Measurement of Optical Density of Blood with Varying Time Using UV-Vis Spectroscopic Method

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The object of the present work is to measure and study the optical density of blood at various stipulated periods of time using ultra-violet and visible spectroscopic method. SL 159 single beam UV-Vis spectrophotometer was used to record the spectra in the wavelength range between 200 and 700 nm. There are six wavelength maxima exhibited at 215, 280, 346, 418, 544 and 578 nm corresponding to proteins, amino acids, NADH and NADPH, CO-hemoglobin and oxyhemoglobin respectively in blood. From the study it is observed that optical density of blood constituents decreases with time except CO-hemoglobin which occurs at the wavelength at 418 nm.

Key Words: Blood, Protein, Amino acids, Hemoglobin, Optical density.

INTRODUCTION

Gunasekaran and Marshell[1] studied the characteristic of blood in normal and diseased subjects using UV-Vis spectroscopic method. Human blood is a fluid tissue consisting of plasma and several types of of cells termed as formed elements. Each element of blood is required to be present in a specific amount for the blood to perform its normal functions. If a physiological or pathological change takes place in the tissue, this leads to changes in the various plasma and cellular constituents. Blood plasma contains a large number of proteins, glucose used in cellular metabolism, amino acids, vitamins, lipids, hormones etc. and many inorganic substances[2, 3]. Plasma carries the formed elements, food materials as well as oxygen to the cells in the various tissues, waste products from tissues and various hormones of the body. It helps to maintain correct water balance and temperature of the body. While more than half of blood volume is plasama, there are other cells suspended in it and are carried by it in circulation around the body. Of these cells the most important are the erythrocytes and leukocytes[4, 5]. In this work we have made an attempt to analyse the variation of light absorption characteristics of the absorption band of blood over a period of 40 days.

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EXPERIMENTAL

Blood samples of different group were obtained from the blood bank of a leading hospital at Chennai, India. The blood sample was diluted with normal saline water and fed into a UV-Vis single beam spectrophotometer and the spectra were scanned in the region between 200 nm and 700 nm. In recording the absorption spectra, two cuvettes were used, one as the reference cuvette and the other as the sample cuvette. The reference cuvette was filled with normal saline water as a solvent and the sample cuvette with the blood. Such an arrangement compensates for solvent absorption and also for losses of radiation by scattering and reflection. Absorption cuvettes of different dimensions made of glass or quartz are available. In deciding the choice of solvents in that, the solvent should not absorb in the same region as the solute[6]. The spectra were recorded under identical conditions.

RESULTS AND DISCUSSION

Generally blood can be stored in a blood bank for a maximum period of 35 days called storage time. A comparative study of the ultra-violet and visible spectra of blood of different subjects with varying periods of time is as shown in Fig. 1. The light absorption characteristics are summarized in Table-1. Six peaks are observed at 215, 280, 346, 418, 544 and 578 nm due to constituents of blood[1]. The protein in blood exhibits strong absorbance at 215 nm and the protein metabolism can be conveniently monitored by changes in the absorbance of wavelength maximum at 280 nm due to tyrosine and tryptophan[2]. The carbohydrate metabolism in blood can be assigned to the absorption maxima at 346 nm
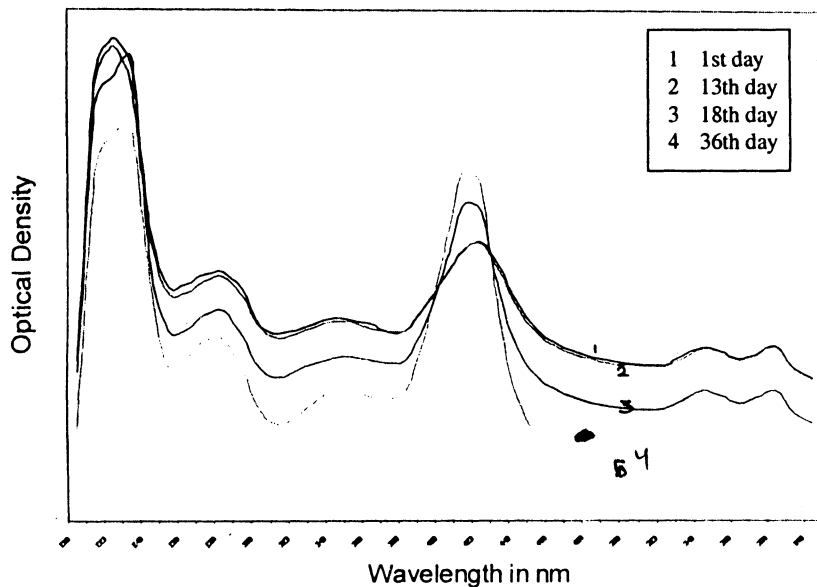

Fig. 1   UV-VIS Spectrum of blood with varying time

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. These molecules contain a nicotinamide group in addition to adenine and upon reduction the structure of the nicotinamide moiety changes substantially to cause the absorption of about 346 nm[7]. Also the absorptions at 418, 544 and 578 nm are due to oxyhemoglobin, particularly, *d-f* transition of CO-oxyhemoglobin[8]. Hence the results obtained are interpreted with respect to the absorbance values of λ_{max} of blood constituents. From Table-1 it is observed that there is a change in the optical density of blood with time.

TABLE-1
ABSORBANCE OF BLOOD CONSTITUENTS WITH VARYING TIME

Sample 1:   Age: 39 yrs.

Days	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6
1	2.880	1.277	1.020	1.440	0.910	0.920
8	2.911	1.231	1.004	1.489	0.854	0.863
16	2.743	1.060	0.811	1.755	0.589	0.591
27	2.692	0.996	0.720	1.858	0.461	0.460
40	2.460	0.912	0.630	1.950	0.327	0.327
50	2.355	0.907	0.595	2.006	0.259	0.247

Sample 2:   Age: 43 yrs.

Days	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6
1	2.689	0.986	0.786	1.202	0.666	0.670
8	2.664	1.063	0.857	1.198	0.731	0.729
16	2.682	0.873	0.671	1.348	0.476	0.475
27	2.692	0.716	0.538	1.501	0.292	0.280
40	2.360	0.682	0.479	1.658	0.209	0.190
50	2.287	0.690	0.500	1.701	0.170	0.150

Sample 3:   Age: 46 yrs.

Days	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6
1	2.833	1.243	1.029	1.551	0.879	0.883
8	2.859	1.204	0.982	1.661	0.799	0.801
16	2.811	1.093	0.854	1.770	0.641	0.641
27	2.679	0.967	0.736	1.862	0.477	0.475
40	2.434	0.869	0.622	1.942	0.298	0.290
50	2.342	0.828	0.569	1.979	0.225	0.211

Sample 4:   Age: 23 yrs.

Days	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6
1	3.152	1.614	1.313	1.808	1.146	1.157
13	3.034	1.578	1.303	1.797	1.133	1.142
18	2.960	1.376	1.087	2.091	0.854	0.859
25	2.662	1.232	0.915	2.206	0.638	0.642
36	2.539	1.208	0.852	2.305	0.501	0.499

Sample 5:   Age: 26 yrs.

Days	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6
1	3.131	1.685	1.396	1.923	1.209	1.220
13	3.016	1.640	1.361	1.978	1.165	1.171
18	2.812	1.341	1.070	2.309	0.803	0.811
25	2.608	1.210	0.894	2.354	0.574	0.574
36	2.531	1.244	0.872	2.404	0.462	0.455

Conclusion

From the study, it is concluded that the stored blood is having significant changes in its optical density with time of collection of blood from the human being in a blood bank. The light absorption character is decreased with varying time except the substance(CO-hemoglobin), which exhibits at 418 nm. The present work is devoted to the study of light absorption characteristics of blood through UV-Vis spectroscopy. The light absorption characteristic of blood samples with varying periods of time was studied by considering blood samples of five different age groups as a model experiment. It is better to use the stored blood as soon as possible to overcome the decrease in concentration of the substances in the blood; otherwise, we have to take necessary steps for solving such kinds of problems.

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