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UV Visible Spectrophotometric Approach and Absorption Model for the Discrimination of Diseased Blood

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In the present investigation, UV-visible spectral characteristics of the whole blood were studied for the classification of the patients with leukemia, anemia, liver cirrhosis, thalassemia and diabetes with reference to the healthy subjects. The optical density of the diseased blood was dramatically different from that of the healthy one. The optical densities and their ratios were used as input variables for the statistical analysis of normal and diseased blood. The observed differences were statistically significant and could be exploited to differentiate between the healthy and diseased blood.

Key Words: Blood, Leukemia, Anemia, Cirrhosis liver, Thalassemia, Diabetes, UV-Visible spectrum, Optical density, Anova, Univariate statistical analysis.

INTRODUCTION

Blood is a fluid tissue, which circulates through every organ of human body and participates in all functional activities of the body. It contains plasma and several types of cells termed as formed elements. Blood has about 55 % of plasma which contains a large number of proteins, glucose used in cellular metabolism, amino acids, vitamins, lipids, hormones *etc.* and many more inorganic substances[1,2]. Plasma carries the formed elements, food materials as well as oxygen to the cells in various tissues, waste products from tissues to excretory organs and maintains hormonal balance of the body[3]. The cellular fraction of blood is composed of three types of cells, namely erythrocytes or red blood cells, leucocytes or white blood cells and thrombocytes or blood platelets. Thus along with the transportation function, blood regulates body temperature and correct water balance[4]. Each element of blood is required to be present in a specific amount for it to perform its normal functions. If a physiological or pathological change takes place in the tissue due to a disease, this leads to changes in plasma and cellular constituents. Thus there is a remarkable difference in the number and function of various formed elements present in the blood of healthy and diseased persons. It has been established that fast, reagent free, non-invasive spectroscopic methods of blood analysis have day-to-

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day clinical use[5]. Spectroscopic analysis provides an alternative method to clinical analysis, since it requires fewer samples and provide more information[6]. In this work, healthy blood samples and those affected with abnormal values of white blood cells, red blood cells, plasma protein and glucose hormone are analyzed by employing UV-visible spectroscopic technique. An attempt has been made to analyze the variation of light absorption characteristics of the absorption band of blood for healthy and diseased subjects. The diseases chosen are leukemia, anaemia, cirrhosis liver, thalassemia and diabetes.

EXPERIMENTAL

Blood samples were collected from about 15 normal healthy persons of the same age group. The samples with abnormal values of white blood cells, red blood cells, plasma protein and glucose were obtained from patients of a leading hospital at Chennai, India. For UV-visible spectral studies, each blood sample was diluted with normal saline at a concentration of 5 μL/mL. The spectra were scanned in the region between 200-700 nm using Elico SL 159 UV-visible spectrophotometer at Spectrophysics Research Laboratory, Pachaiyappa's College, Chennai, India. The sources that produce ultra violet rays in the spectrometer were deuterium and tungsten halogen lamps with the monochromator as Czerny-Turner type with 1200 lines/mm holographic grating. The detector has a wide range of photodiode with greater efficiency and stray light loss is about < 0.1 % at 220 nm with NaI 10 g/L. The scanning range capability of the device was 200-1000 nm with an accuracy of ± 0.5 nm. Quartz cuvettes of 10 mm path length were used. The device compensates for solvent absorption and losses of radiation by scattering and reflection. The decision of the solvent is made in such a way that, the solvent should not absorb in the same region as the solute[7]. The spectra were recorded under identical conditions.

RESULTS AND DISCUSSION

Absorption spectroscopy is ideally suited for quantitative measurements as the absorbance of a solute depends linearly on its concentration. The light absorption properties of a molecule depend on the molecular environment and the mobility of chromophores. Multiwavelength UV-visible spectroscopy is relatively simple technique that can provide considerable quantity of information. The UV-visible spectrum of blood contains information on the light absorption and scattering properties of suspended particles in it. Many workers have successfully employed UV-visible spectroscopy for characterization and confirmation of many proteins and nucleic acids. The UV-Vis spectral study of blood in healthy and diseased subjects has already been reported[8-11].

As blood undergoes physical and biochemical changes in many diseases, the patho physiological changes in the healthy and diseased blood are analyzed by the characteristic absorptions in the UV-visible spectral region. The UV-visible spectra of the healthy and diseased subjects are presented in Figs. 1-5. Six peaks are observed at 210, 278, 344, 417, 543 and 578 nm due to constituents of blood[8,9]. The amide chains of the proteins present in the blood absorbs strongly at *ca.* 210 nm. Also the strong absorption peak at around 280 nm is due to amino acids tyrosine and tryptophan[10]. The carbohydrate metabolism in blood can be assigned to the absorption maxima at 344 nm as due to NADH and NADPH. These are reduced forms of the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) which function in the action of many dehydrogenases[9]. While getting reduced these enzymes undergo structural change which causes the absorption at around

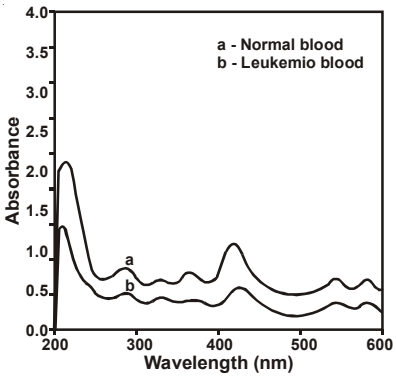


Fig. 1. Comparative UV Visible spectra of normal and leukemia blood

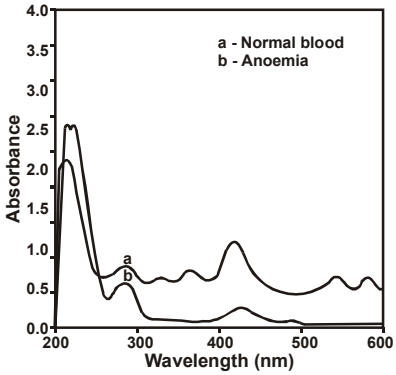


Fig. 2. Comparative UV Visible spectra of normal and anemia blood

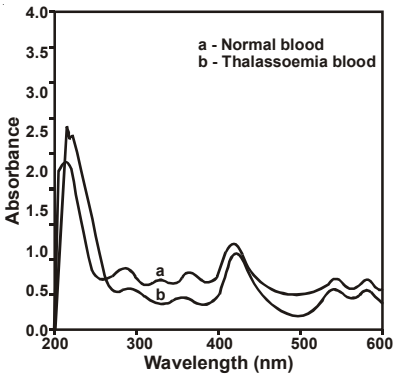


Fig. 3. Comparative UV Visible spectra of normal and thalassemia blood

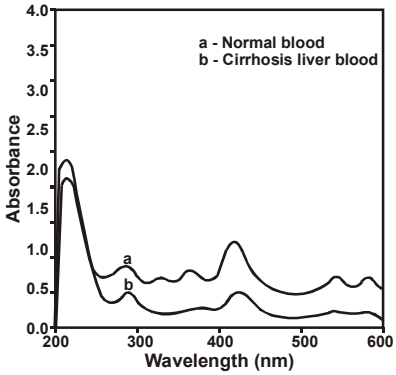


Fig. 4. Comparative UV Visible spectra of normal and liver cirrhosis blood

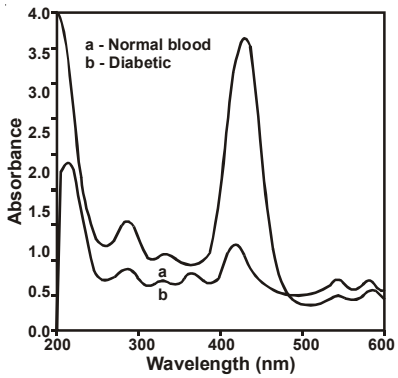


Fig. 5. Comparative UV Visible spectra of normal and diabetes blood

344 nm[12]. The absorptions at 417, 543 and 578 nm are due to *d-f* transition of CO–oxy hemoglobin[13]. There is a great change in the optical density of healthy and diseased blood. The Table-1 summarizes the average ± SD values of optical density. The statistical analysis one way Anova and Tukey-HSD test[14] were carried out with significance level 0.05 and the results are presented in Table-1. The ** in the Table-1 shows that the 'p' value is less than 0.01 and hence there is a significant difference between normal and the diseased blood samples at 1 % error level. The differences were statistically significant at p values of less than 0.01. Based on Tukey-HSD test, the different alphabets between bloods denote significant difference at 5 % level.

The Q-factor or the ratio of optical densities among the peaks is also calculated. This internal standard among the absorption peaks were used for the diagnostic discrimination of diseased blood from normal blood based on Univariate statistical analysis. The results of the statistical analysis are presented in Table-2. Considerable spectral differences have been observed between the normal and diseased blood in this study. Based on these differences in the spectral signatures, three absorbance ratio parameters were introduced. They were A_{278}/A_{210} in the protein region and A_{543}/A_{417}, A_{578}/A_{417} in the oxy hemoglobin region. Fig. 6 shows the histogram presenting the variation in the absorbance ratio between normal and the diseased blood samples in both protein and oxy hemoglobin regions. It is also perceived from the scatter plot in the protein region presented in Fig. 7, the critical ratio 0.3682 classifies the cirrhosis liver blood from the healthy one. Also the absorbance ratio of the cirrhosis liver blood is much different from that of the normal blood in the protein region, since the disease cirrhosis liver affects the protein content of the blood[15]. Similarly the scatter plots in the oxy haemoglobin region in Fig. 8 discriminates the blood samples affected by the diseases like leukemia, anemia, thalassemia and diabetes from the normal blood.

TABLE-1
RESULTS OF ONE WAY ANOVA AND t TEST FOR THE UV VISIBLE SPECTRAL DATA OF BLOOD SAMPLES

Wavelength (nm)	Optical density						f value	p value
	Healthy blood	Leukemia blood	Anemia blood	Cirrhosis liver blood	Thalassemia blood	Diabetes blood		
210	$2.204^{ab} \pm 0.502$	$2.483^{bc} \pm 0.248$	$2.342^{abc} \pm 0.374$	$2.128^{a} \pm 0.265$	$2.594^{c} \pm 0.198$	–	4.920	0.002^{**}
278	$1.901^{b} \pm 0.250$	$0.588^{a} \pm 0.094$	$1.086^{b} \pm 0.100$	$0.513^{a} \pm 0.078$	$1.202^{b} \pm 0.115$	$1.186^{b} \pm 0.331$	41.138	0.000^{**}
417	$1.333^{c} \pm 0.356$	$0.808^{ab} \pm 0.210$	$0.727^{ab} \pm 0.117$	$1.089^{bc} \pm 0.285$	$0.486^{a} \pm 0.160$	$2.814^{d} \pm 1.052$	45.359	0.000^{**}
543	$0.811^{d} \pm 0.211$	$0.646^{cd} \pm 0.165$	$0.107^{a} \pm 0.019$	$0.624^{c} \pm 0.206$	$0.205^{a} \pm 0.068$	$0.451^{b} \pm 0.158$	46.617	0.000^{**}
578	$0.814^{d} \pm 0.213$	$0.654^{c} \pm 0.166$	$0.114^{a} \pm 0.019$	$0.659^{cd} \pm 0.159$	$0.210^{a} \pm 0.068$	$0.463^{b} \pm 0.160$	53.096	0.000^{**}

Different alphabets denote significance @ 5 % level
*Denotes $0.011 \leq$ p value ≤ 0.05 significance @ 1 % level; **Denotes p value ≤ 0.01 significance @ 1 % level

TABLE-2
RESULTS OF ABSORBANCE RATIO PARAMETERS FOR THE TWO REGIONS OF HEALTHY AND DISEASED BLOOD SAMPLES

Region	Absorbance ratio parameter (Average ± SD)					
	Healthy blood	Leukemia blood	Anemia blood	Cirrhosis liver blood	Thalassemia blood	Diabetes blood
Protein A_{278}/A_{210}	0.4950 ± 0.00790	0.2367 ± 0.0274	0.4507 ± 0.0137	0.2413 ± 0.0256	0.4632 ± 0.0239	–
Oxy hemoglobin A_{543}/A_{417}	0.6100 ± 0.01186	0.7978 ± 0.0165	0.1464 ± 0.0057	0.6001 ± 0.0289	0.4222 ± 0.0102	0.1903 ± 0.126
A_{578}/A_{417}	0.6149 ± 0.01190	0.8073 ± 0.0165	0.1572 ± 0.0057	0.6081 ± 0.0297	0.4330 ± 0.0101	0.1954 ± 0.131

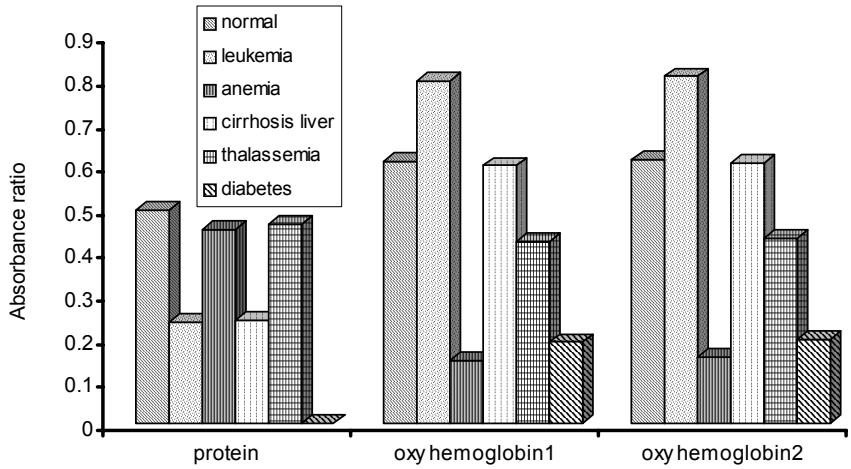


Fig. 6. Histogram to show the variation in the absorbance ratio between healthy and diseased blood samples

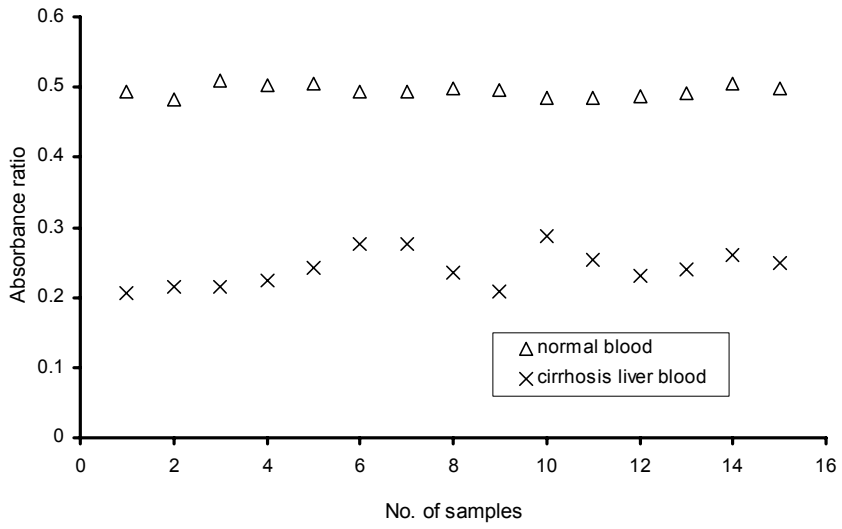
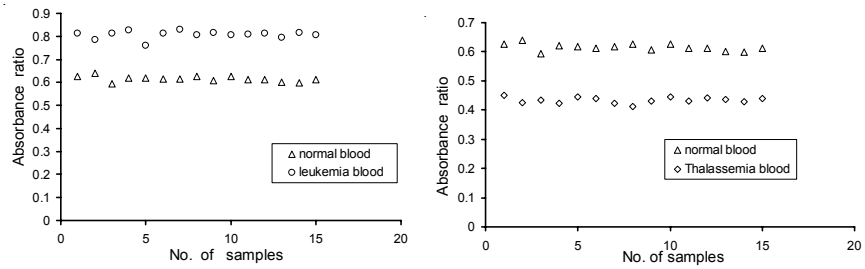


Fig. 7. Scatter plot in protein region



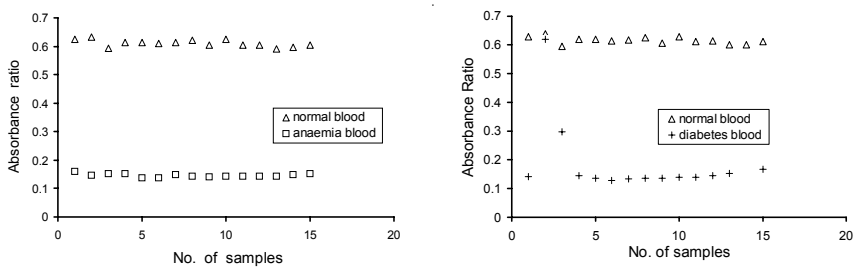


Fig. 8. Scatter plot in oxy hemoglobin region

Conclusion

UV-visible spectroscopic method has been employed to study the healthy and diseased blood samples. The optical density ratios among the peaks of the light absorption characteristics of the blood were calculated and Univariate statistical analysis has been made. The scatter plots and the histogram clearly indicate how a diseased blood sample is different from the normal one. Thus spectroscopic techniques can be effectively employed as a diagnostic tool in clinical chemistry and it can be an alternative method in clinical analysis.

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