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Development of an Automatic Electronic System to Human Blood Typing

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Abstract— **Blood typing has a vital contribution to the success of life-saving procedures, such as blood transfusions, and it can be critical, especially in emergency situations. For that, in this paper the main principles to the development of a miniaturized, low cost, portable and automatic system to human blood typing, in emergency situations, are presented. In a previous study, the authors validated a general experimental protocol to be applied in the automatic system. Now, the implementation of a specific light source system by using standard Light Emission Diodes (LEDs) was studied. Moreover, the specification of all electronic components to be used in the prototype device was performed.**

I. INTRODUCTION

Blood is the most important body fluid [1]. It presents fundamental functions, such as body temperature control and cell viability. Blood has many types of elements, such as plasma, red blood cells, and others [2, 3]. If a person loses a large quantity of blood, this quantity of blood must be immediately regained. Otherwise, there is a risk to human life [1]. Before a blood transfusion, it is necessary to determine the blood type. This situation represents a critical test, especially in emergency situations [1, 3]. A person blood type is defined by the antigens presented in red blood cells' surface. The most important and common blood groups are the ABO and Rh blood systems [1]. The ABO blood system can be classified with four blood types: A, B, AB and O. Someone with an A blood type has type A antigens in the red blood cells' surface, and type B antibodies in the plasma. A person with a B blood type has types B antigens and A antibodies [1, 4]. Thus, the plasma has always the natural antibody, which matches to the antigen that is missing in the red blood cells' surface [5]. The Rh blood system can be classified as Rh positive, if the type D antigen is present in red blood cells' surface; or Rh negative, if the same antigen is missing [1, 4].

For that, a blood transfusion can bring some risks to a patient, particularly, if he/she receives antigens that could be attacked by his/her own antibodies [5]. For all these reasons, the blood transfusions are usually done between patients with the same ABO and Rh blood types [1, 5]. However, sometimes in emergency situations there is no time to determine the blood type, and the blood administered is the O negative, which is the universal donor, because it does not have any type of antigens in the red blood cells [4]. Nevertheless, some reactions between the antibodies present in donor's blood and the antigens still present in the receptor's blood can happen and may risk patient's life [1, 4].

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Concerning the tests available nowadays to determine the blood type, there are the manual tests, such as plate and tube tests; and the automatic systems, full automatic or semi automatic. In spite of the speed of the manual tests, which are suitable to emergency situations, they are particular subjective, since the tests results depend on the technical assistant, who performs the task of mixing blood with specific reagents and visually check if there is agglutination, i.e., antigen-antibody interaction [6]. The automatic systems reduce part of this subjectivity. However, these systems are inconveniently large and they cannot produce results in a short time [6], which could be critical in emergency situations.

In the last years, many approaches to determine a person blood type are emerging, such as the use of spectrophotometry to human blood typing [7, 8]. This approach was initially described by Narayanan et al. [7, 8], who showed that human blood typing is possible by mixing blood samples with specific reagents (commercial antibodies) and then, measuring the optical density (OD) spectrum of this mixtures. They verified that an agglutinated sample produces a spectrum distinct from a non-agglutinated sample, and with this feature it is possible to distinct several blood groups. After that, some works have emerged in an attempt to automate this approach [3, 9, 10]. Thus, taking into account the work already done, it is desirable to develop a device that reduces the limitations of the existing systems.

In this paper, the principles for the development of a miniaturized, low cost, portable and automatic system, based on a spectrophotometric approach, are presented. The system will be able to determine ABO and Rh blood types in a short time and in-situ, which is suitable to emergency situations and allow the blood typing outside a conventional clinical laboratory. For that, the basic elements of the system should be: a light source, a light receptor and a microcontroller.

II. WORK DEVELOPED

This section presents the work developed considering the validation of a universal protocol to be applied in the automatic system. Moreover, some steps to the miniaturization of the device are presented, for instance the

specification of the electronic components that will be used in the prototype system.

A. Validation of the universal test protocol

The experimental tests considered the application of two different methodologies. The first methodology was a standard protocol, based on the cuvette test, similar to the protocol applied by Narayanan et al. [7, 8], with some changes that enable to produce a faster approach. In the second methodology a simpler approach, based on the plate test, was developed to verify its convenience to blood typing. The tests protocols, the results and discussion are presented in a previous study [11] performed by the research team. In [11], it was concluded that the second methodology is a simpler, more precise and more convenient approach to be applied in the automatic system, especially because it is possible to distinct the ABO and Rh blood types with the same principle.

The protocol applied in the system use blood samples (from the Portuguese Blood Institute) and commercial antibodies as reagents (monoclonal Anti-A, Anti-B, Anti-AB and polyclonal Anti-D from HosLab Diagnostic). Four test samples need to be prepared for each blood sample. Each test sample is obtained by mixing blood with a specific antibody. The quantity of blood must be approximately ¼ of the quantity of antibody, for example 50 µL of antibody with 12.5 µL of blood. With a manually mixing stick, antibody and blood are mixed over an area of approximately 2.5 cm^2 during about 5 seconds. Mixing blood with the reagents it can be obtained two types of samples: agglutinated, if there is antigen-antibody interaction; or non-agglutinated, if there is no interaction. For example, mixing A positive blood type with Anti-A, Anti-AB or Anti-D it is obtained an agglutinated sample because this blood type has the A and D antigens. With the Anti-B, it is obtained a non-agglutinated sample.

In the previous study, the spectra were then measured in the range from 400 nm to 1000 nm (ten samples, $n = 10$), in a homemade experimental system aiming to miniaturization. The system (shown in [11]) is based on the use of a commercial tungsten light source, a monochromator and a photodiode measuring device (S1336-5BQ from *Hamamatsu*).

B. Miniaturization of the experimental system

After the validation of the spectrophotometry measurements to human blood typing, with the application of a fast and simple protocol, the next step was the implementation of a specific light source system by using Light Emitting Diodes (LEDs) and a photodiode measuring device (S1336-5BQ photodiode from *Hamamatsu*), e.g., avoiding the bulky and expensive system based on a light source and monochromator. For that, analyzing the spectra previously obtained [11] it was chosen a group of LEDs with emission peaks in a specific wavelengths range, in order to increase the differences between agglutinated and non-agglutinated samples (400 nm – 430 nm, 530nm – 575 nm and > 750 nm). Therefore, three standard LEDs (RS components) were chosen with a peak emission at 406 nm, 566 nm e 956 nm [12-14]. This choice was based on the following features: low consumption, high efficiency, tight band width and emission angle, low cost and small dimensions. Concerning the experimental setup used for spectrophotometric measurements (seven samples, $n = 7$), figure 1 shows an example, with the use of the green LED (peak emission in 566 nm).

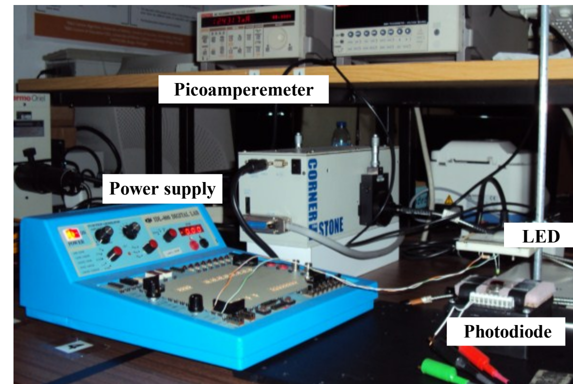

Figure 1. Experimental setup for spectrophotometric measurements with LEDs.

III. RESULTS AND DISCUSSION

This section presents some results obtained by using the experimental protocol presented in [11]. The results obtained after replacing the light source and monochromator by LEDs are also presented. Comparing the results, it is possible to conclude that the setup with three LEDs (three discrete values of optical density (OD)) is appropriate to differentiate an agglutinated sample from a non-agglutinated sample, and consequently, determine several blood types.

A. System with a light source

Figures 2 and 3 present the results obtained with the use of two blood samples and following the experimental protocol presented in [11]. A non-agglutinated sample (A positive blood type in the presence of type B antibodies (figure 2) and AB negative blood type in presence of type D antibodies (figure 3)) has an OD spectrum similar to a spectrum of red blood cells suspension, with a strong presence of the hemoglobin absorption peaks. An agglutinated sample (other samples in the figures 2 and 3) has a flattened OD spectrum, where hemoglobin absorption peaks are not present. The agglutinated sample has aggregated cells with high dimensions and is less turbid, enabling the scattering light. These results are in accordance to Mie scattering theory [15], which relates the scattering light with the state of agglutinated cells; and with the presence of chromospheres in blood samples, which affects the OD spectrum, especially the spectrum of a non-agglutinated sample.

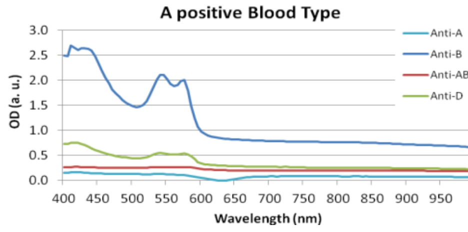

Figure 2. OD spectrum of A blood type in the presence of: Anti-A, Anti-B, Anti-AB and Anti-D. It can be seen the agglutination with Anti-A, Anti-AB and Anti-D, that leads to a lower OD.

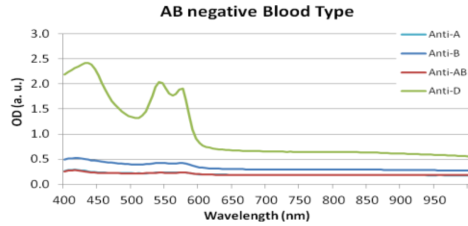

Figure 3. OD spectrum of AB blood type in the presence of: Anti-A, Anti-B, Anti-AB and Anti-D. It can be seen the agglutination with Anti-A, Anti-B and Anti-AB, that leads to a lower OD.

B. *System with LEDs*

Figures 4, 5, 6 and 7 show the results obtained with the use of 3 LEDs to identify all ABO-Rh blood types. It can be noted that a non-agglutinated sample has discrete OD values with a strong variation between them. Moreover, the OD values are high, especially in 406 nm and 566 nm, due to hemoglobin absorption peaks. An agglutinated sample has low discrete OD values as a result of the large quantity of scattering light due to aggregated cells. In addition, the OD values are very similar between them, with a small slope. These results are in accordance to the theory and to the results previously obtained with the use of a monochromator and light source (OD spectrum in the range of 400 nm to 1000 nm).

Table I shows the mean variation of OD values between the discrete wavelengths (first range 406-566 nm and second range 566-956 nm) relative to the LEDs emission peaks, in non-agglutinated and agglutinated samples, obtained with the use of blood samples (seven samples, n=7). The values confirm the spectral differences between non-agglutinated and agglutinated samples. The differences between the 2 wavelengths ranges are higher in the case of non-agglutinated samples. In the agglutinated sample there are small variations as a result of the similarity between the OD values.

For that, it can be concluded that human blood typing is possible with the application of a specific light source system by using standard LEDs.

The design of the prototype system for human blood typing involves the selection and specification of all electronic components. Figure 8 shows a block diagram of the system to implement. The prototype system should accommodate four test samples (one for each reagent – Anti-A, Anti-B, Anti-AB and Anti-D).

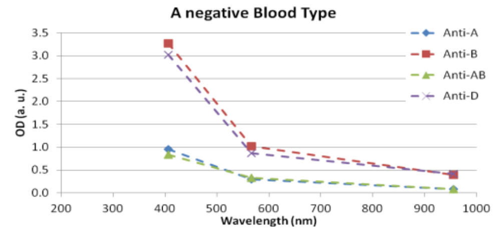

Figure 4. Discrete values of OD of A negative blood type in the presence of: Anti-A, Anti-B, Anti-AB and Anti-D. It can be seen the agglutination with Anti-A and Anti-AB, that leads to a lower OD.

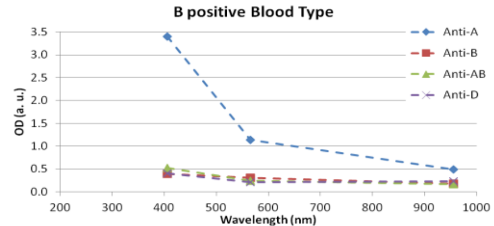

Figure 5. Discrete values of OD of B positive blood type in the presence of: Anti-A, Anti-B, Anti-AB and Anti-D. It can be seen the agglutination with Anti-B, Anti-AB and Anti-D, that leads to a lower OD.

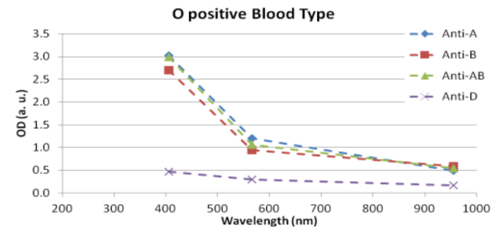

Figure 6. Discrete values of OD of O positive blood type in the presence of: Anti-A, Anti-B, Anti-AB and Anti-D. It can be seen the agglutination with Anti-D, that leads to a lower OD.

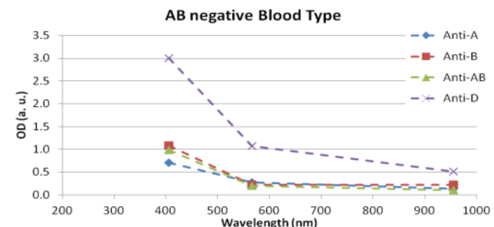

Figure 7. Discrete values of OD of AB negative blood type in the presence of: Anti-A, Anti-B, Anti-AB and Anti-D. It can be seen the agglutination with Anti-A, Anti-B and Anti-AB, that leads to a lower OD.

TABLE I. MEAN VARIATION OF OD VALUES IN NON-AGGLUTINATED AND AGGLUTINATED SAMPLES.

	Mean Variation of OD (a. u.)	
	406 nm - 566 nm	***566 nm - 956 nm***
Non-agglutinated samples	2.011	0.550
Agglutinated samples	0.443	0.116

For this, four groups of three LEDs each need to be used, which makes the total amount of twelve standard LEDs. A low-voltage 16 channel multiplexer, (ADG706) from *Analog Devices* [16] is chosen to select which LED is working. The low-power consumption and operating supply range of 1.8 V to 5.5 V make the ADG706 suitable for portable devices [16]. A photodiode is selected to be used as a light detector, to produce an output current directly proportional to the light intensity received. This choice has to be done in accordance

to LEDs emission peaks, in other words, the photodiode spectral response range must include the wavelengths 406 nm, 566 nm e 956 nm. Thus, a *Hamamatsu* model is chosen (S2386-8K) with features suitable to analytical instruments: high sensitivity, low dark current, high shunt resistance and low terminal capacitance [17]. A common current to voltage converter (figure 9) need to be used, where the output voltage (V) is directly proportional to the photodiode current. For that, operational amplifiers (AD8694) from *Analog Devices* are chosen [18], with features suitable to the photodiode amplification. The resistance value (R) must be adjusted according to the desired gain. The microcontroller should obtain the voltage values and calculated the discrete OD values, based on the voltage obtained with pure reagents that formed the baseline.

Besides controlling the multiplexer and monitoring the OD values, the microcontroller has also to interpret the result and show the blood type in a display. A microcontroller of the STM32F10 family will be used, specifically the STM32F103VET6, which is a microcontroller with ARM® technology that incorporates high-speed embedded memories (Flash memory up to 512 Kbytes) [19].

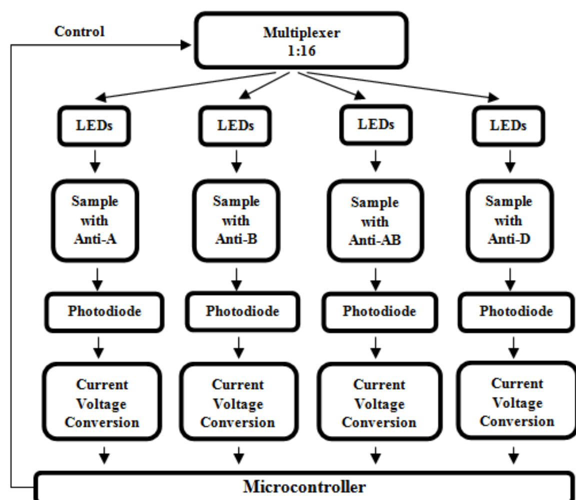

Figure 8. Block diagram of the automatic system.

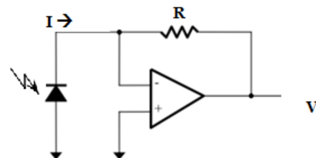

Figure 9. Current to voltage converter.

IV. FINAL COMMENTS

The results presented in this paper allow concluding that human blood typing is possible and viable with the application of a specific light source system by using LEDs, which permits the implementation of an automatic, low cost, miniaturized and portable device. Moreover, the specification and individual test of all electronic components was performed.

In the near future, the prototype system, with all the components assembled, as well as the automatic control system, will be tested in laboratory environment. After this validation, the system will be tested in clinics.

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