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Experimental Research

Biochemistry Section

Estimation of Ideal Visible Spectrum Wavelength for Optimal ABO Blood Grouping using Spectrophotometry

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ABSTRACT

Introduction: The most widely accepted and used method of blood typing is the agglutination test with the antibodies A and B for identifying ABO blood group and a separate antibody D is used for giving Rh (Rhesus) type of the sample under study. The problem faced by this method is that it consumes more time and technician's alertness in case of large number of samples. Thus, optical ABO blood grouping system with appropriate wavelength will improve the blood grouping with better accuracy and time efficiency.

Aim: The aim of this paper is to determine the ideal wavelength of visible spectrum which has maximum absorbance with the agglutinated blood sample using spectrophotometry so that the estimated wavelength can be implemented as light source in an optical blood typing system for better results.

Materials and Methods: An observational study has been performed on nine subjects which was conducted at Sri Sivasubramaniya Nadar college of engineering (SSNCE) Tamil Nadu, India. The subject's blood were collected and mixed with saline. Antibodies A, B, D are added and the data of specific blood absorbance values were measured using Spectrometry. Obtained data were tabulated and graphically represented using Microsoft Excel.

Results: The highest absorbance of sample corresponds to the wavelength in the range of 425-475 nm. Thus, this wavelength range is most suitable and ideal visible spectrum range for optimal ABO blood grouping using spectrophotometer. Significant difference of absorbance between various types of positive and negative samples is obtained.

Conclusion: A difference in absorbance of 0.3 is seen between negative and positive samples. With further research and study, it is possible to separate the specific blood group after identification of the positive and negative types.

Keywords: Absorbance, Agglutination test, Antibodies, Blood group

INTRODUCTION

The blood has large number of constituents out of which the red blood cells and plasma [1] serves the key for the studies associated with the blood grouping because plasma and red blood cells have a contrast antigen-antibody assembly [2]. For example a person with blood type A will have antigen A over the surface of the RBC and corresponding contrast antibody of the plasma is antibody B. Red blood cell in ABO blood grouping system has three types of antigen attached to their surface namely A, B and both and their contrast antibodies on the plasma [3].

Identification of the blood group is done through the application of external monoclonal antibodies A, B and D [4]. In which A and B are very useful in determining the ABO factor and antibody D is used to determine the Rh factor whether positive or negative for the given sample under study. Agglutination of blood is the process by which human blood loses its structure and forms into agglutinated molecule due to the interaction of surface antigen with similar antibodies which is being introduced in the blood stream [1,2]. The antibody which agglutinize the sample gives the blood group for the sample. It is considered that the same example in blood sample containing a surface antigen A and plasma antibody B, when introduced with an external monoclonal antibody A results in agglutination due to reaction between the surface antigen and external antibody [1,4].

Since the external antibody A used to cause the blood sample to agglutinate the blood sample is termed as A group. Further, if the new sample of same blood group shows a similar reaction with external antibody D then the term positive is used for denoting the Rh factor. Thus the sample is collectively called as A+ve. The blood typing and spectrometer has a certain principles associated with them which are very crucial in understanding the entire purpose of the study.

An Absorbance spectrophotometer is used to measure the amount of light absorbed by the sample under study to that of the incident light [5,6]. There are three important parameters that can be analysed using this absorbance spectrophotometer [7,8]. They are the amount of light absorbed or transmitted by number of cells in the suspension, concentration of compounds in the solution. All this involves many fundamental principles such as transmittance, absorbance and concentration [9]. Out of which transmittance is the main parameter from which all other parameters are obtained.

$$\text{Transmittance, } T = I/I_0$$

$$\text{Percentage transmittance, } \%T = I/I_0 \times 100 \text{ [9]}$$

where I, is the light intensity measured through spectrophotometer.

I_0, is the light intensity incident over the sample under study.

The amount of light absorbed is related in logarithm with the transmittance through this equation.

$$\text{Absorbance, } A = -\text{Log } T \text{ [10,11]}$$

Absorbance of the sample indeed depends on another factor called as concentration. Lambert - Beer law gives the fundamental relationship between the absorbance and the concentration of the sample [9].

$$A \propto C$$

$$A = elC$$

Where C, Molar concentration of the sample has unit M.

e, the extension coefficient of the substance has units of $M^{-1}cm^{-1}$.

L, the sample path length measured in cm.

As the title suggests visible spectrum of light from the wide electromagnetic spectrum [10,11], the study will only concentrate with the wavelength of 400-700 nm which falls under the visible range. For this measurement the spectrophotometer is used which has a

single incandescence lamp to produce the entire spectrum of light with the help of prism or diffraction grating assembly. As the angle of contact between the collimated light source from the incandescence lamp and the diffraction grating or prism changes with the rotation or tilt there is a change in the production of visible light wavelength. Ideal wavelength of absorbance is the wavelength at which maximum absorbance takes place. This wavelength provides the maximum sensitivity for measurement in case of optical blood group detection [12,13]. Therefore, time efficient, cost effective and portable device is designed with the preset absorbance range and it paves way in life saving tool without delaying the blood detection process.

MATERIALS AND METHODS

Study Population and Design

The study was conducted during June 2018 with healthy recruited subjects at SSNCE in Tamilnadu, India. The samples absorbance was calculated using Beer-Lambert law using the formula Absorbance, A = - Log T; where T is transmittance. Therefore, approximately two subjects were selected for A+, B+, O+, A- blood group and one O-.

Ethics Approval and Consent to Participate

The study design was reviewed and approved by the Ethical Committee of Sri Sivasubramaniya Nadar college of engineering (SSNCE) Tamilnadu, India. The study was conducted in accordance to the guidelines of SSNCE ethical committee.

Inclusion/Exclusion Criteria

Subjects above 19 years of age who were physically fit without any clinical disinfection were recruited for the study. Haemophilia and leukaemia patients were excluded from the study. Healthy individuals were considered as subjects and their blood was collected and study was conducted.

Data Collection

Nine subjects having different blood groups namely two A+ve, two B+ve, two O+ve, two A-ve and O-ve blood were selected for performing the test and each subject have been numbered from S1, to S9 according to their blood group A, B, O positives and negatives respectively. Blood taken from subjects were added with standard antibodies and their visible light absorbance values were obtained using spectrophotometer and tabulated.

Blood Collection and Laboratory Analysis

Swab of cotton dipped in Ethanol were used to rub the site of puncture before taking the blood sample from the subject. A 3 mL of blood was taken from each subject under similar environmental conditions. Saline was added to the sample as plasma volume expander to increase the volume of solution with the saline to blood ratio of 10:1. Now, equally divided solution containing the saline and blood into three separate 4 mL cuvettes was placed. The remaining solution was kept preserved for future experiment. Another cuvette was filled with distilled water and was kept as blank which is essential to fix the absorbance value to zero and reduce the error during measurement. Now the three cuvettes containing sample were introduced with few drops of monoclonal antibodies A, B and D for intialising the agglutination reaction.

A tabulation with details of selected wavelength, transmittance, absorbance and the antibody used was made for observation. Once the reaction starts its time for us to load the cuvette containing the specific external antibody onto the spectrophotometer unit and take their respective absorbance value with the changes made in the wavelength of the source. The entire range of wavelength used is of visible Electromagnetic spectrum of wavelength ranging from 400 nm to 700 nm with a steep increase of 25 nm from the initial value of 400 nm wavelength and finally, it reaches a wavelength of 700 nm. Thus, for a particular value of wavelength both absorbance and transmittance were measured. Similarly insert the other cuvette one by one containing remaining antibodies and follow the same protocol. The time taken for the overall protocol is an hour per individual.

Finding Peak Absorbance Values

Three columns of absorbance and transmittance value for a single subject were obtained. Each column represents each antibody used. This is done for the two subjects of same blood group and there mean was taken for further calculation. Analysis of values obtained in table was done through grouping the each antibody column of all the nine subjects into three tables with each table containing a particular antigen's absorbance and wavelength characteristic. Thus, three tables were formed using separate tabulations for anti A, B, D.

The six tabulations each carrying a particular set of information which can be analysed by visualizing them graphically and infer the peaks in the graphs. The manner by which the curve slopes down is the relationship between the wave forms of different blood groups. Thus providing the ideal peak absorbance for corresponding wavelength is used in an optical blood grouping setup as a source.

STATISTICAL ANALYSIS

Data was analysed using Microsoft Excel. Results were expressed as Mean wavelength vs absorbance. Mean absorbance value of corresponding positive and negative blood groups were compared with corresponding Anti-A, Anti-B, Anti-D using blood sample of A, B, AB and O. Transmittance and absorbance was found by the corresponding formulae,

$$\text{Transmittance, } T = I/I_0 \quad \text{Absorbance, } A = -\text{Log } T$$

where I, is the light intensity measured through spectrophotometer.

$I_{0,}$ is the light intensity incident over the sample under study.

Novelty of the study:

The novelty of the present work is that if executed under:

- The study involves a novel sample preparation for optical estimation.
- Compared to conventional blood grouping method, the present study plays major part in time reduction as well as in less computing error instead of manual error and the device plays a key role in life saving factor with increased number of training data set.

Sensitivity and Specificity

In medical diagnosis, both specificity and sensitivity are equally important. However, this overall experimental study focuses only on specificity, because only healthy blood groups absorbance was considered to show variance in different blood groups. So, Diagnostic accuracy calculated based on specificity and sensitivity can be achieved with the sample size increased to a greater extend. Therefore, in future, along with healthy blood groups, diseased blood groups will be tested to obtain its absorbance which is helpful in determining diagnostic accuracy of diseased patients.

RESULTS

Baseline Characteristic of Participants

A total of nine subjects participated in the study of which are A, B, AB, O blood group with an age above 19 years old. Among these subjects, there were seven females and two males who were physically fit for the study.

For positive samples one can see the range of absorbance to be 0.8 to 0.9 and the negative absorbance ranges from 0.50 to 0.55. Thus, a difference of 0.3 is seen for the negative and positive samples.

Now different types of positive blood group samples like A, B and O groups can be differentiated using various antibodies. Namely one can differentiate O blood group using antibody B by observing the absorbance value at a wavelength range of 650 to 750 nm, for O

blood group the values lies in 0.75 to 0.77 range for a non- O blood group the value in the same range of wavelength is 0.85. Thus, nearly a difference of 0.1 is seen in that range of wavelength. Thus, O positive blood can be distinguished from A and B blood group.

A and B blood groups are typed by antibody A which are used in the wavelength of 400 to 475 nm. Therefore, the absorbance difference of 0.1 is observed between the sample of A and B. The one having the lower value is the A blood group and the remaining blood group is B. Thus we can easily distinguish positive groups like A, B and O using the study performed using spectrometer.

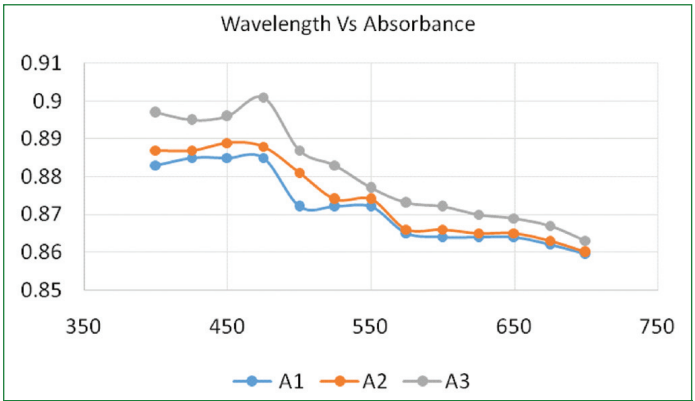
DISCUSSION

This section speaks about the characteristics of each and every graphs right from [Table/Fig-1-9]. The discussion will throw lights on the useful hidden features of those data represented graphically. S1 and S2 mean is taken and number as 1, S3 and S4 mean is taken and number as 2, S5 and S6 mean is taken and number as 3, S7 and S8 mean is taken and number as 4, S9 is numbered as 5, with respect to antibodies (A,B,D), it act as A1, A2,.. A5,B1…B5,D1….D5 respectively. [Table/Fig-2,5,8] represents the wavelength vs absorbance obtained using various antibodies A, B and D respectively over the positive blood groups. Similarly graphs [Table/Fig-3,6,9] describe the antibodies over the negative samples of study. [Table/Fig-1,4,7] describes the tabulation of wavelength vs absorbance for positive and negative blood group using anti-A, anti-B and anti-D respectively.

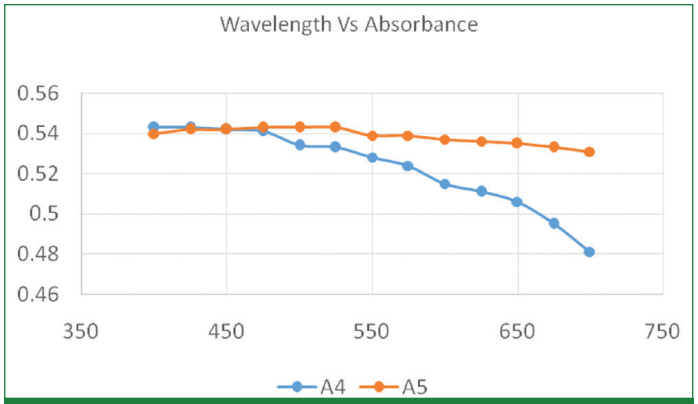
Noticeable similarities over all the graphs drawn are the appearance of the absorbance peaks for the same wavelength range. Peak absorbance indicates that the maximum source light radiation has been observed at those intensities. The major advantage of the peak absorbance is the sensitivity of the medium offered during the usage

Wavelength (nm)	A1	A2	A3	A4	A5
400	0.883	0.887	0.897	0.543	0.54
425	0.885	0.887	0.895	0.543	0.542
450	0.885	0.889	0.896	0.542	0.542
475	0.885	0.888	0.901	0.541	0.543
500	0.872	0.881	0.887	0.534	0.543
525	0.872	0.874	0.883	0.533	0.543
550	0.872	0.874	0.877	0.528	0.539
575	0.865	0.866	0.873	0.524	0.539
600	0.864	0.866	0.872	0.515	0.537
625	0.864	0.865	0.87	0.511	0.536
650	0.864	0.865	0.869	0.506	0.535
675	0.862	0.863	0.867	0.495	0.533
700	0.859	0.86	0.863	0.481	0.531

[Table/Fig-1]: Wavelength vs Absorbance using Anti - A for positive (A1, A2, A3) and negative (A4, A5) blood groups.



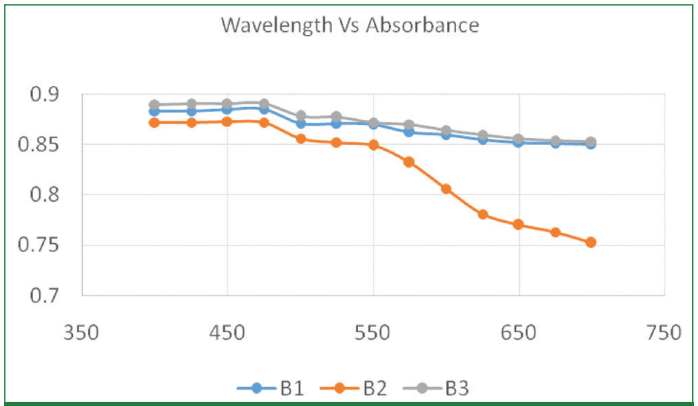
[Table/Fig-2]: Graph of Wavelength vs Absorbance using Anti - A for positive blood groups.
A1, A2, A3 - represents mean Absorbance value of corresponding positive blood groups obtained using Antibody-A



[Table/Fig-3]: Graph of Absorbance vs Wavelength using Anti - A for negative blood groups.
A4, A5 - represents mean Absorbance value of corresponding negative blood groups obtained using Antibody-A

Wavelength (nm)	B1	B2	B3	B4	B5
400	0.883	0.872	0.89	0.54	0.536
425	0.883	0.872	0.891	0.541	0.538
450	0.885	0.873	0.891	0.542	0.539
475	0.885	0.872	0.891	0.542	0.539
500	0.871	0.856	0.878	0.538	0.539
525	0.871	0.852	0.877	0.536	0.539
550	0.87	0.849	0.872	0.534	0.535
575	0.862	0.832	0.87	0.533	0.534
600	0.86	0.806	0.8645	0.53	0.532
625	0.855	0.781	0.86	0.53	0.528
650	0.852	0.77	0.856	0.526	0.527
675	0.851	0.763	0.854	0.525	0.526
700	0.850	0.752	0.853	0.526	0.525

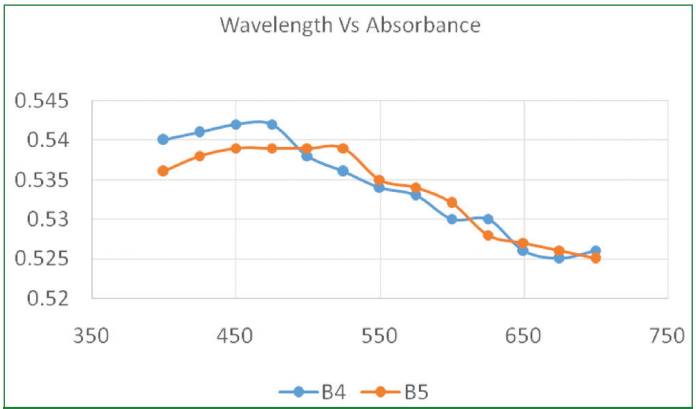
[Table/Fig-4]: Absorbance vs Wavelength using Anti-B for positive (B1, B2, B3) and negative (B4, B5) blood groups.



[Table/Fig-5]: Graph of Absorbance vs Wavelength using Anti-B for positive blood groups.
B1, B2, B3 - represents mean Absorbance value of corresponding positive blood groups obtained using Antibody-B

of source of that ideal peak wavelength. The sensitivity shows a remarkable difference in the various blood type differentiated though they are more or less similar in appearance and texture.

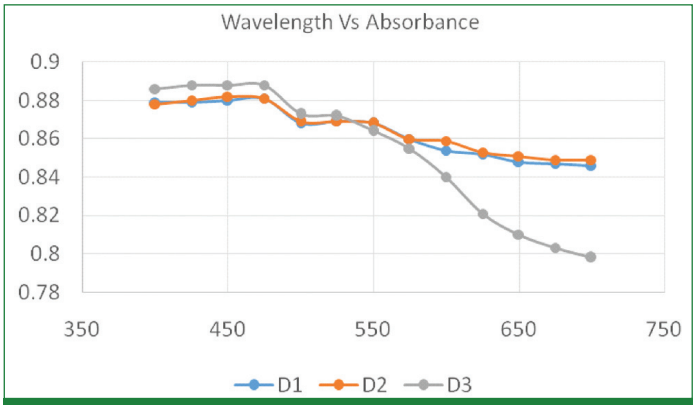
During this wavelength the difference between those samples are very larger when compared to the source of other wavelength, which helps in the accuracy of the blood group detection process. Now in our example there are two clearly visible peaks namely P1 and P2. P1, is the larger of two peaks observed at a wavelength of 425 to 475 nm is in the positive blood group and P2, is smaller in comparison with P1 observed at a wavelength of 550 to 570 nm, out of those two most preferable is the range of 425 to 475 nm which falls under sky blue colour series than the green range of 550 to 575 nm because of its greater absorbance over other wavelength range and will be more suitable for the objective of



[Table/Fig-6]: Graph of Absorbance vs Wavelength using Anti-B for negative blood groups.
B4, B5 - represents mean Absorbance value of corresponding negative blood groups obtained using Antibody-B

Wavelength (nm)	D1	D2	D3	D4	D5
400	0.879	0.878	0.886	0.54	0.536
425	0.879	0.88	0.888	0.541	0.538
450	0.88	0.882	0.888	0.542	0.539
475	0.881	0.881	0.888	0.542	0.539
500	0.868	0.869	0.873	0.538	0.539
525	0.869	0.869	0.872	0.536	0.539
550	0.868	0.868	0.864	0.534	0.535
575	0.86	0.86	0.855	0.533	0.534
600	0.854	0.859	0.84	0.53	0.532
625	0.852	0.853	0.821	0.537	0.528
650	0.848	0.851	0.81	0.526	0.527
675	0.847	0.849	0.803	0.525	0.526
700	0.846	0.849	0.798	0.526	0.525

[Table/Fig-7]: Tabulation of Absorbance vs Wavelength using Anti-D for positive(D1, D2, D3) and negative (D4, D5) blood groups.

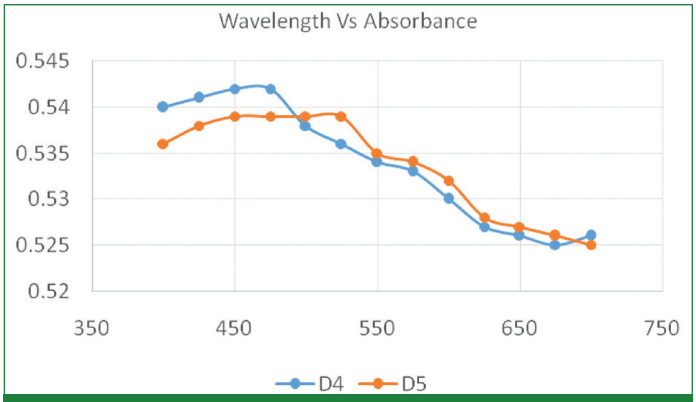


[Table/Fig-8]: Graph of Absorbance vs Wavelength using Anti-D for positive blood groups.
D1, D2, D3 - represents mean Absorbance value obtained of corresponding positive blood groups using Antibody-D

study. Thus, it can be said that the peak P1 is the most suitable and ideal visible spectrum wavelength for optimal ABO blood grouping using spectrophotometer.

Future Recommendations

Apart from the detection of ideal optimal visible wavelength for blood grouping this study further helps in grouping the blood samples directly observing the absorbance vs wavelength characteristics obtained by the above mentioned experiment using spectrophotometer for a greater sample study of positive and negative groups which can be used for framing a protocol for differentiating the blood samples if the following changes are observed in the graphical representation which includes the difference in the range of absorbance obtained, antibody used, wavelength observed. By closely selecting and



[Table/Fig-9]: Graph of Absorbance vs Wavelength using Anti-D for negative blood groups.
D4, D5 - represents mean Absorbance value of corresponding negative blood groups obtained using Antibody-D

monitoring these parameters one can predict the blood sample through spectrophotometer readings.

Some of the notable difference obtained by studying the characteristics of various blood groups are listed below. Since this study involves the majority of subjects being positive blooded it would not be used for typing the negative blood samples however this study helps in differentiating the negative from the positive subjects by observing the range of absorbance given by the sample. From this the blood typing for all kind of peoples of different blood grouping can be performed. Non contact devices for blood grouping can be developed like one which is available for measuring temperature using Non-contact digital IR temperature.

Clinical Significance

- The designed device is helpful in detecting ABO blood group with Rh+ve or Rh-ve in less time which will acts as an life saving factor in accident or emergency cases.
- Clinically, it diagnosises the blood related disorder with predefined wavelength range.
- Pre-analysis of blood using optical method reduces the side effects of blood transfusion mismatch.
- Spectrophotometer can be a potential equipment for blood typing through standardising the various blood group outputs in laboratories.

CONCLUSION

Thus, it can be concluded that the peak in wavelength range of 425 to 475 nm is the most suitable and ideal visible spectrum wavelength for optimal ABO blood grouping using spectrophotometer. 425 to 475 nm wavelength range of light spectrum is a good source of light for the blood detection kit involving in an optical method of blood group detection.

Moreover, the mean values are appreciable when it is performed over a larger population.

LIMITATION

- The sample size in the present study is less in quantity to compute basic difference in results which will be helpful to estimate values in larger samples.
- The sample results depends on the nature and mass of the working environment like chemical industries, atomic power plant, etc., and healthy diet habits of people which shows blood thickness variations, due to generation gap- the past and current generation shows heredity variation in blood count and red blood cell lifetime, due to all these factors the absorbance value varies. So large number of trials (sample) are required to increase the power of study to show accurate result to determine specificity and sensitivity rate.

• Antigen-antibody tests need a lot of requirements such as proper sample preparations and reagent usage. Factors affecting the antigen-antibody reactions like optimal room conditions, time duration and optical properties should be considered to establish the suitable reaction in every sample of interest. If the reaction conditions are not followed, false negative or false positive results can occur, which can lead to incorrect blood group determination.

Author's contributions: SL: Coordinated the entire research activity; VS: Data analysis, representation and documentation; SB: Framing the protocol for carrying out the study; OS: Participant preparation, data collection and documentation.

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REFERENCES

[1] Seeley RR, Stephens TD, Tate P. Essentials of anatomy and physiology. McGraw-Hill; 2005 Jan.
[2] Malomgré W, Neumeister B. Recent and future trends in blood group typing. Analytical and Bioanalytical Chemistry. 2009;393(5):1443-51.
[3] Nonoyama A, Garcia-Lopez A, Garcia-Rubio LH, Leparc GF, Potter RL. Hypochromicity in red blood cells: an experimental and theoretical investigation. Biomedical Optics Express. 2011;2(8):2126-43.
[4] Ravindran G, Titus TJ, Pravin M, Pandiyan P. Determination and classification of blood types using image processing techniques. International Journal of Computer Applications. 2017;157(1):12-16.
[5] Fernandes J, Pimenta S, Soares FO, Minas G. A complete blood typing device for automatic agglutination detection based on absorption spectrophotometry. IEEE Transactions on Instrumentation and Measurement. 2015;64(1):112-19.
[6] Anthony SR, Ramasubramanian MK. Visible/Near-Infrared Spectrophotometric Blood Typing Sensor for Automated Near-Patient Testing. In Engineering in Medicine and Biology Society, 2005. IEEE-EMBS 2005. 27th Annual International Conference of the 2005 (pp. 1980-1983). IEEE.
[7] Lambert JB. A miniaturized device for blood typing using a simplified spectrophotometric approach.
[8] Narayanan S, Orton S, Leparc GF, Rubio LH, Potter RL. Ultraviolet and visible light spectrophotometric approach to blood typing: objective analysis by agglutination index. Transfusion. 1999;39(10):1051-59.
[9] Shah RS, Shah RR, Pawar RB, Gayakar PP. UV-visible spectroscopy-a review. International Journal of Institutional Pharmacy and Life Sciences. 2015;5(5):
[10] Ramasubramanian M, Anthony S, Lambert J. Simplified spectraphotometric method for the detection of red blood cell agglutination. Applied Optics. 2008;47(22):4094-105.
[11] Fernandes J, Soares FO, Minas G. A spectrophotometry based blood typing device. InBioengineering (ENBENG), 2015 IEEE 4th Portuguese Meeting on 2015 Feb 26 (pp. 1-6). IEEE.
[12] Ashiba H, Fujimaki M, Awazu K, Fu M, Ohki Y, Tanaka T, et al. Hemagglutination detection for blood typing based on waveguide-mode sensors. Sensing and Bio-Sensing Research. 2015;3:59-64.
[13] Pimenta S, Nobrega JM, Duarte FM, Minas G, Soares FO. Automatic electronic system to human blood typing. Procedia Engineering. 2012;47:647-50.

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