and quick response when exposed to light. In the proposed system, the TSL230R sensor is used; it precisely measures light using an array of photodiodes and converts it into frequency. A brief description of some principles of sensor and convertor used is made in what follows.

II.4.1 TSL230R light-to-frequency convertor

II.4.1.1 Description of TSL230R

The TSL230R is programmable light-to-frequency converters combining a configurable silicon photodiode and a current-to-frequency converter on single monolithic CMOS integrated circuits. The output can be a square wave with a frequency closely correlated to light intensity or a pulse train with a 50% duty cycle. Device sensitivity can be adjusted over a two-decade period by choosing between three settings. One of four predetermined values may be used to scale the output frequency at full scale. All inputs and the output are TTL compatible, allowing direct two-way communication with a microcontroller for programming and output interface (details of this interface with the Arduino Board is provided in the next chapter). An output enable (OE) is provided that places the output in the high-impedance state for multiple-unit sharing of a microcontroller input line.

The device has been temperature-adjusted for the ultraviolet to visible light range of 320 nm to 700 nm and responds over the light range of 320 nm to 1050 nm[39].

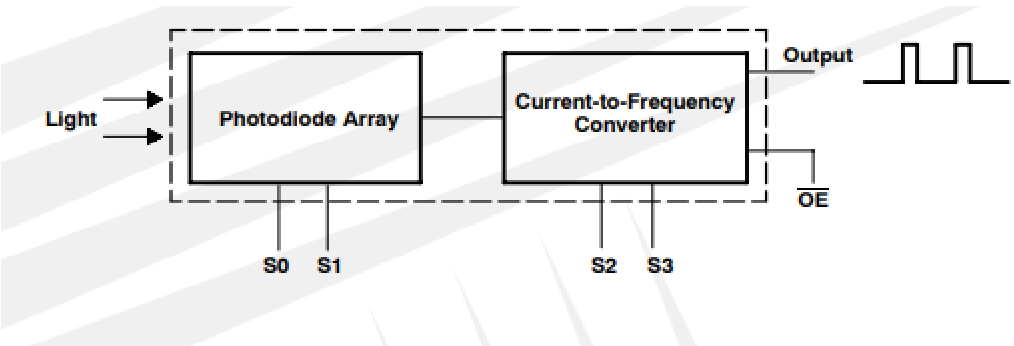


Figure II.16: Functional Block Diagram of TSL230R [39]

II.4.1.2 TSL230R working principle

A block diagram of the TSL230 programmable light-to-frequency converter can be viewed in Figure II.17. The 8-pin DIP IC package is transparent, allowing light to shine on it to illuminate a number of photodiodes. Part or all of this array is connected to the "analog magic" current-to-frequency converter, which is identified on the picture by an electronic sensitivity control. Under total darkness, the frequency is close to 0 Hz, whereas under bright light, it is closer to 1.4 MHz. A programmable divider receives the output and can split the signal by 2, 10, or 100 by regulating two logic inputs, S2 and S3 [40].

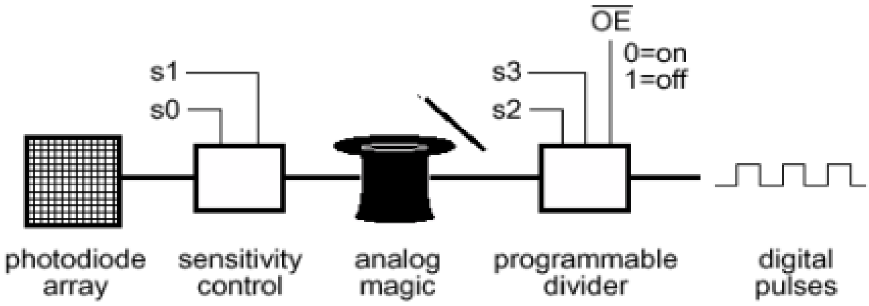


Figure II.17: Simplified block diagram of the TSL230R [40]

II.4.1.3 The TSL230R sensitivity

S0 and S1 are the two logic inputs that regulate sensitivity. An electronic iris approach, or aperture control, is used to change the device's sensitivity in order to alter how it reacts to a specific amount of light. The sensitivity can be adjusted over a period of two decades to one of three levels: 1×, 10×, or 100×. In doing so, the device's responsiveness can be tuned to a specific light level while still maintaining its complete output-frequency range. The effective photodiode area changes proportionally to the change in sensitivity [39].

As illustrated in the table below, the sensitivity is set by adjusting the levels on control pins S0 and S1.

S1	S0	Sensitivity
L	L	Power down
L	H	1x
H	L	10x
H	H	100x

Table 1: sensitivity setting [39]

II.5.1.5 The TSL230RFeatures:

- ✓ Non-intrusive high-resolution conversion of light intensity to frequency

- ✓ Absolute output frequency tolerance of +/- 20%;
- ✓ Programmable sensitivity and full-scale output frequency
- ✓ Direct communication with a BASIC Stamp or SX microcontroller
- ✓ Nonlinearity error is typically less than 2% at 100 kHz
- ✓ It is stable at a wide range of temperatures [39]

II.6 The frequency to voltage convertor LM2917-8N

II.6.1Description of LM2917

The monolithic frequency-to-voltage converters LM2917 and LM2907 have high gain operational amplifiers and are intended to operate a relay, light, or other load when the input frequency meets or exceeds a predetermined rate. The frequency to voltage conversion employs the charge pump approach, comes in two versions (8-pin LM2907 and LM2917), and offers frequency doubling for low-ripple, full-input protection. Its output swings to ground for a zero frequency input.

II.6.2 Applications

- Speedometers
- Over- and Under-Speed Sensing
- Frequency-to-Voltage Conversion (Tachometer)
- Automotive Door Lock Controls
- Touch or Sound Switches
- Hand-Held Tachometers
- Speed Governors, Cruise Controk
- Automotive Door Lock Control, Clutch Control, and Horn Control [41]

II.6.3 Frequency to voltage conversion

The specific IC model that we will analyze is the LM2917-8N, a 8-pin DIP package that contains a comparator, capacitor charge pump, and op-amp/transistor amplification stage and zener diode. The schematic for the device can be seen below:

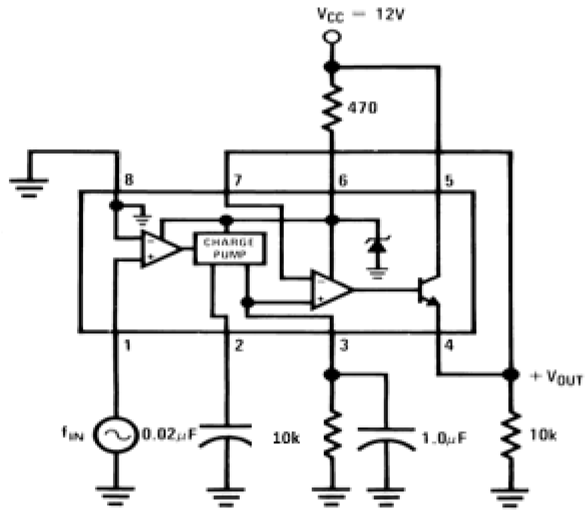


Figure II.19: frequency to voltage conversion [41]

We create a zero-crossing detector by grounding the comparator's negative differential input. The output will be a square wave if an AC signal is applied to the positive wire with common grounding and no DC offset.The output will be a square wave if an AC signal is applied to the positive wire with common grounding and no DC offset.

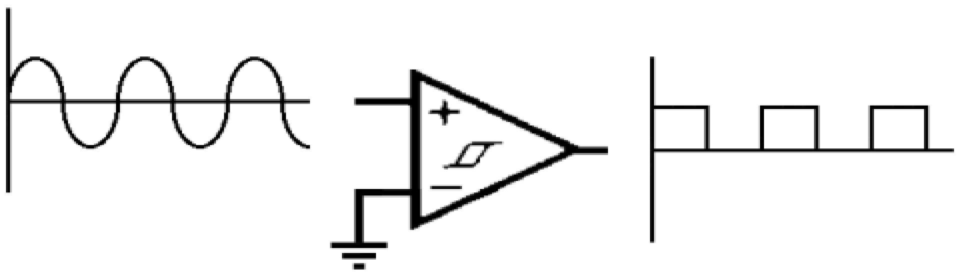


Figure II.20: Output response of a zero-crossing detector [42]

The charge pump generates the charge and discharge for the charge pumping action from this squarewave using a timing capacitor. Pin 2 is where the charging capacitor is connected.

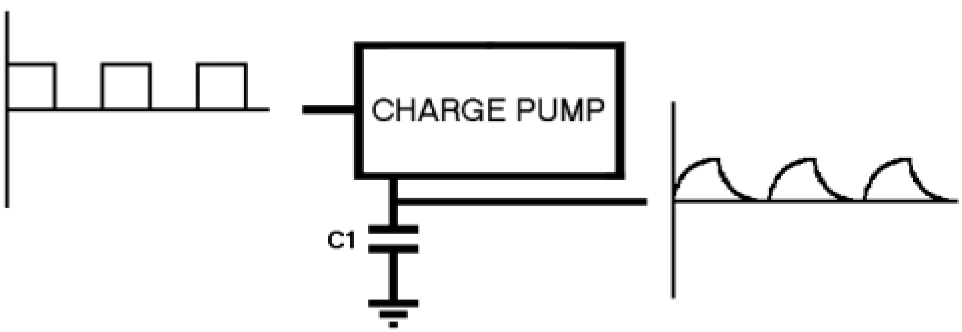


Figure II.21: Voltage response of the timing capacitor C1 [42]

C1 is a filter capacitor used to cut down on charge pump ripple. It should maintain the measurements' stability.

The resulting signal has an amplitude of, and the capacitor experiences a change in voltage of this size once per 1/(2f0) seconds. As a consequence, the capacitor experiences an average current throughout time:

I_{avg} = C1 x 2f0 x Vcc/2 = c1 x f0 x Vcc (1)

This current is then reversed on the charge pump's output so that it can be supplied to a grounding resistor and filtered by a second capacitor for a more consistent response. It is therefore possible to determine the output voltage across the resistor using Ohm's law and Eq (1):

V_{out} = I_{avg} x R1 = C1 x R1 x f0 x Vcc (2)

The output voltage is totally influenced by the frequency of the input signal in a relatively straightforward linear relationship, as seen in the following figure.

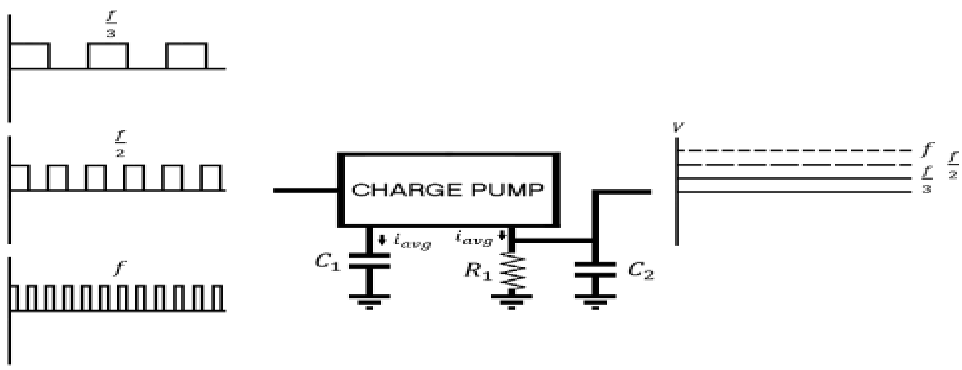


Figure II.22: Expected output response of a charge pump [42]

The second op-amp is driving the output transistor while buffering the signal stored on C2, and it also has feedback from the output to its -ve input, which is a typical setup for a unit-gain buffer.

Open emitter and collector are included on the output transistor to enable use in a variety of setups.

The 470 ohm resistor limits the input current, making advantage of the Zener regulator. In spite of changes in input voltage, this produces very steady outputs [42].

In our example, the pulse-to-voltage conversion is carried out by converting the input pulse frequency into the matching voltage using a frequency-to-voltage converter.

Since the output voltage of the converter is proportional to the frequency of the input pulses, the output voltage can be managed by adjusting the duty cycle of the input pulses.

To make sure that the converter can precisely convert the incoming pulses into a voltage, we must modify the signal level. The Arduino's ADC subsystem converts this voltage into digital data, which provides a calibration curve that we can use to determine the hemoglobin concentration.

II. 5 The interface and processing part:

As specified above the interface and processing part mainly consists on a microcontroller (ArduinoMega Board)responsible for digitizing the electrical signal obtained in the Analog part, the calculation and the execution of commands to control the stepper motor, the limit switches, the pump, the solenoid valves, the led, the buttons, the LCD displayer...

II.5.1 Description of the Arduino Board

Arduino is an open-source prototyping platform (a microcontroller based prototyping board) in electronics based on easy-to-use hardware (of which the most popular type is the Arduino Uno) and software required to be run on a computer (to program the board) and some peripheral shields that can be plugged into it and ready for use [43].

II 5.1.1 Themincrocotroller

The microcontroller used in the Arduino board is the ATmega2560 (8-bit Microcontroller) . Its most important part is the Central Processing Unit (CPU) as seen in figure II.23 with a Memory spaces such as RAM, ROM, EEPROM, EPROM are there to store data and programs. Volatile memory, such as RAM, is used to store data, while other memory spaces, such as ROM, are used to store programs and operational parameters.

The central processing unit (CPU), often referred to as the brain of the microcontroller, retrieves, decodes, and executes the instructions. It coordinates different microcontroller-based operations [44].

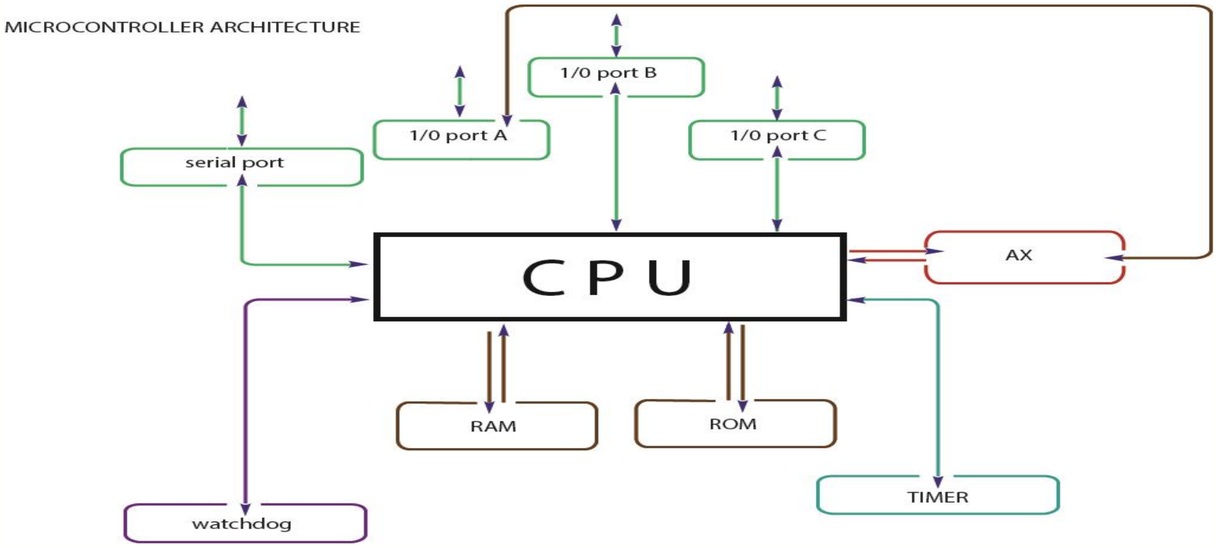


Figure II.23: microcontroller architecture [44]

II.6.1.2 Arduino architecture

The 54 digital input/output pins on the Arduino Mega, for instance, can be used as input or output pins by utilizing the pinMode(), digitalRead(), and digitalWrite() functions in arduino programming. Each pin operates at 5 volts and includes a 20 to 50 kOhm internal pull-up resistor that is by default unconnected. It can deliver or receive a maximum of 20 mA of current. Some of these 54 pins have particular uses, which are detailed below:

- Serial 0 (RX) and Serial 1 (19 RX and 18 TX); Serial 2 (17 RX and 16 TX); and Serial 3 (15 RX and 14 TX). used to transmit and receive TTL serial data (RX and TX). Additionally, pins 0 and 1 are connected to their equivalent ATmega2560 USB-to-TTL Serial pins.

- External Interrupts: two (interrupt 0), three (interrupt 1), eighteen (interrupt 5), nineteen (interrupt 4), twenty (interrupt 3), and twenty-one (interrupt 2). These pins can be set up to initiate an interrupt in response to low levels, rising or decreasing edges, or level changes. Details can be found in the attachInterrupt() function.

- PWM: 2–13, and 44–46. The analogWrite() method outputs an 8-bit PWM signal.

- SPI: 50, 51, 52, 53 (MISO, MOSI, SCK, SS). The SPI library is supported by these pins for SPI communication. The ICSP header, which is physically compatible with the Arduino /Genuino Uno and the vintage Duemilanove and DiecimilaArduino boards, is also broken apart to include the SPI pins.

- LED: 13. Digital pin 13 is wired to a built-in LED. The LED is on when the pin has a HIGH value; it is off when the pin has a LOW value.
- TWI: 20 for SDA and 21 for SCL. Utilize the Wire library to support TWI communication. It should be noted that these pins are not located where the TWI pins were on the previous Duemilanove or DiecimilaArduino boards.

There are 16 analog input pins in addition to 54 digital pins, each of which has 10 bits of resolution, or 1024 distinct values. They can measure between 0 and 5 volts, however by using the AREF pin and analog Reference() function, this limit can be raised.

Additional pins on the Arduino Mega 2560 are described below:

- AREF: Used with the analogReference() function to supply a reference voltage for analog inputs.
- Reset Pin: When this pin is low, the microcontroller is reset. [45].

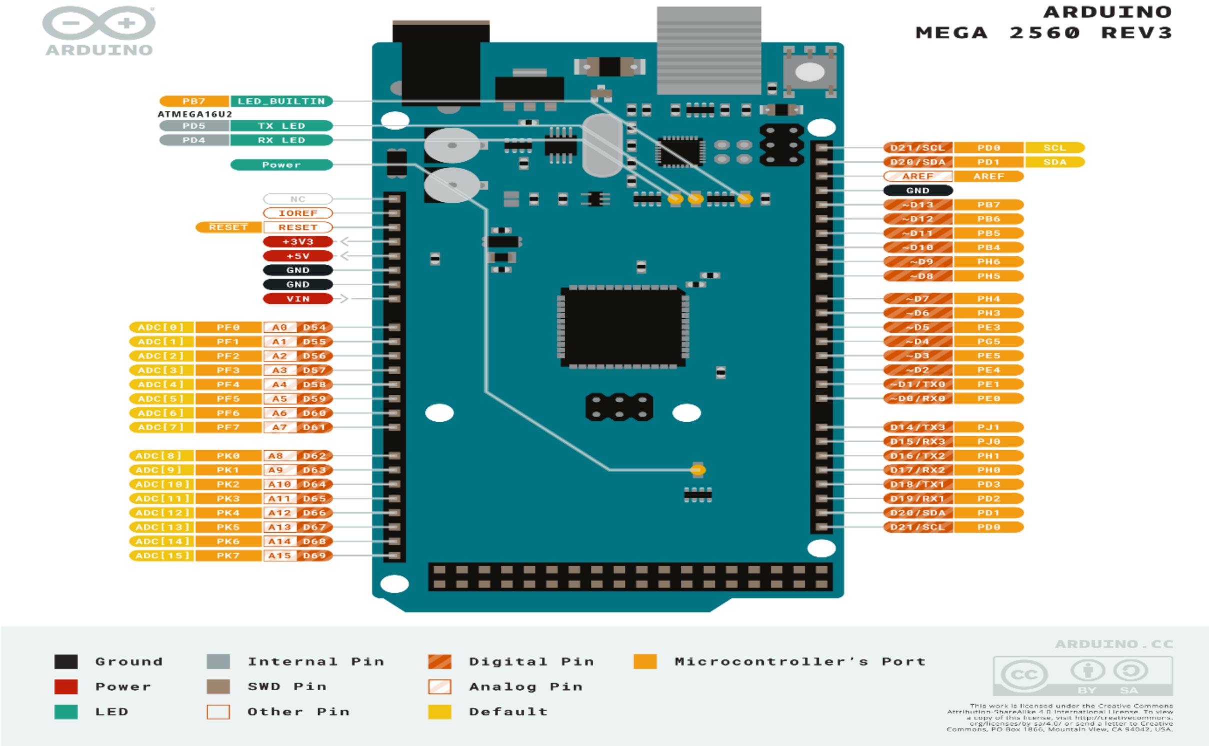


Figure II.24: Arduino pins diagram[45]

II.6.1.3 Arduino IDE software

Arduino IDE (Integrated Development Environment) is required to program the Arduino Uno board.

Once Arduino IDE is installed on the computer, the Arduino Board should be connected to the computer using an USB cable. Then the Arduino IDE should be opened; the correct board selected by selecting Tools>Boards>Arduino/GenuinoMega, and the correct Port selected by selecting Tools>Port. As an example,ArduinoUno programmed using Arduino programming language based on Wiringas shown in figure II.25 and figure II.26 .

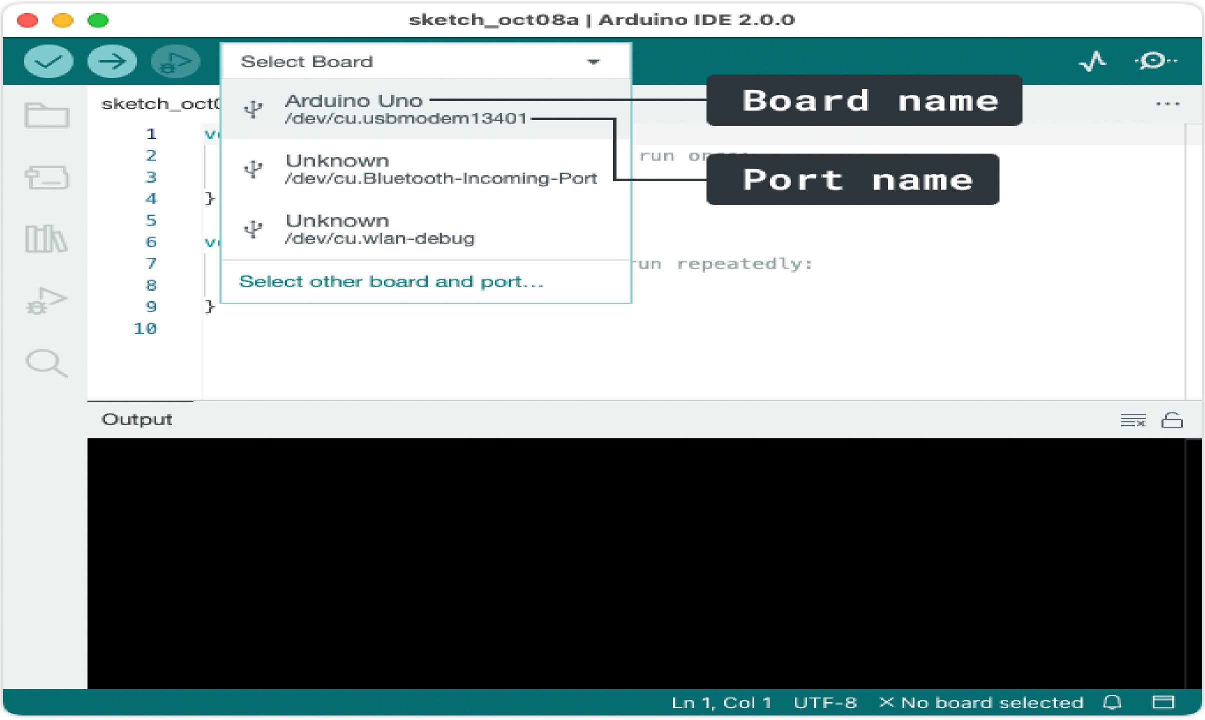


Figure II.25: Arduino board and port selection

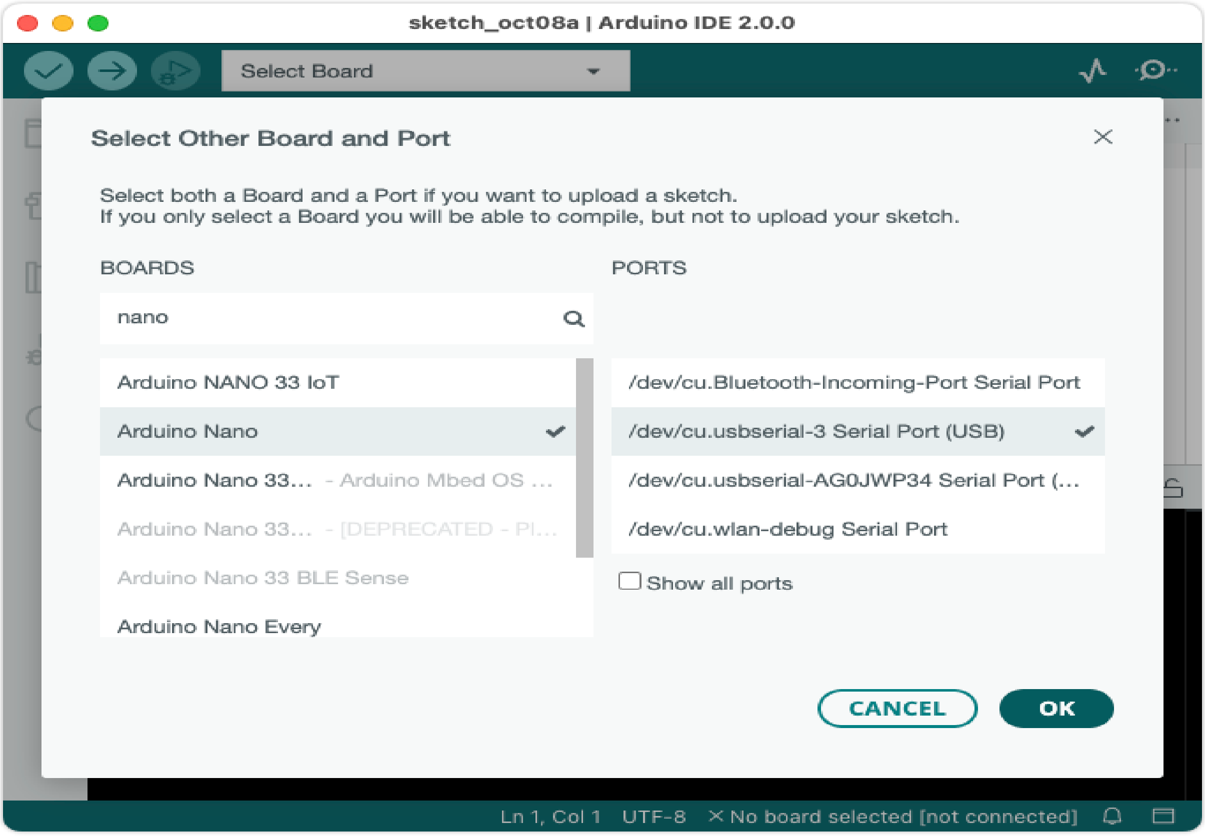


Figure II.26: Arduino boards and portslist

II. 7 The I2C LCD interface:

A sort of display that makes use of liquid crystals is the LCD (Liquid Crystal Display).

Here, we'll take the computer's serial input and upload the Arduino sketch. On the LCD, the characters will be shown.

The Liquid Crystal Library, which is covered in more detail below, is the library that enables us to control the LCD display.

Declaring the library is as follows: #include "LiquidCrystal.h"

The Hitachi HD44780 chipset, which is compatible, provides the foundation of the library. It can be found on the majority of text-based LCDs. Either an 8-bit mode or a 4-bit mode will function with it. Here, the enable, rs, and rw control lines are optional, and the bit mode also refers to the data lines.

II.7.1 The LCD structure

There is a 16-pin interface on the LCD display.

The LCD's construction is displayed below:

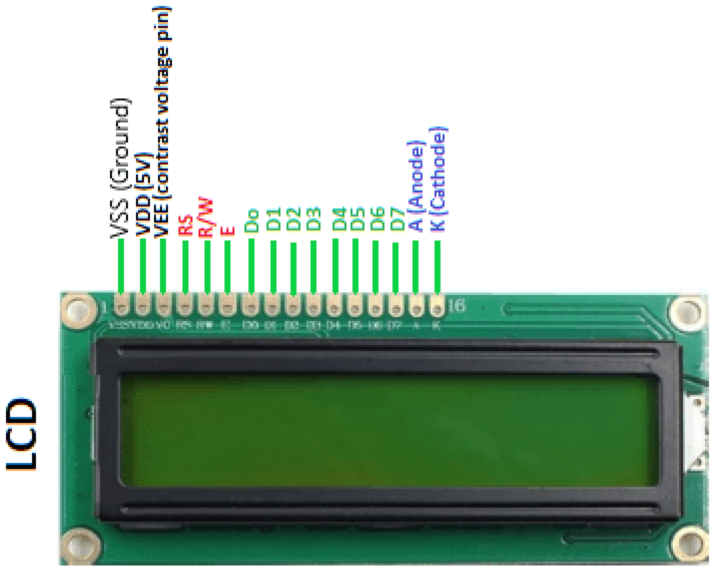


Figure II.27: LCD structure

The parallel interface of the liquid crystal display is present. This indicates that the microcontroller controls the LCD display by simultaneously operating several pins.

The following is a discussion of the 16 pins on the LCD display:

- RS: the memory of the LCD that we write the data to is controlled by the Register Select (RS) pin. Either the data register or the instruction register are options. The upcoming instruction, which is stored in the instruction register, is sought after by the LCD.

- R/W: the reading or writing mode is chosen by the Read/Write pin.

- E: the writing to the registers can be enabled by using the Enable (E) mode. When the mode is HIGH, it transmits the data to the data pins.

- D0 to D7: These eight data pins have the following designations: D0, D1, D3, D3, D4, D5, D6, and D7. We have the option of setting the data pin's status to HIGH or LOW.

The LCD's Ground pin is pin 1, and the Vcc, or voltage supply pin is pin 2.

The VEE, or contrast pin, is located on LCD pin 3. For instance, by connecting the output of the potentiometer to the VEE, we may modify the LCD's contrast.

Backlight pins (Bklt+ and Bklt-) are another name for the A and K pins.

II.7.2 The Principle of LCD

The procedure entails loading the data into the data registers that will be displayed on the LCD panel. The instruction register is used to store the instructions from the Register Select. The procedure for displaying characters on the LCD has been made simpler by the liquid crystal library.

The 4-bit or 8-bit modes of controlling the LCDs require 7 and 11 Input/Output pins from the specific Arduino board, respectively.

Since an LCD uses a lot of microcontroller pins, we'll utilize serial communication to cut down on that requirement. Serial communication basically involves delivering "packages" of data one after another using only two microcontroller pins, either SDA and SCL or analog pins like A4 and A5.

Using the LCD piggy-back board and the I2C bus, the desired data can be shown on the LCD. The PCF8574 (from NXP), a general-purpose bidirectional 8 bit I/O port expander that uses the I2C protocol, serves as the conceptual foundation for these backpacks. The PCF8574 is a silicon CMOS circuit that provides general-purpose remote I/O extension (an 8-bit quasi-bidirectional) over the two-line bidirectional bus (I2C-bus) for the majority of microcontroller families. The PCF8574T (SO16 package of PCF8574 in DIP16 packaging), which has a default slave address of 0x27, is the core of the majority of piggy-back modules.

Figure II.29: The I2C module

II.8 Conclusion

Through this chapter, we have been able to comprehend the fundamental principles underlying the operation of the many mechanical and analog components essential to the hemoglobin measurement system, including stepper motors, solenoids, voltage converters, frequency to light converters, and.

In the following chapter, a detailed description of the practical realization of the proposed system is discussed along with some results obtained through different tests carried out.

II.9The references

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Chapter III

The practical realization of the system and tests

Chapter III:

III.1 Introduction

After providing a general overview of the various design phases and the elements that make up our system, we will now go into more detail and explore how its many components are actually implemented in practice. Examples of mechanical and analog components are the stepper motor, solenoid valves, and the system's entire electrical circuit. The Arduino Mega board, a digitizing and acquisition board, is used to operate every component of every part that makes up the system, as indicated in the previous chapter and illustrated by the bloc diagram.

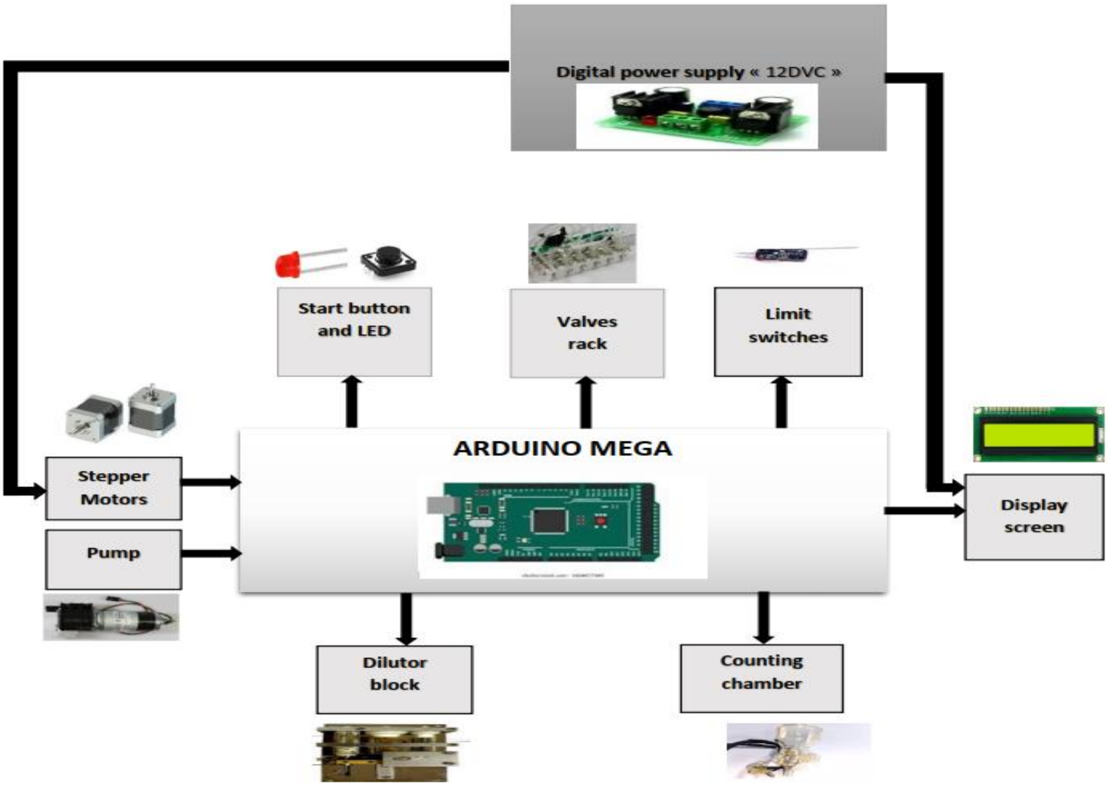


Figure III.1: bloc diagram of the system

The idea behind this diagram is to develop an automated system that aspires blood using a needle that is controlled by a stepped motor, it can also add the diluent and lyse to the counting chamber, then measuresthe Hb rate and displays the result immediately.

III.2 Practical realization of the mechanical part

As was describedin the previous chapter, the mechanical component of the system that is needed to control the movement of the needle for sampling and the syringes for reagent priming consists of connecting stepper driver A4899 with Arduino to control the stepper motor.

III.2.1 Stepper motor interfacing with Arduino Mega

The NEMA 17 stepper motor with a rated current/phase of 1.5A was selected for this realization.

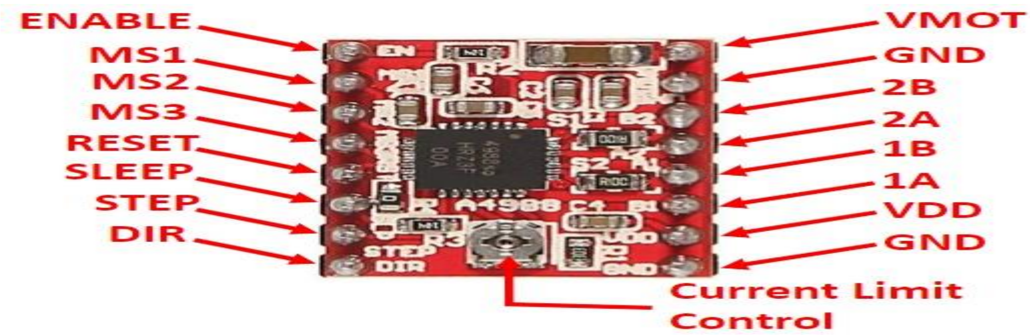
Due to the ease of step motor control and the variety of stepping modes offered by the A4988 driver, this is used to control the NEMA 17 bipolar stepper motor. Two pins are required to control the speed and direction of the NEMA 17 bipolar stepper motor. We'll discuss stepper motor control in this section using an Arduino Mega 2560 and an A4988 driver. The A4988 driver module will first be discussed, after which its connection with the stepper motor will be presented.

III.2.1.1 The description of the stepper motor driver A4899:

While using Arduino mega 2560 interfacing with a stepper motor is comparatively easy using versatile stepper driver called the A4899 driver to control the stepper motor in a highly precise and efficient manner. It contains a chip made by Allegro MicroSystems: the A4988 DMOS Microstepping Driver with Translator and Overcurrent Protection.

The stepper motor driver has a maximum output drive capacity of 35 V and ± 2 A which is great for controlling bipolar stepper motors such as NEMA 17 bipolar stepper motor, at up to 2A output current per coil.

The stepper motor has 16 pinouts as illustrated in the figure III.2 below



FigureIII.2: the stepper motor driver A4988

ENABLE: is to enable the A4988 stepper motor driver. It is enabled by default when it's set to LOW.
MS1, MS2, MS3:Micro stepping resolution select pins. The specific setting of step resolution of the motor is doneby setting the appropriate logic levels for the input pins shown in the table below.

MS1	MS2	MS3	Microstepresolution
LOW	LOW	LOW	**Full step**
HIGH	LOW	LOW	**Half step**
LOW	LOW	LOW	**Quarter step**
HIGH	HIGH	LOW	**Eighteenth step**
HIGH	HIGH	HIGH	**Sixteenth step**

Table III.1: Micro stepping resolution select pins setting

RESET : When the RESET pin is activated, it ignores all inputs sent to the "STEP" pin until it's deactivated.
SLEEP: this pin is to minimize power consumption when the motor is not used.
STEP: this pin is for the micro stepping setting of the motor. One signal on HIGH equals a one step.
DIR: pin of the direction of rotation (clockwise or counter-clockwise).
VMOT: the voltage supply for the motor being driven. The motor I use in this project is a 12V motor.
1A, 1B, 2A, 2B :output pins that should be connected to the stepper motor being controlled.
VDD – To power the internal circuit of the A4988. The voltage range is from 3 – 5.5V.
GND – GND pin(next to VDD pin) must be connected to a common ground.

III.2.1.2 The wiring of driver A4899 with the stepper motor and Arduino:

Here we are using a bipolar that has four wires (A+, A-, B+, B-) which need to be connected to the stepper motor driver (A4899) four input pins (2B, 2A, 1B, 1A), the RST/RESET pin should also be connected to the adjacent SLP/SLEEP pin To keep the A4988 driver enabled,

The DIR and STEP input pins should be connected to the Arduino's digital output pins as shown in the diagram circuit below designed by fritzing.

Fritzing is an open source design software that can be used to design circuits on breadboards, create schematics, and even develop PCBs.

It includes many popular components such as the Arduino range of boards, stepper motors, LCD, components and modules.

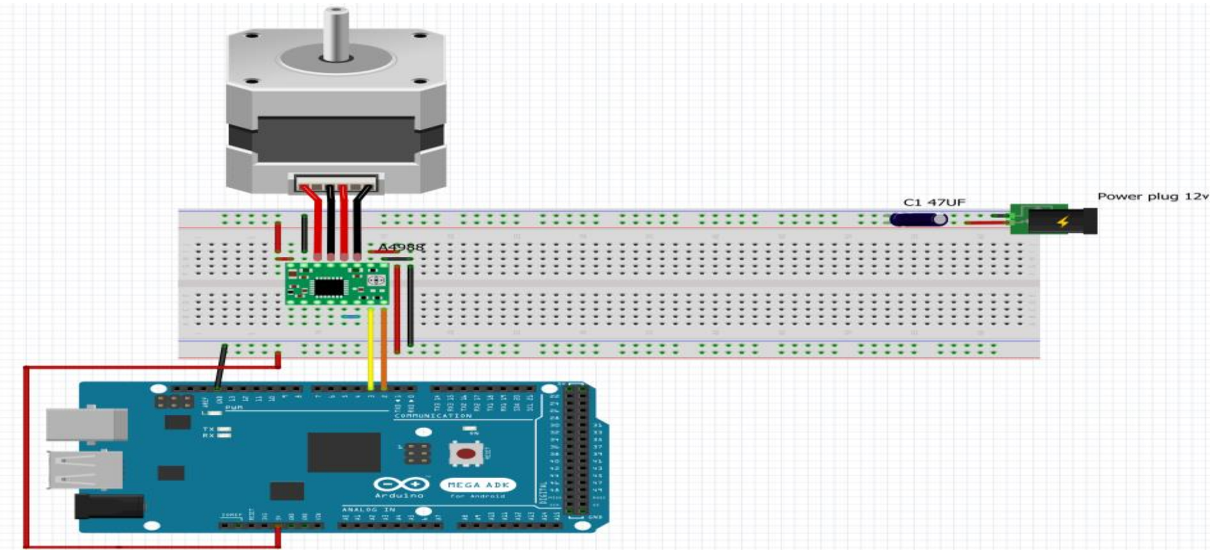


Figure III.3: stepper motor driver wiring with ArduinoMega

In our case, we are going to use six drivers A4988 to control six stepper motors, one motor for horizontal movement, two stepper motors for vertical movement (figure III.4), one stepper motor for blood syringe, one stepper motor for lyse syringe and the last one is for diluent syringe (Figure III.5).

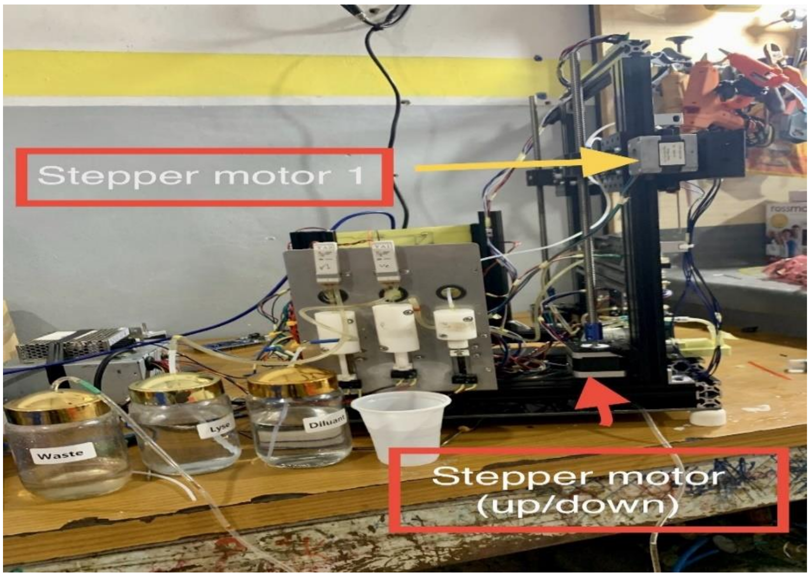


Photo 1: stepper motors for horizontal and vertical movement

Photo 2: the stepper motors of the dilutor bloc

The AccelStepper library should be added to Arduino using the library manager before you proceed.

The AccelStepper library operates by having a position value of zero at startup and moving to a position relative to that value when instructed to do so.

It also gives us the ability to set the acceleration, the initial speed and the maximum speed too.

As an example, the stepper will move 300 steps away from the zero position if you set the position to "move to" to 300 and then give the "run" command.

In our case we'll use a maximum position because the motor runs until the limit switch is pressed, it stops.

Here is a code example of the stepper motor names “stepper” responsible for the forward slide of the needle.

```
#include<AccelStepper.h>

#defineMAX_POSITION 0x7FFFFFFF // maximum of position we can set (long type)

AccelStepperstepperD(1, 2, 3);// step = pin 2; direction = pin 3

voidsetup() {
Serial.begin(115200);

 // 1st motor for forward and backward slide
  stepper.setMaxSpeed(1000.0);   // set the maximum speed
  stepper.setAcceleration(500.0); // set acceleration
  stepper.setSpeed(150);         // set initial speed
  stepper.setCurrentPosition(0); // set position
}

voidForwardSlide (){

  stepper.moveTo(-MAX_POSITION);
 stepper.run(); // MUST be called in loop() function
}

voidloop() {
  // put your main code here, to run repeatedly:
 ForwardSlide();
}
```

III.2.2 The limit switch interfacing with Arduino

Limit switches are one of the most frequently used electronic components that are used in a situation where the limits need to be defined. As in this project, the movement of the stepper motor can be controlled using the limit switch as demonstrated in the figures below.

The motor will run when the Limit switch is released, and will stop when it is pushed in.

The same Arduino Mega microcontroller board is used to monitor the state of ten limit switches used to detect the end of horizontal/ vertical movement of the mechanism and then perform specific actions based on that state.

III.2.2.1 The limit switch wiring with Arduino

We can connect the limit switch to the Arduino board by connecting the normally open (NO) contact of the limit switch to a digital input pin of the Arduino, and the common contact of the limit switch should also be connected to the ground.

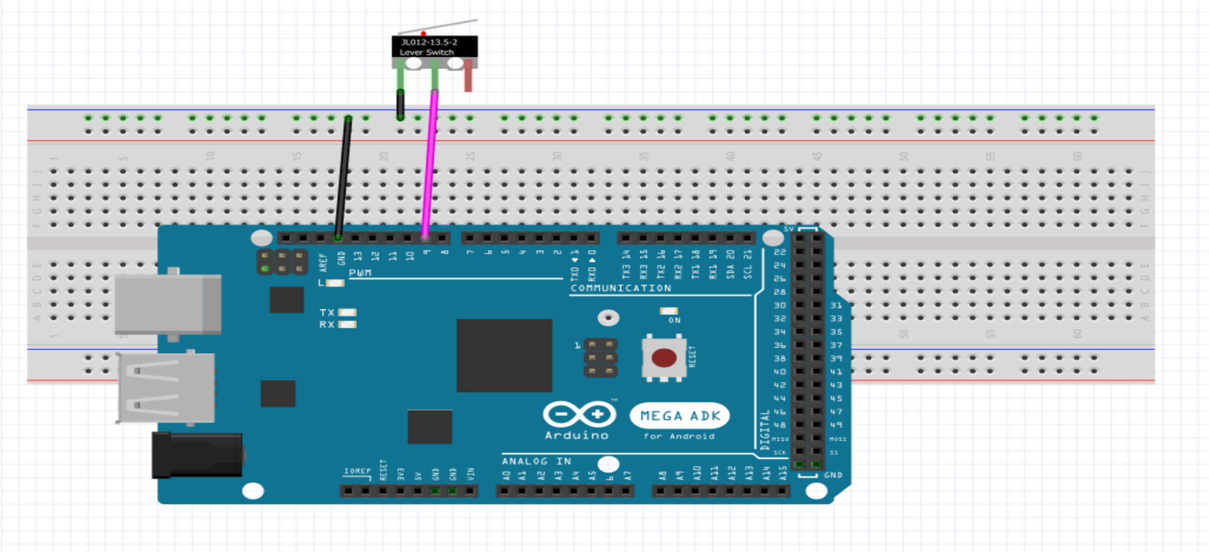


Figure III.4: limit switch wiring with ArduinoMega

The code belowuses the library “J_C button” that should be included and it allows the stepper motor that moves the needle forward to run as long as the limit switch is released; once the limit switch is pressed, the stepper motor stops as well the stepper motors that control the syringes.

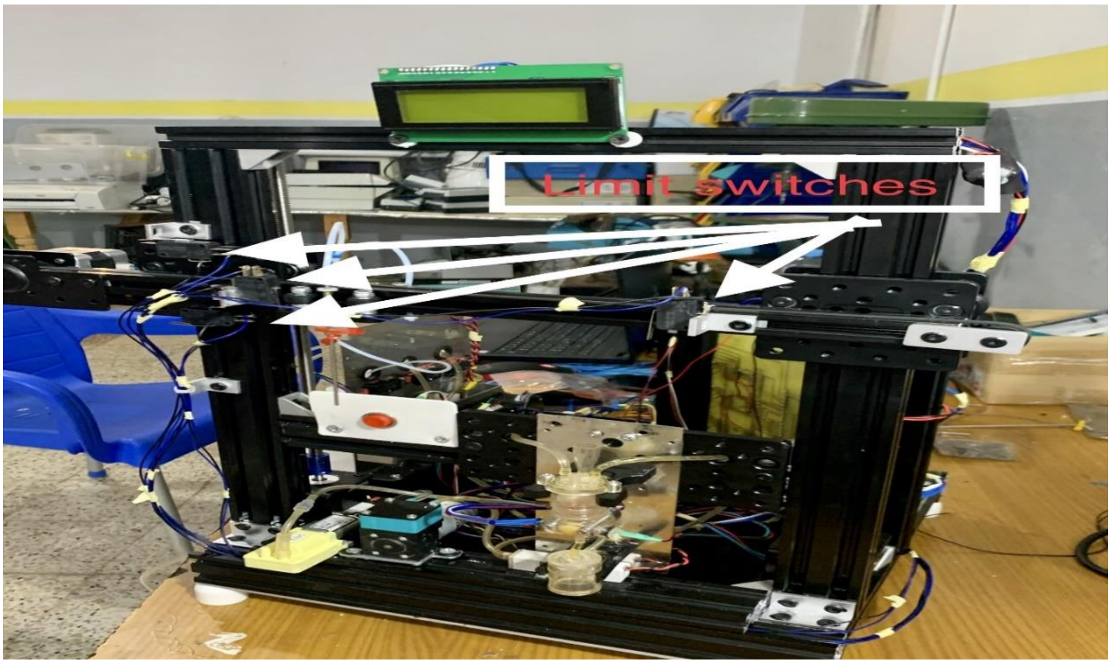


Photo 3: limit switches responsible for stopping the horizontal/vertical movement of the motor[2]

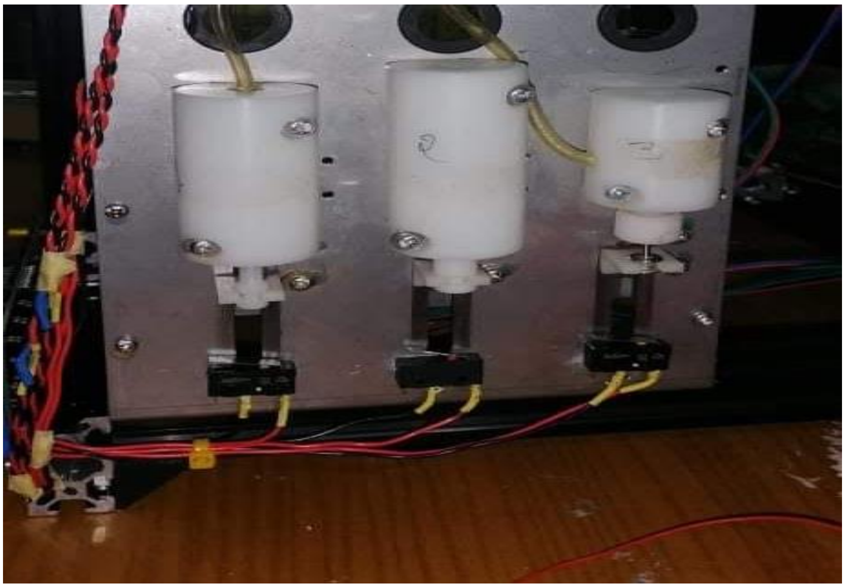


Photo 4: the limit switches of dilutor bloc

```
#include<AccelStepper.h>
#include<JC_Button.h>

#defineMAX_POSITION 0x7FFFFFFF // maximum of position we can set (long type)
Button limitSwitchFor(18); // create J_C Button object that attach to pin 18;
boolstopForward = false;
AccelStepperstepperD(1, 2, 3);// step = pin 2; direction = pin 3

voidsetup() {
Serial.begin(115200);

 // 1st motor for forward and backward slide
  stepper.setMaxSpeed(1000.0);   // set the maximum speed
  stepper.setAcceleration(500.0); // set acceleration
  stepper.setSpeed(150);         // set initial speed
  stepper.setCurrentPosition(0); // set position

}

voidForwardSlide (){

  stepper.moveTo(-MAX_POSITION);
  limitSwitchFor.begin(); // MUST call the loop() function first

  if (limitSwitchFor.isPressed()) {
    Serial.println(F("The limit switch: TOUCHED"));
    stopForward = true;
  }
   if (limitSwitchFor.isReleased()){
    stopForward= false;
```

```
    }

  if (stopForward == false) {

    // without this part, the move will stop after reaching maximum position
    if (stepper.distanceToGo()>0) { // if motor moved to the maximum position
      stepper.setCurrentPosition(0);   // reset position to 0
      stepper.runToNewPosition(0);
    }

    stepper.run(); // MUST be called in loop() function
  }
  else
  {
    // without calling stepper.run() function, motor stops immediately
    // NOTE: stepper.stop() function does NOT stops motor immediately
    Serial.println(F("The stepper motor is STOPPED"));

  }
}
voidloop() {
  // put your main code here, to run repeatedly:
     do{
       ForwardSlide();
     } while(limitSwitchFor.isReleased());// it stops once it’s pressed
}
```

III.3 The realization of the pneumatic part

The pneumatic component of this project is made up of solenoid valves that control the flow of blood, lyse, and diluent needed for measurement as well as a pump for draining the counting chamber.

III.3.1The solenoid valves and pumps interfacing with Arduino:

The solenoid valve and pump can be connected to the Arduino Mega by integrating with the 5 Volt eight-channel relay module since each of the devices draw 500 mA at a 12 Volt DC supply, but the Arduino Mega is only able to supply 40 mA of current at a time, which is insufficient to power the solenoid valve.

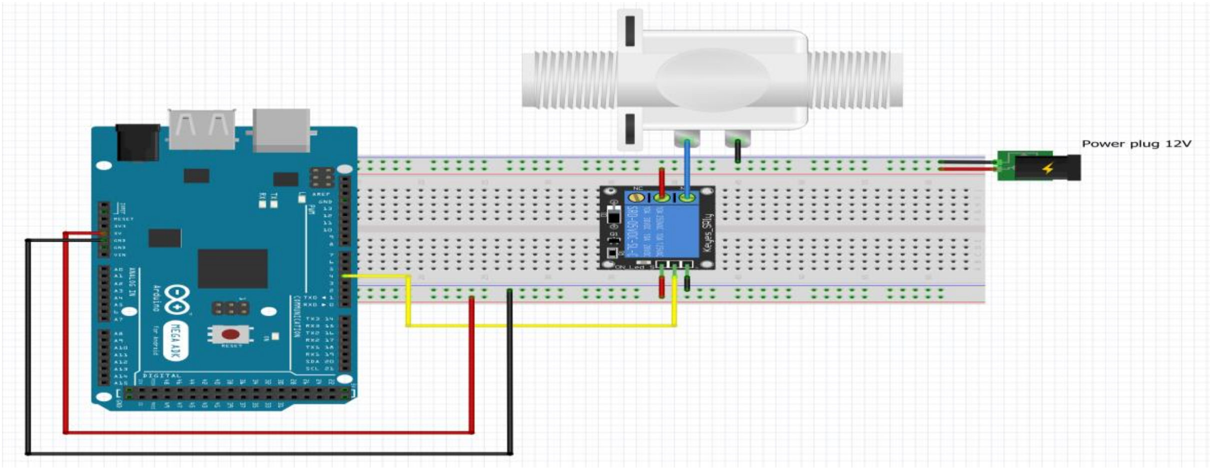


Figure III.5: solenoid valve wiring with ArduinoMega

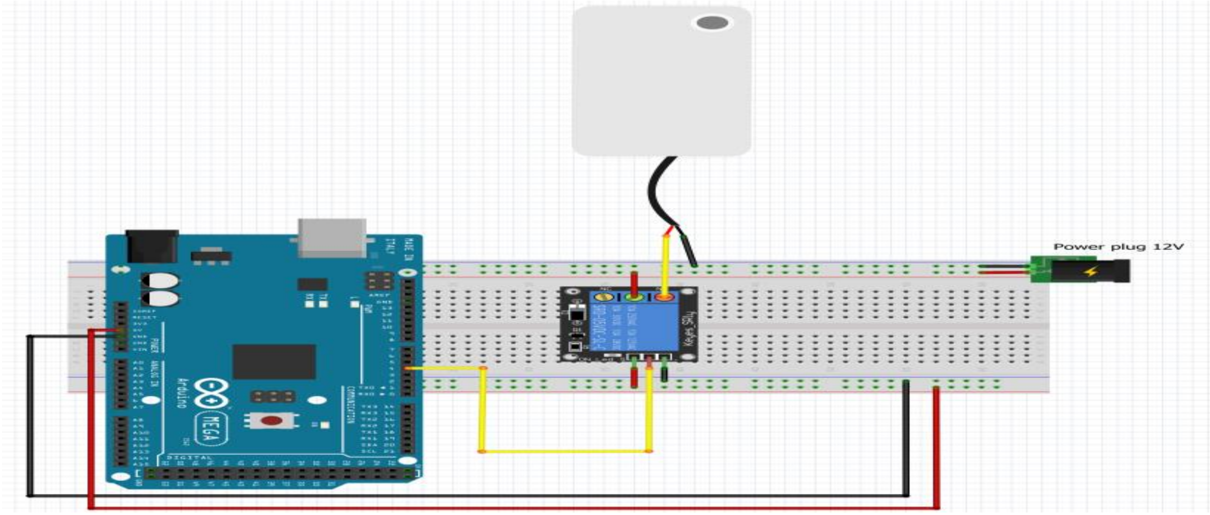


Figure III.6: pump wiring with ArduinoMega

We made the connections in accordance with the circuit diagrams shown above by attaching the ArduinoMega's 5-volt pin to the relay module's VCC pin, the Arduino's GND pin to the relay module's GND pin, and finally, the Arduino's digital pin to the relay module's IN pin.

As shown in the diagram, the positive wire from the 12 volt DC supply should be connected to the common contact on the other side of the relay module, along with the positive wires from the solenoid valve and pump. We should also connect the negative wire from the 12 volt DC supply to the negative wires from the solenoid valve and pump.

```
intvalveL=29;// relay attached to the lyse valve is connected to the Pin 29 of
the Arduino
int pump=31;// relay attached to the pump is connected to the Pin 31 of the
Arduino
```

```
voidsetup() {
 pinMode(valveL,OUTPUT);//set the relay attached to the lyse valve as an
output
 pinMode(pump,OUTPUT);//set the relay attached to the pump as an output

}
voidloop() {
  // put your main code here, to run repeatedly:
     digitalWrite(valveL, LOW);// set the relay of the valve LOW to switch the
valve on, set it high it switch it off
    digitalWrite(pump, LOW);// set the relay of the pump LOW to switch the
pump on
}
```

III. 4 the realization of the analog part:

The analog component has been largely described in the previous chapter. As previously stated, hemoglobin measurement is based on photometry, which necessitates a photodetector. This photodetector plays an important role in the calculation of the hemoglobin concentration obtained from the voltage measured from the output of the LM2917-8N.

III.4.1The light/frequency convertor TSL230R interfacing with Arduino:

The basic idea is to employ a photo detector (TSL230R) that converts light to frequency and a light source (LED) with a wavelength of 540 nm. The TSL230R can be easily interfaced with using an Arduino Mega 2560.

It simply has power and signal out, so we're connecting its pins to our Arduino as seen in the diagram below. But it also has a number of pins that let us adjust the sensitivity and output type.

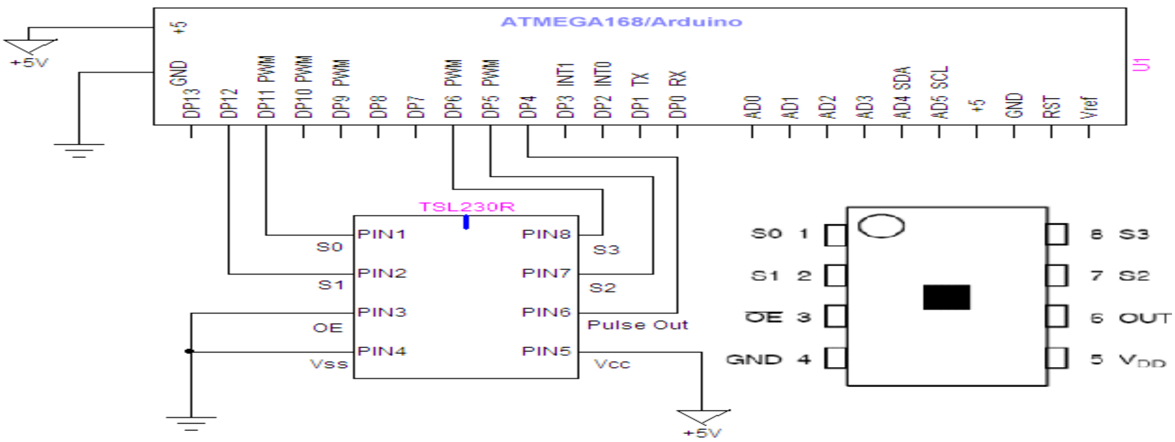


Figure III.7: TSL230R connection to Arduino [46]

S0: Sensitivity select input

S1: Sensitivity select input

S2: Scaling select input

S3: Scaling select input Fo

To regulate the sensitivity levels, we should connect each sensitivity/scaling choose input to an Arduino's digital pins.OE: Output Enable should connected to ground

GND: Ground 0 V

VCC/VDD: +5 VDC supply voltage

OUT: The scaled-frequency output must also be connected to an Arduino digital pin and the LM2917-8N's input.

The code below shows how to control the sensitivity pins, scaling pins and the frequency output.

```
constint led=13;
#defineS036;
#defineS137;
#defineS238;
#defineS339;
#definepulsePin40;

unsigned longpulseCount;
unsignedintval;
voidsetup() {
  pinMode(S0, OUTPUT); // to S0
  pinMode(S1, OUTPUT); // to S1
  pinMode(pulsePin, INPUT);  // freq input
  pinMode(S2, OUTPUT);  // to S2
  pinMode(S3, OUTPUT);  // to S3

    /* set sensitivity
   S1     S0     sensitivity
   L      L      power down
   L      H      1X
   H      L      10X
   H      H      100X
   Higher sensitivity creates
   higher frequency out.
   */

  digitalWrite(S1, 1);
  digitalWrite(S0, 1);

  /* Frequency divide
   S3      S2          (divide-by)
   L       L              1
```

```
   L      H              2
   H      L              10
   H      H              100    */

  digitalWrite(S3, 1);
  digitalWrite(S2, 1);
}

voidtsl(){
int a= 0.9 ;
int b= -8.97;
  pulseCount = pulseIn(pulsePin, HIGH);
  val = pulseCount * 2; // get the period
  // play with 900000 for better accuracy
  val = 900000 / val;

  lcd.print("Freq = ");
  lcd.print(val);
  lcd.println(" Hz       ");
  delay(500);
   lcd.clear();
 // lcd.home();
  // Mesure la tension sur la broche A0
  int val = analogRead(A0);

  // Transforme la mesure (nombre entier) en tension via un produit en croix
  float voltage = val * (5.0 / 1023.0);  // ADC conversion
 int HGB = (voltage-b)/a ;

  Lcd.print(HGB);
  delay(250);
  }
voidloop() {
tsl() ;   // call the function in loop

}
```

The output signal of the light-to-frequency converter TSL230R is depicted in the image below; it is a pulse signal with a frequency that is proportional to light.

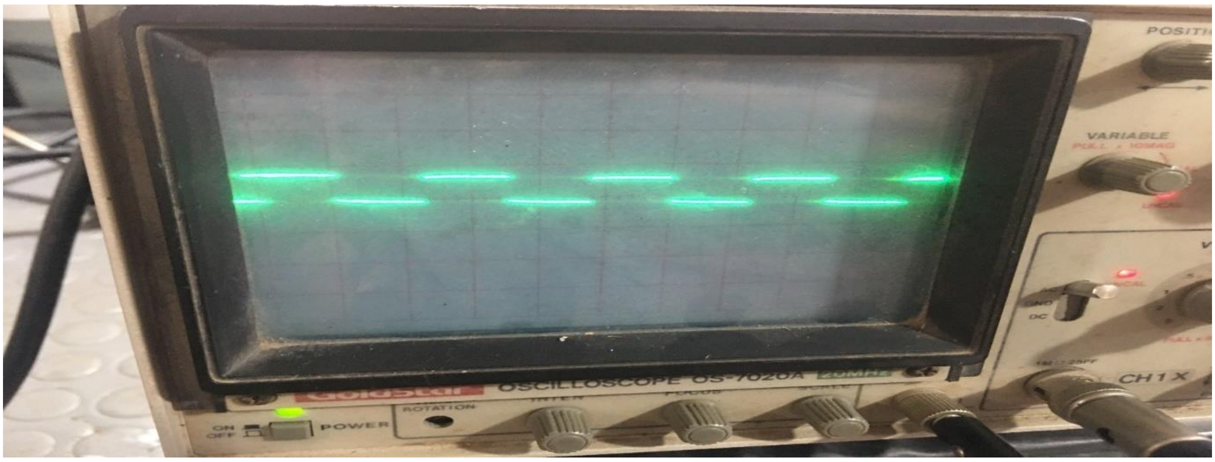


Photo 5: The TSL230R output signal

III.4.2 The power supply:

We should be aware that the supply voltage we will use to power the circuit's components is 12 VDC output, but that the Arduino requires 5 VDC. Therefore, in order to produce a 5 volt output, we should employ a regulator circuit.

Here is a regulator circuit (figure III.8) simulated by proteus software. It consists of a regulator (LM7805) and two capacitors, and its input voltage ranges from 7 to 15 volts.

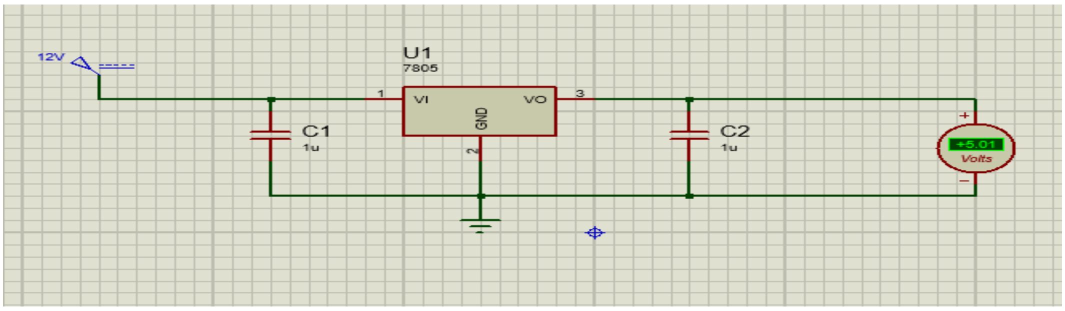


Figure III.8: regulator circuit of 5v output

III.5 The interface and processing part:

This phaseis primarily made up of an Arduino Mega Board microcontroller, which is in charge of digitizing the electrical signal obtained in the analog part, computing and carrying out commands to control LED, buttons, and LCD displayer and the rest of the components.

III.5.1 The frequency/voltage convertor LM2917-8N interfacing with Arduino:

The TSL230R output frequency is converted by the frequency to voltage converter LM2917 to the proportional output voltage, which is interfaced to Arduino.

The frequency to voltage convertor outputs an analog signal when connected to the ArduinoMega'smicrocontroller, however the microprocessor only handles digital signals.

Because of this, this analog signal is converted to digital one through the ADC of the ArduinoMega's. and the Atmega 2560 microcontroller.

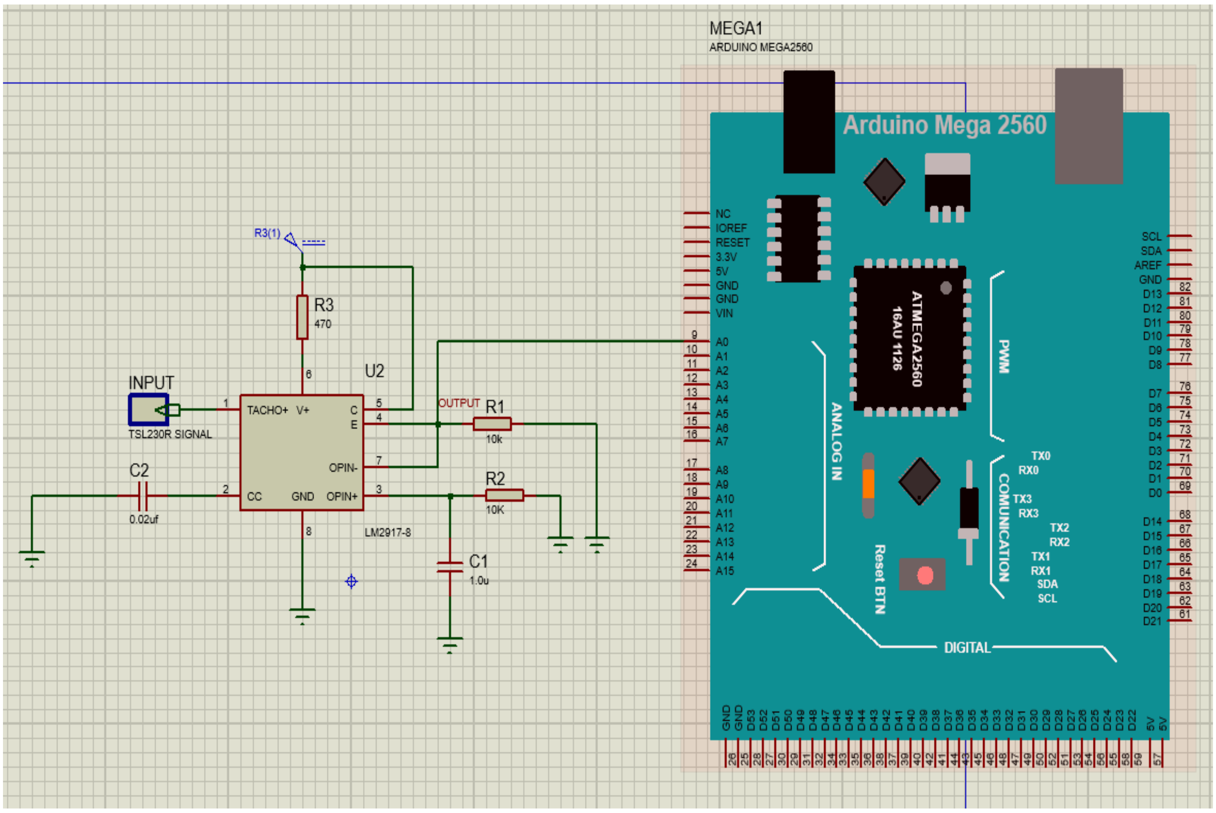


Figure III.9: Analog to Digital Converter (ADC)

The 16 board ADC channels on the Arduino Mega can be utilized to read analog signals between 0 and 5 volts using the function analogRead. The 10-bit ADC on the Arduino can output a digital value between 0 and 1023 (210) because it has this capability.

It may produce throughout the range of analog levels and is referred to as a resolution, which denotes the number of discrete analog levels.

A frequency to voltage converters LM2917-8N, converts the photodetector's frequency output signal to voltage figure III.13. The **analogRead** function on an Arduino analog pin is used to detect the voltage output signal.

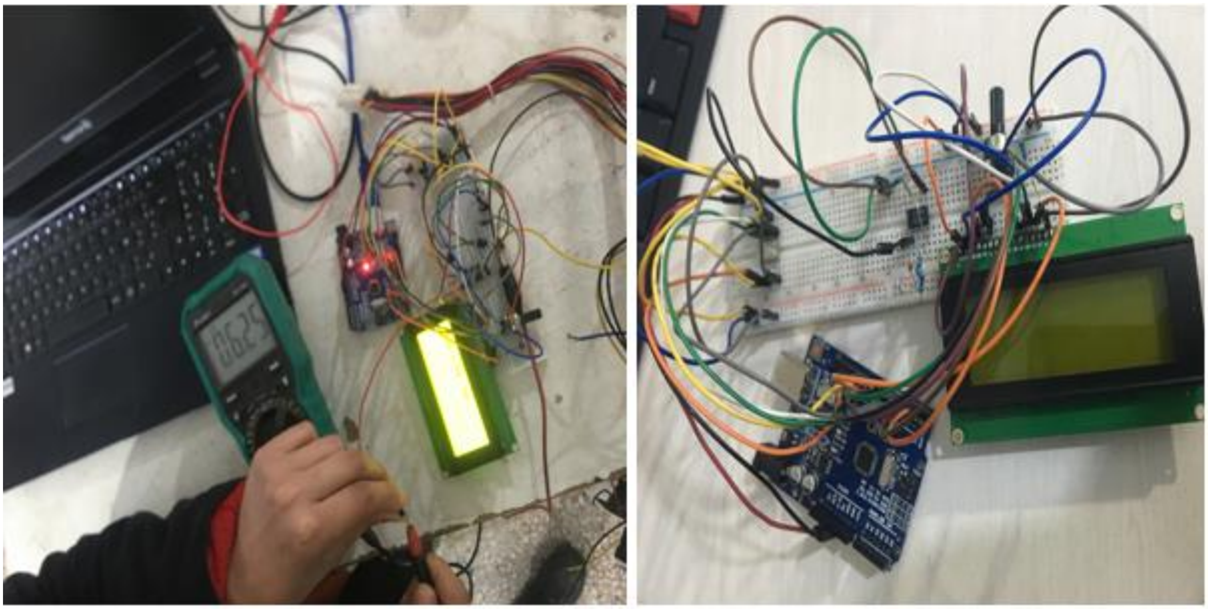


photo 6: the TSL230R and LM2917-8N test

These observed voltages will result in a calibration curve. The straight line equation for this calibration curve is:

Y=aX+b

Y: represents the voltage which is the analog input of the Arduinoboard which must be proportional the frequency converted into voltage.

X: represents the different calibration of hemoglobin concentrations.

a and **b** are coefficients which will be determined by the following formulas :

$$a = \frac{Y2-Y1}{X2-X1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

b= Y1 - a*X1………………….…….(2)

Then we will deduce the equation of the hemoglobin concentration as follows:

X=Y–b/ a…………………………...(3)

These calculations will be carried out manually in order to have the values of the coefficients “a” and “b” and then declare them in the calculation program ofArduino.

III.5.2 The i2c LCD interfacing with Arduino:

Compared to a standard LCD display, connecting an I2C LCD display to an Arduino is fairly straightforward. Only four wires need to be connected to the Arduino.

We wire up the i2c pins module in accordance with the Fritzing-designed schematic. The VCC and Ground pins of the LCD module must be wired to the Arduino's 5 volt and ground pins, respectively.

As indicated in the diagram below, the final two pins of the i2c module, SCL and SDA, should be connected to the SCL and SDA pins of the Arduino. The Arduino is connected to USB, and we are now ready to program. To make the LCD function, we first import the LCD library for Arduino.

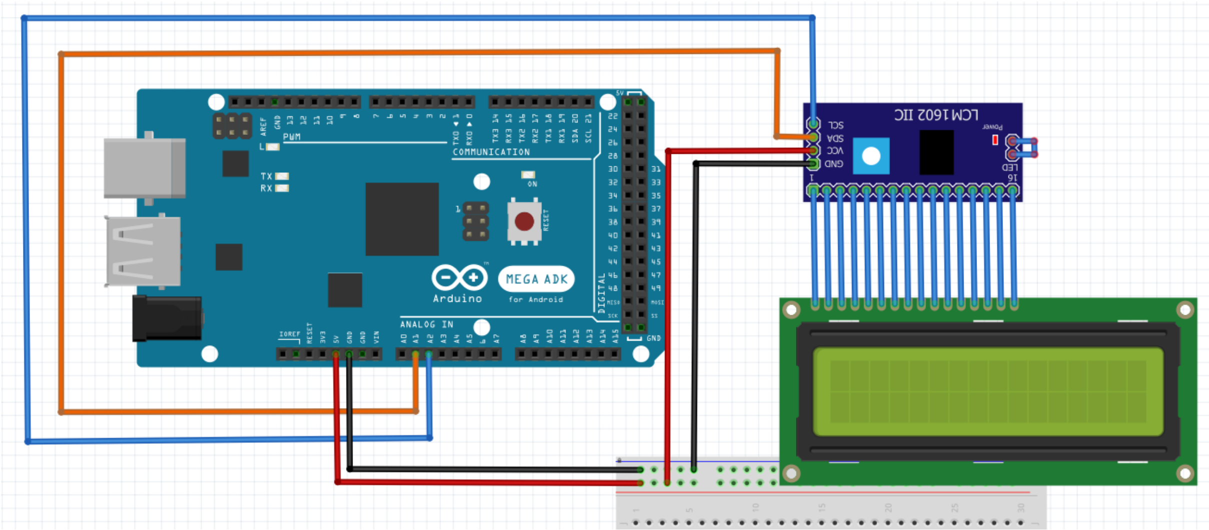


Figure III.10: I2c LCD wiring with ArduinoMega

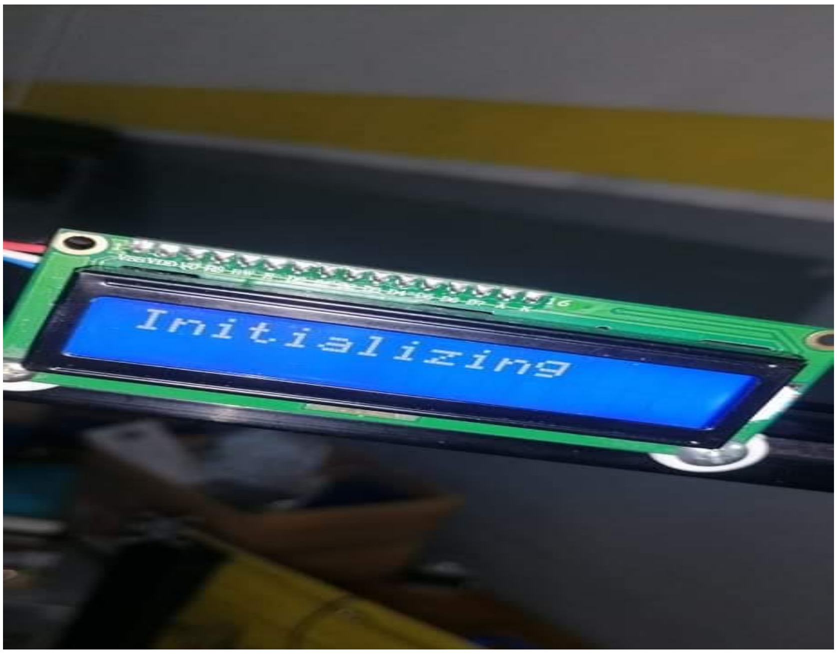


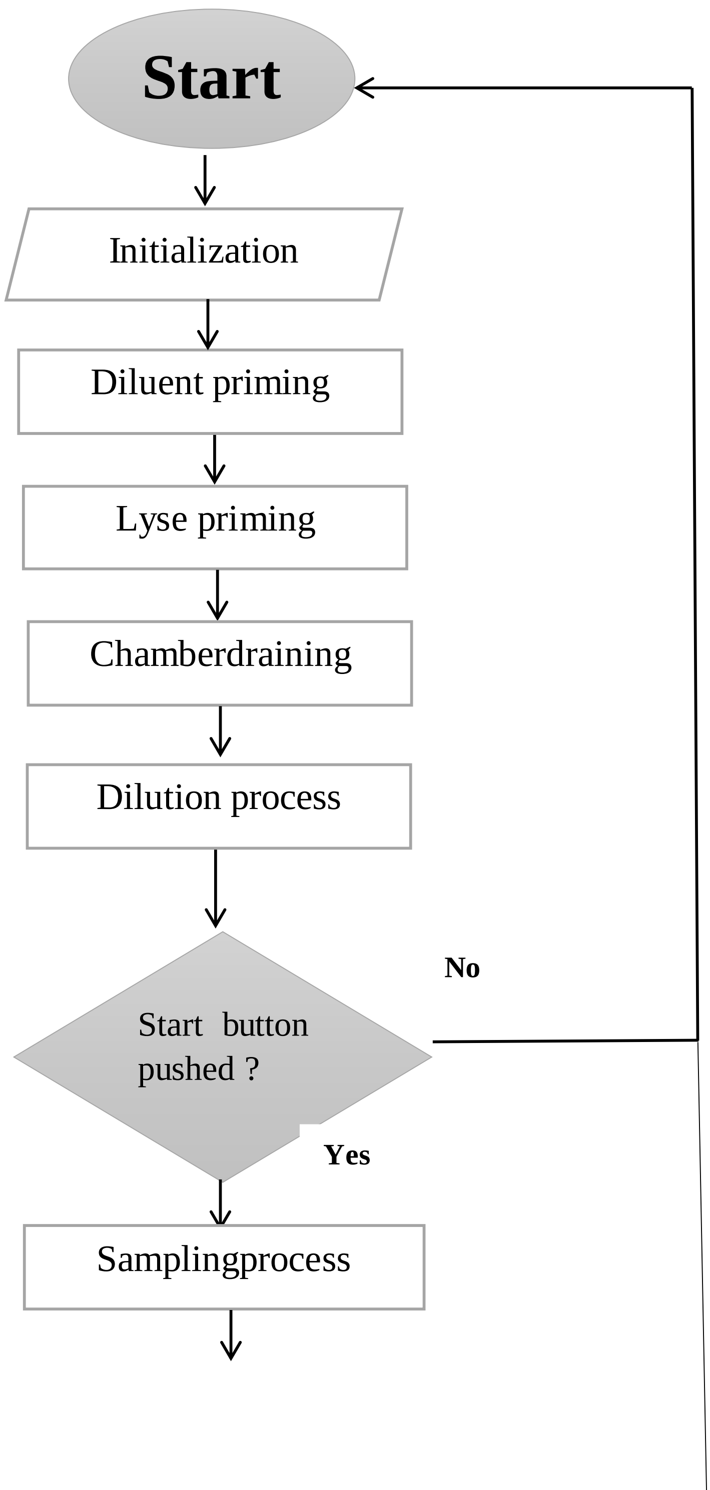
Photo 7: LCD display

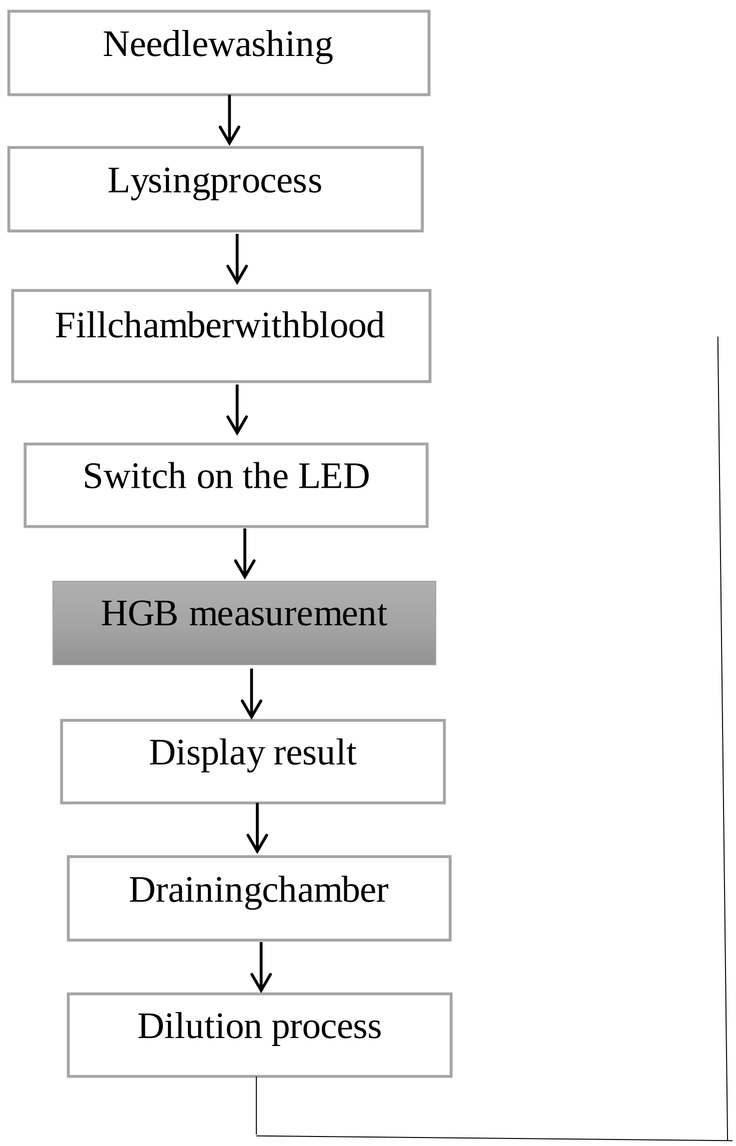
```
#include<LCD_I2C.h>
LCD_I2C lcd(0x27, 16, 2); // Default address of most PCF8574 modules, change
according

voidsetup() {

 Serial.begin(9600);
```

```
  lcd.begin(); // If you are using more I2C devices using the Wire library use
lcd.begin(false)
                 // this stop the library(LCD_I2C) from calling Wire.begin()
lcd.backlight();
}
voidloop() {
lcd.print(HGB);
}
```

III.7Measuring diagram :

The system has its own procedure for measuring hemoglobin; the block diagram of the procedure is shown below, along with a description of every step of it





III.8 Explanation of the measurement diagram

Initialization:consists of putting everything in place mechanically by scanning the movement range (using a limit switch) and checking the valves by turning them all on and off.

Reagent (diluent and lyse) priming: Once the needle is in its initial position, the priming and cleaning menu can automatically prime the reagent (diluent and lyse) syringe. This is done by turning the three-way valves (V1 and V2) on and off and managing the limit switches and stepper motors that move the diluent syringe and lyse syringe up and down. With a slight delay, that will automatically fill the counting chamber.

Draining chamber: Before and after measurement, a pump empties the counting chamber of reagent and blood.

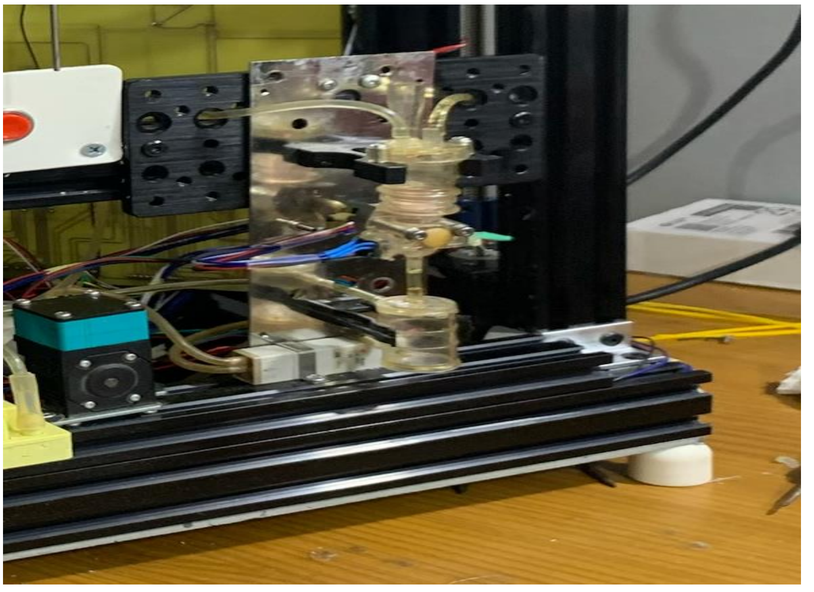


Photo 8: draining pump and couting chamber

Dilution process: the second Dilutor motor rises upward as the counting chamber is filled with 3 ml of diluent before the button start is pressed. This diluent is delivered from the Macro syringe through V1 (On) and the Micro-dilutor.

Stepper motor moves the diluent syringe upward as long as the limit switch is released.

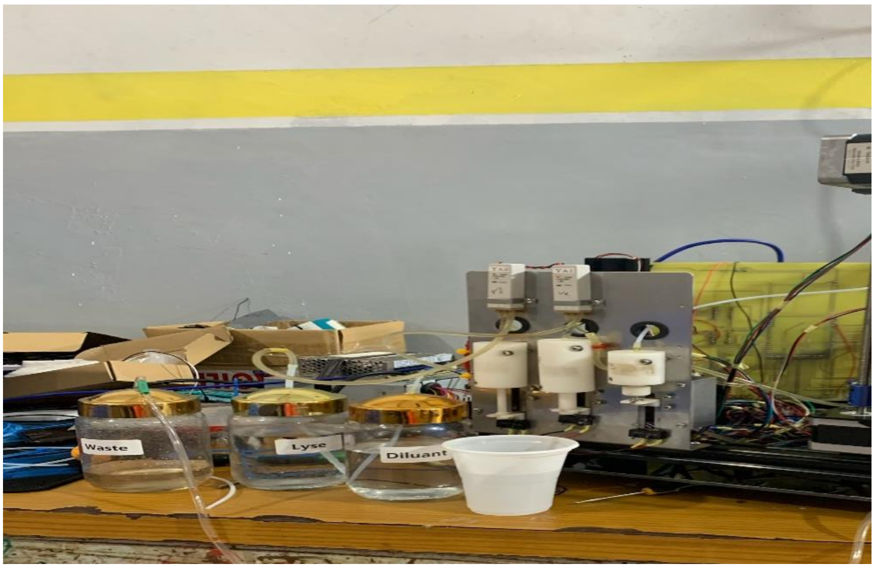


Photo 9: dilutor bloc with reagents

Switch the LED: Turn on an LED with a 540 nm wavelength after the start button is pressedso that a photodetector can detect the light.

Sampling process: A sample of 25 l of blood is aspirated using the aspirating needle. While the third Micro-dilutor motor descends, the Micro-dilutor syringe aspirates.

To prevent erroneous sample, it's crucial to clean the sampling needle's outside surface. The pump removes the diluent from the washing head while the Macro syringe doses.

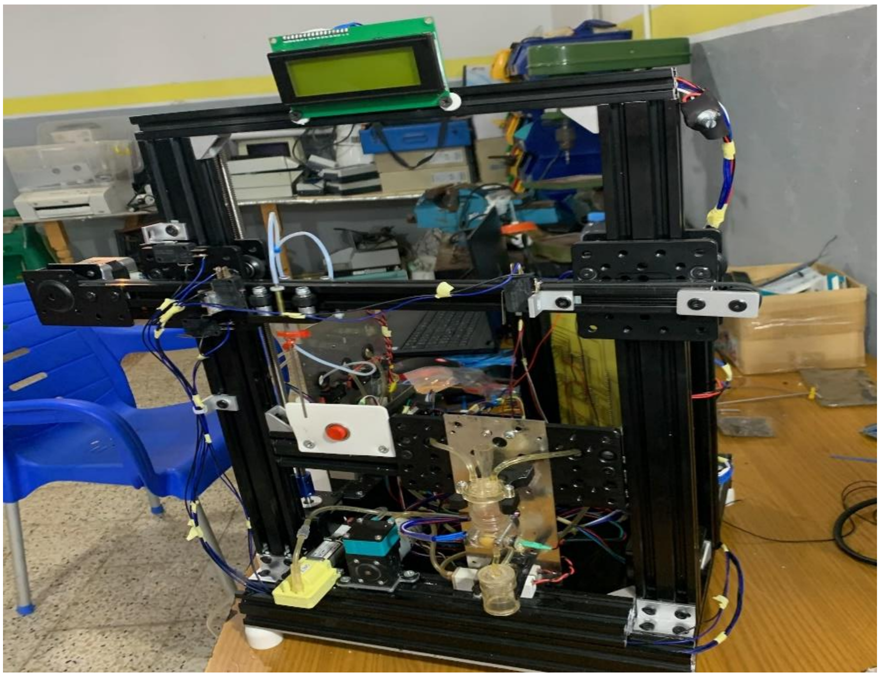


Photo 10: the aspirating needle

Lysing process: In this phase, the Lyse syringe is moved upward while the set lysing reagent is introduced into the counting chamber through V2 (On)as long as the limit switch is released as shown in the figure below.

Photo 11: Dilutor bloc

For better mixing, the pump generates some air bubbles after the lysing process.

HGB Measurement: when the blood sample and lyse are thoroughly combined for a full reaction, allowing the sample's parameter voltage to be evaluated. Using the calculations listed below, HGB may then be computed using the parameter voltage that was measured as explained in the interface and processing part.

When the needle is ready for sampling, we should press the start button, and the blood syringe descends and aspirates a sample; after that, the needle descends and fills the counting chamber with the sample; the led is on, and the photodetector detector detects the light and converts it to frequency; the frequency is then converted to voltage; the voltage is then converted to a digital signal, and the Hb is determined in 13 seconds as described in The frequency/voltage convertor LM2917-8N interfacing with arduino part.

This device is linear and rapid compared to the portable hemoglobinometers that have non linear response to light.

Display result: the result will be displayed immediately on the i2c LCD.

Photo 12: Hb rate measured result

III. 9The electrical diagram of the proposed HGB device design

The Proteus Design Suite is a piece of software that electronic design engineers primarily use to produce schematic capture, simulation, and electronic prints manufacturing PCB (Printed Circuit Board) layout designs. The two key characteristics of this software that we are interested in are:

Schematic capture: Proteus schematic capture combines an effective design environment with full support for design reuse, assembly variants, and a comprehensive BOM reporting sub-system. It has all the electronic design tools required for circuit design.

The suggested HGB device design's electrical schematic is shown in Figure III.23 below.

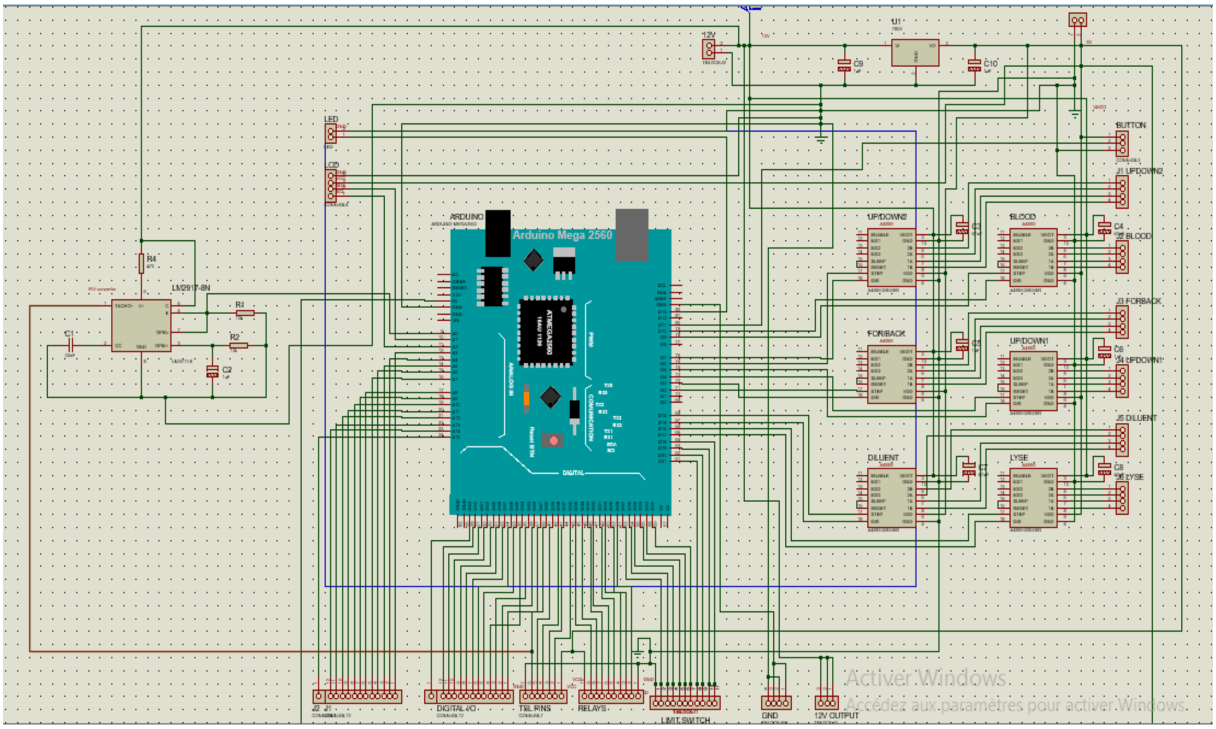


Figure III.11: theelectrical diagram of the proposed HGB device design

PCB design: Proteus PCB Software offers a flexible, powerful, and user-friendly set of tools for designing various circuits on PCBs by combining schematic capture and PCB Layout modules.

The suggested HGB device design's PCB board layout is shown in Figure III.24 below.

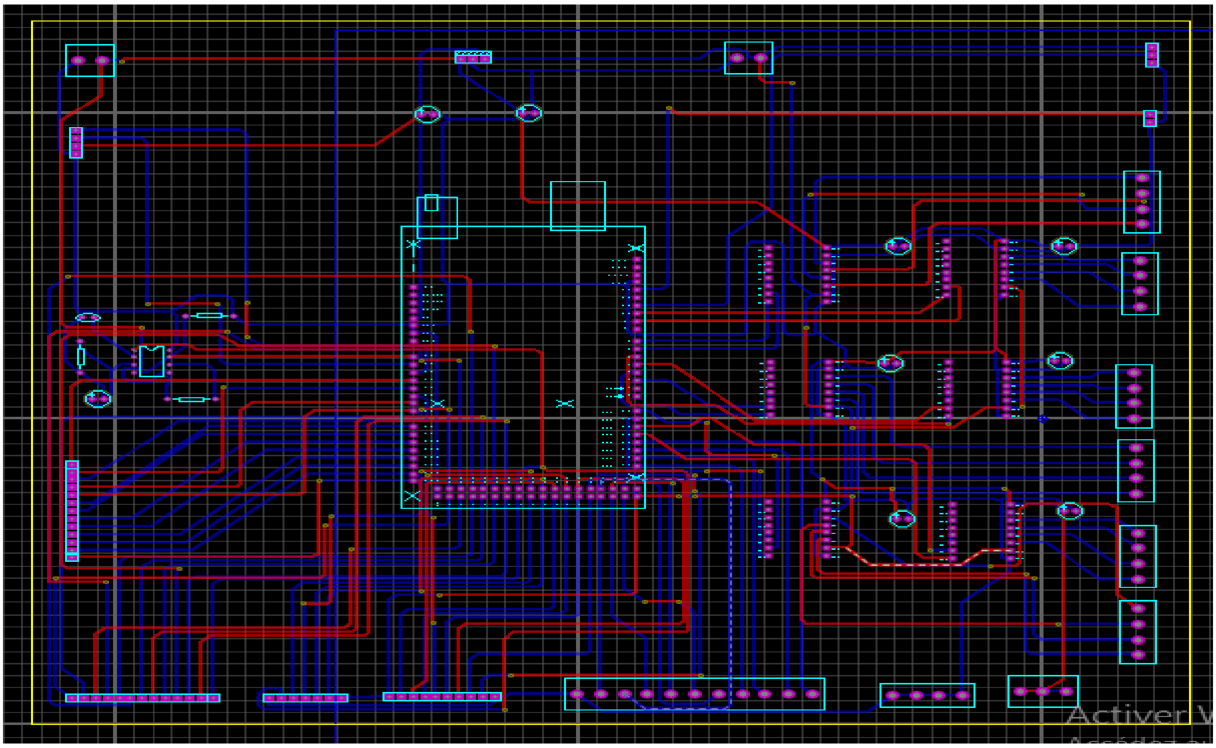


Figure III.12: the PCB board layout of the proposed HGB device design.

The circuit is printed in SNC ALMITech that is one of Algeria's top companies for the sale, production, and design of all kinds of electronic cards. North Africa is home to it. Their services are tailored to our requirements and embellished with creative know-how and unwavering resolve, and they guarantee top-notch goods and unquestionable dependability.

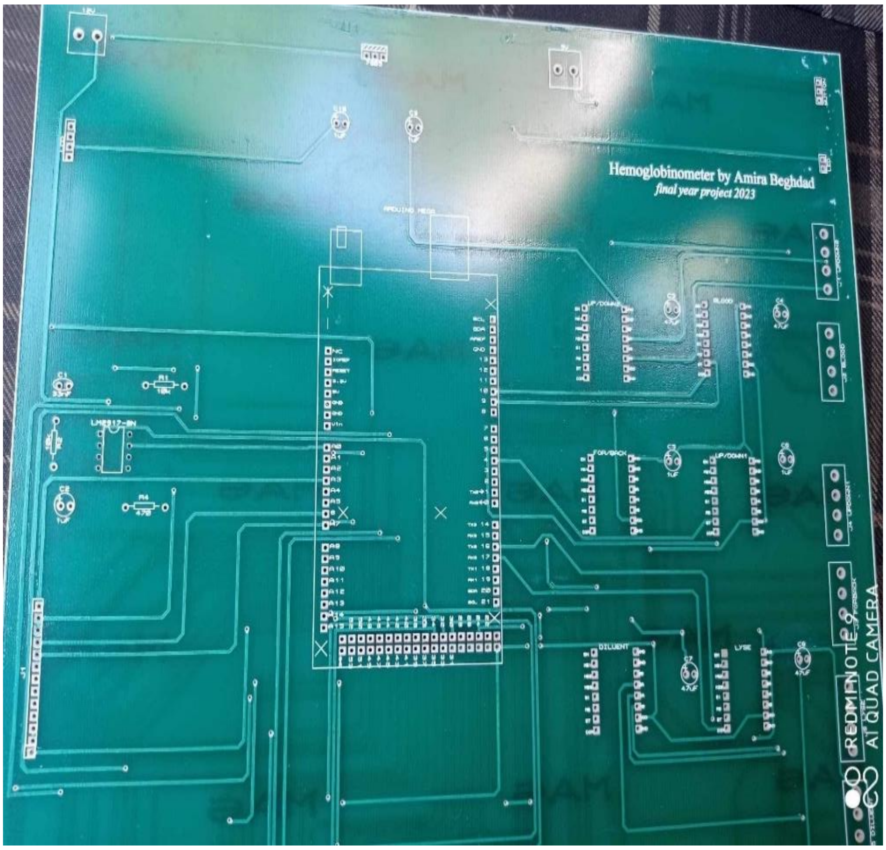


Photo 13: the printed circuit of the system

III. 9 conclusion

The components and materials that can be utilized to assemble our device are all covered in this chapter, along with the wiring of each component with an Arduino with the design software fritzing to assure its proper operation.

Additionally, we have described how hemoglobin is calculated and provided a diagram showing how it is measured. We have also provided an electrical diagram and a pcb board layout of our system.

III.10 The reference

[46] :https://www.instructables.com/Arduino-Interfacing-TSL230R-Light-Frequency-Conver/

Conclusion and perspective:

The present study has described the several hemoglobin estimation techniques that are available, each with advantages and disadvantages of its own. Thus, in order to develop our hemoglobinometer, we concentrated on the cbc analyzer method as the fundamental concept for hemoglobin estimation after describing the fundamentals of each approach and resolving the issue with hematological labs.

We were able to understand the fundamental principles underlying the operation of the numerous mechanical and analog components necessary to the hemoglobin measurement system, including stepper motors, solenoids, voltage converters, frequency to light converters, and even programming. We also discussed some results that should be reached through various tests and provided a detailed description of how the proposed system would actually be put into practice.

As perspectives, we advise interested parties to complete this device and making it rapid, dependable clinical tools for determining blood's hemoglobin concentration and frequently utilized in a variety of therapeutic settings, including community hospitals, private labs, healthcare facilities, and primary care clinics.