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MEASURING OF CLOTTING TIME OF BLOOD USING AN ARDUINO PLATFORM AND AN IMPEDANCE CONVERTER.

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Abstract: *Monitoring blood coagulation time is crucial for diagnosing and managing hematological disorders, including coagulation disorders such as hemophilia, and for monitoring anticoagulant treatment plans in patients with cardiovascular conditions. Standard laboratory devices, although precise, require the patient to be present in a clinical setting, are bulky, and often expensive. The creation of a portable monitoring system offers numerous advantages, allowing for constant and real-time monitoring of coagulation parameters, providing additional comfort for the patient, facilitating rapid intervention in case of problems, and reducing the need for clinic visits. Considering these aspects, we decided to develop a portable blood coagulation time monitoring device to assist individuals with hematological disorders.*

Keywords: *Arduino, impedance, converter, AD5933, blood, coagulation, clotting time.*

1. INTRODUCTION

Coagulometry is a key component in the diagnosis and treatment of conditions related to blood coagulation. This analytical action provides information about the coagulation process, significantly contributing to the correct management of therapy and the prevention of complications associated with these disorders. The importance of coagulometry is manifested in its ability to provide accurate data on clotting time, the concentration of clotting factors, and the effectiveness of anticoagulant treatments [1]. Over the years, coagulation tests have been introduced, essential for monitoring patients with specific disorders and for evaluating the effectiveness of anticoagulant treatment. To date, various advanced laboratory techniques and technologies have been developed, tests that are used to adjust the dosage of anticoagulants to ensure an optimal balance between the prevention of clots and the risk of bleeding, these more accurate and faster assessment, monitoring, and treatment. Thus, this technique becomes essential in ensuring a personalized and efficient approach in the treatment of patients with cardiovascular diseases, coagulopathies, or before major surgical interventions.

Due to its capacity and role in the clinical environment, the coagulometer can be integrated into different medical units, such as transfusion centers, emergency units (UPU), cardiology clinics, etc. Also, the use of nanomaterials and biotechnologies in coagulometry devices improves the sensitivity and specificity of analyses, bringing innovation to the monitoring and management of blood coagulation [1]. The coagulation process is triggered in the event of an injury to a blood vessel and aims to stop the bleeding and repair the affected area. Thus, it follows several stages until the proposed goal is completed:

- Vasoconstriction: contraction of vessels to reduce blood flow [2].
- Primary hemostasis plaque formation: platelets move to the injured site to release activating chemicals.
- Formation of secondary hemostasis: plasma proteins are activated, respectively the coagulation factors that help to form the fibrin clot.

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- Transformation of fibrogen into fibrin: fibrin forms a network that strengthens the clot formed.
- Blood vessel repair: after the bleeding stops, the repair process begins [2].
- Inhibition of excessive coagulation: coagulation around the affected area is limited and further clots are avoided.
- Negative control of coagulation: the fibrinolytic system triggers the process by which it dissolves clots to restore normal circulation [2].

2. MATERIALS AND METHODS

2.1 Method of determining blood coagulation time using capillary glass tubes

Clotting Time is the time required for blood to form a clot. The normal coagulation time in glass tubes is 5 to 15 minutes. The whole blood clotting time is a rough measure of all intrinsic clotting factors in the absence of tissue factors. Clotting Time is used in the diagnosis of hemophilia and its chief application is in monitoring anticoagulant therapy. The process of determining the coagulation time, in the case of people undergoing anticoagulant treatment, can also be done in the laboratory, not only with the help of some devices. For this experiment, we need the following materials [3]:

1. Capillary tubes of uniform size, containing no anticoagulant.
2. Petri dish, used for cultivating microorganisms or for other biological experiments.
3. Alcohol swabs for skin sterilization.
4. Plasticine, moldable, and non-toxic materials to seal samples.
5. Water bath set at 37°C, for incubating or maintaining the sample at a certain temperature, respectively at the temperature of the human body. The following stages will be completed:

- Disinfect the index finger with an alcohol swab and prick it with a lancet, noting the minute the puncture was made. The first drop of blood is wiped off, and one end of the capillary tube is placed on the next drop [3].
- The capillary tube is held horizontally, allowing it to fill, then the end is closed with plasticine and placed in the water bath.
- At an interval of 2 minutes after performing the puncture, break the capillary tube and separate it into two halves carefully, repeating this process at an interval of 30 seconds and with the remaining tubes.
- The moment when the blood forms a continuous, thread-like clot between the two ends of the tube, the endpoint has been reached [3].
- The time interval from the moment of puncture to the formation of a blood clot is the coagulation time. By using this method, the measured clotting time falls within a range of 3-6 minutes.

2.2 Ivy Method

A standardized method is the sphygmomanometric one, also called the Ivy Method. This consists of applying a cuff to the subject's arm and inflating it to 40mmHg. Two small incisions are made on the forearm, 5-10 cm apart, deep enough to cause bleeding, and the event is noted. When bleeding starts, the cuff is deflated. With a sterile absorbent paper touch the cuts every 30 seconds until the bleeding stops [4]. Thus, the duration of bleeding from the time of the incision to its end represents the coagulation time, respectively the time required for the blood vessels to constrict and for the vanes to act in the area of the incisions. In a non-diseased subject, bleeding should stop within 1-9 minutes (Fig.1.).

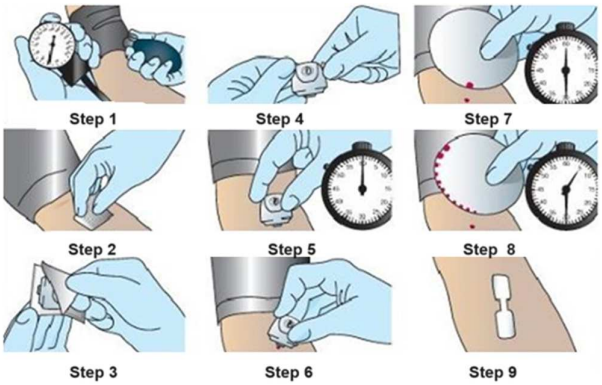

Fig. 1. Measurement steps by the Ivy method.

If bleeding lasts more than 15 minutes, stop the procedure and apply pressure over the lesions.

2.3 Duke Method

Another well-known measurement method is the Duke method, which works on the principle of making a standard incision in the finger or the earlobe. The following materials are used for this: Alcohol, 70% ethanol; Sterile lancet or needle; Filter paper; Timer clock, and Cotton pads. The measurement procedure is similar to the Ivy method, following the following steps:

1. Disinfect the surface with alcohol and perform the puncture. Start the timer and record the time.
2. Wipe the drop of blood with the circular filter paper every 30 seconds. When the bleeding stops, stop the timer.
3. Finally, count the drops on the filter paper and multiply by 30 seconds, then report the time in minutes [4].

2.4 Analysis of coagulation time measuring methods

Following several studies and research on these procedures, the Ivy method is more accurate than the Duke method, while also presenting a lower risk of hematoma or infection. In this field, Point-of-Care tests have an important role in the detection of coagulopathies and the monitoring of anticoagulant treatments.

Prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), and prothrombinase-induced clotting time (PiCT) are the most useful tests for investigating patients suffering from such diseases. The PT test is sensitive to deficiencies of factors II (Prothrombin), V (Proaccelerin), VII (Proconvertin), X (Stuart-Power factor) and measures coagulation from the extrinsic and common pathways, while aPTT measures blood coagulation from the intrinsic and common pathways, being sensitive to factors XII (Hageman factor), XI (Antecedent of plasma thrombo-plastin), IX (Christmas factor) and VIII (Antihemolytic factor) [5]. The International Normalized Ratio (INR) is used to standardize PT values, including reagent and instrument sensitivity to maintain patients within normal limits. The INR range is between 2.0 - 3.0, with a result greater than 4.9 being considered critical and with an increased risk of bleeding, and a value less than 2.0 is used to reduce the risk of bleeding [6].

$$INR = \left[\frac{PT(pat)}{Pt(n)}\right]^{ISI} \quad (1)$$

where: *PT(pat)* represents the patient's prothrombin time; *Pt(n)* represents the normal reference range; ISI stands for International Sensitivity Index [7]. Currently, the *aPTT* test is the standard test used in the hospital to be a performance indicator that measures the effectiveness of the coagulation pathways. In addition to detecting abnormalities, it is also used in monitoring the effects of anticoagulant treatment. To perform the test, samples are collected in tubes with oxalate or citrate to prevent clotting by binding calcium, then the plasma is separated by centrifugation in the laboratory (Fig.2.) and mixed with reagents including phospholipids, activators such as silica, celite or ellagic acid, and calcium to activate the intrinsic way [8]. Fibrin formation is the result of a complex reaction involving several clotting factors. For its detection, several measurement methods have been reported such as: Ultrasonic method; Optical measurement; and Detection of changes in the mechanical and electrical properties of blood. These methods require bulky equipment in the laboratory, and obtaining plasma in the *aPTT* assay is time-consuming and labor-intensive.

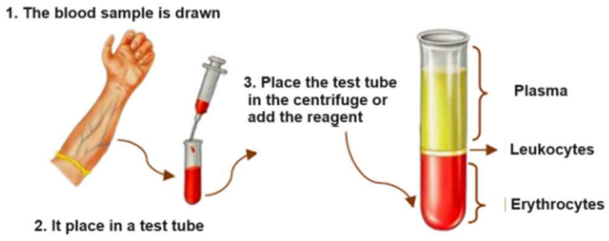

Fig. 2. Separation process [9]

After analyzing the previously mentioned detection methods, it was reported that the most efficient method for measuring the clotting time is by measuring the electrical impedance. Moreover, this form of measurement can be used in the development of a coagulation time determination device because the electrical signal resulting from the measurements can be interpreted by a compact electrical circuit.

From the literature, it has been found that the magnitude of impedance change is associated with plasma fibrinogen concentration. Thus, the impedance represents the erythrocyte sedimentation rate (ESR) and evaluates the quality of the stored blood. The electrical impedance method has been reported to be reproducible and shows a good correlation with other standard methods [8]. Miniaturization and single-use properties of a coagulation testing device are important point-of-care requirements for routine testing.

3. DESIGN, CONSTRUCTION AND PROGRAMMING OF THE DEVICE

3.1 The structure of the measuring device

The measuring device is designed to facilitate fast and accurate blood clotting time testing, it uses a combination of modern electronic components to provide an efficient alternative to traditional laboratory methods. The microfluidic channel enables precise handling of blood samples, the Arduino Uno R3 board handles the data processing and user interface, the AD5933 converter measures impedance precisely, and the LCD text display displays the results in a clear format. The device works like this:

- A drop of the blood sample is placed on the slide, then it is inserted into the microfluidic channel.
- The plate controls the blood flow, it receives the signal at the time of coagulation.
- The AD5933 converts the measured impedance and the result is displayed in seconds on the LCD text display.

An advantage of this system is its portability, being compact and easy to transport, it allows testing in almost any condition. The low cost of the components, the speed of receiving the results in a few minutes, and the accuracy provided by the converter are advantages of the chosen device.

3.2 Electrical circuit design

The creation and simulation of the electric circuit were done with the help of the EAGLE ver.9.6.2 software (Fig. 3).

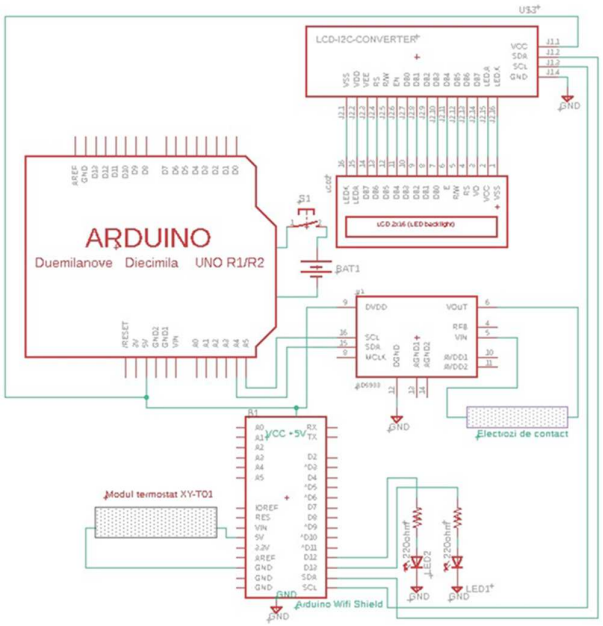

Fig. 3. Electrical circuit of the device

The electrical circuit of the device consists of: the Arduino Uno R3 platform, Arduino WiFi Shield (Fig.4), the AD5933 impedance convertor, LEDs, LCD 2x16 display, LCD I2C Converter, Ag-AgCl surface electrodes, digital thermostat and 9V battery. For the integration of the coagulation time measuring device in a telemedicine or IOT system, we decided to use a shield with WiFI, Bluetooth, and memory card facilities (Fig.4.). To simplify the way to connect the 2x16 LCD module to the Arduino Uno R3 platform I used an I2C 1602 LCD converter.

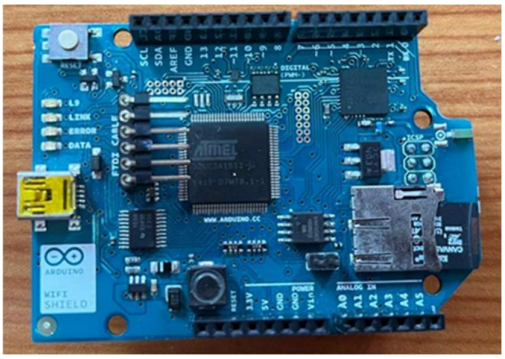

Fig.4. Arduino WiFi Shield compatible with Arduino Uno R3

3.3 Construction of device

After the design and simulation of the electric circuit (with the help of the EAGLE 9.6.2

software), we moved on to the practical realization of the electric circuit.

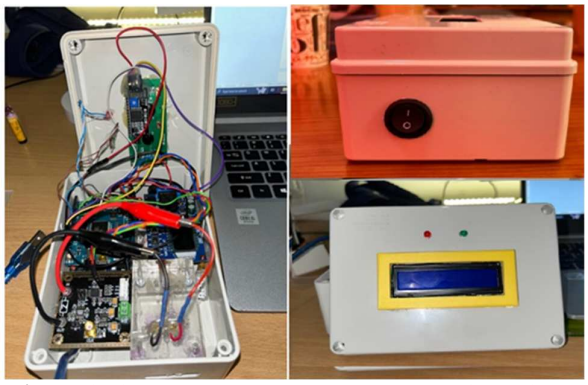

Fig.5. The internal and external appearance of the blood clotting time-measuring device

The following components were connected to the Arduino Uno R3 platform one by one: the 2x16 LCD together with the I2C 1602 converter, the Arduino WiFi shield, the AD5399 impedance converter, a 9V battery for power, an ON/OFF switch, 2 LEDs and a digital thermostat (Fig.5). All these components were placed in a plastic case of appropriate dimensions.

3.4 Programming of the device (The software subsystem)

When programming the device (of the Arduino Uno R3 platform), the Arduino IDE programming environment was used, a popular and easy-to-use platform for projects of this kind. The libraries used in making the source code of the device were: Wire.h; LiquidCrystal_I2C.h and AD5933.h.

To begin with, several local variables were declared for coagulation detection:

```
bool probeIntrodusa = false;
unsigned long startProbeTime = 0;
unsigned long deviceStartTime = 0;
bool deviceReady = false;
```

For the AD5933 impedance converter, several variables and values for frequency and reference resistance have been declared:

```
#define START_FREQ  (80000)
#define FREQ_INCR   (1000)
#define NUM_INCR    (40)
#define REF_RESIST  (10000)
```

When the ON/OFF switch is pressed, the entire electrical circuit is powered (from the 9V battery) and the 1602 LCD will display the message "*Insert Sample- Introdu Proba*" and the green LED will light up. The following lines of code were used to execute this statement:

```
void loop(void)
{
  if (!deviceReady && (millis() -
deviceStartTime >= 30000)) {
    deviceReady = true;
    digitalWrite(ledVerde, HIGH);
    lcd.clear();
    lcd.setCursor(0, 0);
    lcd.print("Introdu proba");
  }
```

To detect the sample inserted into the microfluidic channel, the following lines of code are used:

```
if (deviceReady && !probeIntrodusa &&
statusXYT01 == LOW) {
    probeIntrodusa = true;
    startProbeTime = millis();
```

When this message appears, the green LED turns off and the red LED turns on (Fig.6).

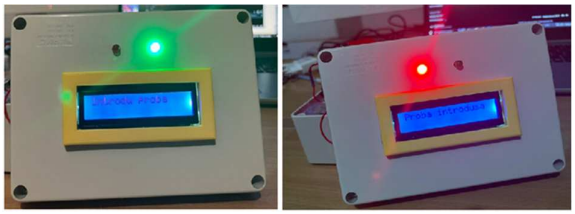

Fig.6. State of the LEDs during the test

The following instructions are used to calculate the coagulation time in minutes and seconds:

```
if (probeIntrodusa && statusXYT01 ==
HIGH) {
    probeIntrodusa = false;
    unsigned long coagulationTime =
(millis() - startProbeTime) / 1000;
    unsigned long minutes =
coagulationTime / 60;
         unsigned   long   seconds   =
coagulationTime % 60;
```

To display the data both in the Serial Monitor and on the 1602 LCD display, the following lines of code were used:

```
lcd.clear();
    lcd.setCursor(0, 0);
    lcd.print("Timp coagulare:");
    lcd.setCursor(0, 1);
    lcd.print(minutes);
    lcd.print(" min ");
    lcd.print(seconds);
    lcd.print(" sec");
    Serial.print("Timp coagulare:");
    Serial.print(minutes);
    Serial.print(" min ");
    Serial.print(seconds);
    Serial.println(" sec");
```

3.5 Calibration of the device

To ensure the accuracy of impedance measurements using the AD5933 converter, it is important to perform a proper calibration. This method allows the system to correct any errors and establish an accurate reference point for subsequent measurements. The calibration was carried out in the Measurements and Instrumentation laboratory (from the Faculty of Product and Environmental Design) using the Tins-ley ZX93 Decadal Resistor (Fig.7.). The Tinsley ZX93 Decadal Resistor has been designed to provide precision and stability measurements, being used particularly in metrology laboratories and the precision industrial sector.

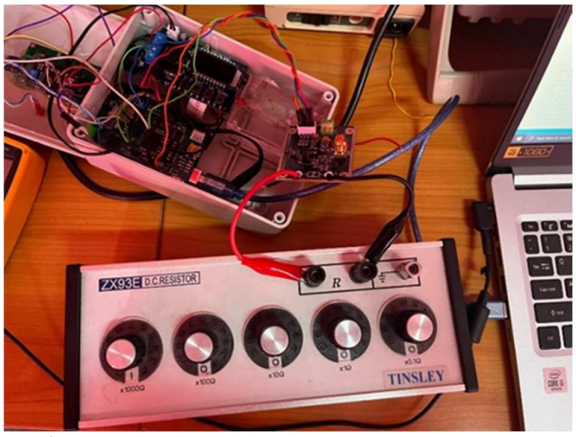

Fig.7. Calibration of the device using Tinsley ZX93 decade resistor

3.6 Carrying out the first tests

The initial state of the parameters:

- Start frequency: 80 kHz;
- Frequency increment: 1kHz;
- Number of increments: 40;
- Reference resistance: 1 kΩ;

The AD5933 has been reset to establish a known initial state. Parameters such as start frequency, frequency step, and total number of steps have been configured. In addition, a 1 kΩ reference resistor was connected between the measurement terminals of the AD5933 device. A frequency scan was performed to obtain the calibration data (gain and phase) associated with each frequency point (Fig.8).

```
15:30:29.636 -> □□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□
15:30:32.251 -> Calibrated!
15:30:32.251 -> Calibration Data:
15:30:32.251 -> Start Frequency: 80000 Hz
15:30:32.284 -> Frequency Increment: 1000 Hz
15:30:32.317 -> Number of Increments: 40
15:30:32.349 -> Reference Resistance: 1000 Ohms
15:30:32.382 -> Increment 0: Gain = 0.00, Phase = 0
15:30:32.416 -> Increment 1: Gain = 0.00, Phase = 0
15:30:32.448 -> Increment 2: Gain = 0.00, Phase = 0
15:30:32.517 -> Increment 3: Gain = 0.00, Phase = 0
```

Fig.8. The first data displayed in the Serial Monitor

```
15:30:33.824 -> Increment 37: Gain = 0.00, Phase = 0
15:30:33.890 -> Increment 38: Gain = 0.00, Phase = 0
15:30:33.922 -> Increment 39: Gain = 0.00, Phase = 0
15:30:33.955 -> Increment 40: Gain = 0.00, Phase = 0
15:30:34.976 -> 80: R=-14841/I=13479  |Z|=999.97
15:30:35.009 -> 81: R=-14993/I=13302  |Z|=1000.09
15:30:35.041 -> 82: R=-15134/I=13130  |Z|=1000.19
15:30:35.073 -> 83: R=-15299/I=12935  |Z|=1000.14
15:30:35.106 -> 84: R=-15440/I=12761  |Z|=999.89
15:30:35.139 -> 85: R=-15559/I=12593  |Z|=999.49
15:30:35.204 -> 86: R=-15735/I=12385  |Z|=999.77
15:30:35.237 -> 87: R=-15876/I=12192  |Z|=999.86
15:30:35.269 -> 88: R=-16009/I=12010  |Z|=999.85
15:30:35.303 -> 89: R=-16154/I=11811  |Z|=999.87
15:30:35.335 -> 90: R=-16285/I=11617  |Z|=999.50
15:30:35.368 -> 91: R=-16419/I=11413  |Z|=999.89
15:30:35.401 -> 92: R=-16544/I=11221  |Z|=999.87
15:30:35.433 -> 93: R=-16666/I=11031  |Z|=999.92
15:30:35.467 -> 94: R=-16796/I=10830  |Z|=999.90
15:30:35.499 -> 95: R=-16913/I=10632  |Z|=999.90
15:30:35.532 -> 96: R=-17040/I=10421  |Z|=999.83
15:30:35.565 -> 97: R=-17153/I=10221  |Z|=999.98
```

Fig.9. Data displayed in the Serial Monitor after calibration

The test stage of the device consisted of the use of a laboratory synthetic compound level 2,

Rapid Point 500, manufactured by Siemens (Fig. 10.) for the calibration of blood analyzers, the procedure for determining the coagulation time was initiated. For placement of the synthetic composite sample, a 75 x 26 mm ground-end microscope slide was used, which was inserted into the pre-prepared slot in the device housing. Turned on the device by pressing the ON/OFF switch and followed the instructions displayed on the LCD screen (green LED is on). Following the displayed message, the sample was prepared and inserted into the microfluidic channel, at which time the warning message was displayed on the screen, indicating that the sample was detected and the measurement started. It should be noted that the temperature at which the tests are carried out is very important (it must be maintained between 36 and 37 degrees Celsius).

Fig.10. Calibration solution

A digital thermostat, an incandescent bulb (which lights up when the temperature drops below the set limit), and a fan (which starts when the temperature rises above the set limit) were used to control the temperature (Fig.11).

Fig.11. Components used to control the temperature in the room where the measurement takes place

4. CONCLUSIONS

A portable coagulometer has been developed that represents a promising tool for rapid testing and uses an innovative bioelectrical impedance technique for measurement. The device can be easily integrated into a telemedicine or IOT system through Wi-Fi and Bluetooth connectivity facilities. Its portability, ease of use, and low cost make it an ideal candidate for use in various environments, starting with Medical Engineering or Medical Electronics Laboratories.

5.Acknowledgements. Given that the electrical safety tests were carried out in collaboration with the team of engineers and technicians of the Brașov County Medical Equipment Center (CJAM-Bv), we would like to thank them in this way.

6.Conflict of Interest. The authors declare that they have no conflict of interest.

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MĂSURAREA TIMPULUI DE COAGULARE A SÂNGELULUI FOLOSIND O PLATFORMĂ ARDUINO ȘI UN CONVERTOR DE IMPEDANȚĂ.

Rezumat: Monitorizarea timpului de coagulare a sângelui este crucială pentru diagnosticarea și gestionarea tulburărilor hematologice, inclusiv tulburările de coagulare, cum ar fi hemofilia, și pentru monitorizarea planurilor de tratament anticoagulant la pacienții cu afecțiuni cardiovasculare. Dispozitivele standard de laborator, deși precise, necesită ca pacientul să fie prezent într-un cadru clinic, sunt voluminoase și adesea costisitoare. Crearea unui sistem portabil de monitorizare oferă numeroase avantaje, permițând monitorizarea constantă și în timp real a parametrilor de coagulare, oferind confort suplimentar pacientului, facilitând intervenția rapidă în caz de probleme și reducând nevoia de vizite la clinică. Având în vedere aceste aspecte, am decis să dezvoltăm un dispozitiv portabil de monitorizare a timpului de coagulare a sângelui pentru a ajuta persoanele cu tulburări hematologice.

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