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Research article

The refractive index of human blood measured at the visible spectral region by single-fiber reflectance spectroscopy

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Abstract: The refractive index is an essential biophysical parameter used in many diagnostic and therapeutic biomedical applications. In the present study, the refractive index of control and anemic blood was measured using the total internal reflection fiber optics technique. For control, the refractive index measured by the indicated method was significantly higher than anemic blood over the wavelengths in the visible spectral region used in this study. Strong linear correlations between refractive index and hemoglobin concentration were obtained for control and anemic blood. These findings could enhance the use of the refractive index in many applications of blood analysis and hematology.

Keywords: refractive index; visible spectra; anemia; blood

1. Introduction

The optical properties of biological tissue could offer much information that are used in medical diagnosis and therapy. The ability of tissues to absorb the different types of spectra is the basis for many therapeutic and diagnostic applications [1]. Optical properties of tissue such as reflection, scattering, and absorption coefficients, scattering phase functions, and irradiance levels (light dosage) at tissue depth are under active investigation. These are essential quantities necessary to describe the transport and interaction of light through tissue [2–4].

One of the fundamental optical properties is the refractive index (n). The refractive index based on the wavelength is known as the refractive index dispersion (IRD) [5]. IRD reflects the physiological condition of biological tissues and cells in therapeutic and diagnostic medical devices [6]. IRD for biological materials such as blood is only available for a few wavelengths [7]. There are many methods used to measure RID, such as examining n at discrete wavelengths, or over a continuous spectrum [8–10].

The optical properties of blood can be studied at the macroscopic and microscopic levels. Blood consists of two main parts, plasma, and cells. Red blood cells make up to 99% of blood cells, so the optical properties of blood depend on the physiological properties of red blood cells [11,12]. As one of the optical properties of blood, the refractive index depends on many physiological factors, including hemoglobin concentration, hematocrit, temperature, and oxygen saturation. Measurements of the optical properties of blood at the visible light and near-infrared spectrum are the basis for the diagnosis of many blood diseases [13–15]. Studying the optical properties of blood helps in determining the optimal wavelength that provides the maximum penetration depth for the radiation used in treatment or diagnosis [16,17].

The study of the optical properties of blood focuses on measurements of absorption, dispersion, and refractive index at specific wavelengths. The blood refractive index (n) could be defined as following [18]:

$$n = n_r + ik \tag{1}$$

n_r is the real refractive index characterizing the wave phase shift, and k specifies the extinction coefficient characterizing the wave absorption.

For multicomponent biological materials such as blood, the refractive index could be calculated by the Gladstone-Dale law. That states that in the absence of the chemical interaction between the components of the medium the refractive index is the average of refractive indices of the components with their volume fractions as weighting factors, i.e., [19]

$$n = \sum_{i=1}^{N} n_i f_i \tag{2}$$

where n_i are the refractive indices of individual components, f_i is the volume fractions, respectively, and N is the number of components.

Since red blood cells and plasma are the most substantial part of the blood, the previous equation could be simplified to be as follow [20]:

$$n_{blood} = n_{RBC} f_{RBC} + n_{PL} f_{PL} \tag{3}$$

where n_{blood}, n_{RBC}, and n_{PL} are the refractive indices of blood, red blood cells, and plasma, respectively; f_{RBC} and f_{PL} are the volume fractions of red blood cells and plasma in the blood, respectively.

One of the most common blood disorders is anemia, which arises from a deficiency of Iron, Vitamin B12, or Folic acid. Hemoglobin concentration of blood drops as a result of anemia [21]. As most of the optical properties of blood depend on hemoglobin concentration, this makes anemia is an ideal model when examining a new theatrical or experimental optical technique for blood [22–24]. In the present study, the method used in the previous study to measure the refractive index of turbid

media has been used to determine the refractive index (n_s) of blood [25]. Compression between the measured n_s for anemic and normal blood was done to evaluate the use of n_s as a diagnostic tool for blood disorder.

2. Materials and methods

2.1. Sample preparation

All experiments in this study were done in compliance with the guidelines set by the Ethics Committee at Medical Research Institute–Alexandria University and were approved by it. Human blood samples were used to prepare tested hemoglobin solutions. Blood samples were collected from healthy volunteers and used as control. Other blood samples were collected from volunteers suffering from iron deficiency anemia. All blood samples were collected on EDTA as an anticoagulant. Blood samples were centrifuged at 3000 rpm for 5 min in order to separate red blood cells. Separated RBCs were then washed three times in phosphate buffer saline. Hemoglobin was obtained by lysis the RBCs. RBCs hemolysis was done by freezing at −20 ℃ for 24 h. The final hemoglobin concentration was determined for all samples spectrophotometrically at 540 nm.

2.2. Refractive index setup and measurement

The total internal reflection apparatus was assembled to measure n_s that was established in a previous study by Zhang X.U. et al. [25]. The setup was constructed from a fiber-optic Y-Bundle, halogen lamp as light source, and spectrophotometer. The light was transmitted from the light source through one branch of the fiber optic that was in contact with the sample. The other branch of the fiber optic cable detected the reflected light. Ends of the fiber optic that immersed into the sample were covered with aluminum jacket cylinder. Since the diluted blood sample is a turbid media, additional fiber with tip polished at 15° was used in order to monitor the backscattered light. The Flat fiber received reflection with the contribution of Fresnel reflection, but for the polished angle fiber, Fresnel reflection was eliminated. The reflectance measured by the flat polished fiber, $R_{\perp}$, composed of Fresnel reflection and backscattering reflection. In contrast the reflectance of polished angle fiber, $R_{\angle}$, the Fresnel reflection is completely eliminated.

The procedure used to calculate the refractive index of the tested sample was the same as used by Zhang X.U. et al. [25] for turbid media. Reflected intensities for air, water, undiluted human hemoglobin standard (as reference), and tested hemoglobin solution were determined by flat polished fiber which were $I_{\perp air}, I_{\perp water}\ I_{\perp HHS}, and\ I_{\perp HGB}$ respectively. The flat fiber was immersed in the previous subjects to perform the desired measurements. The absolute reflection of the undiluted human hemoglobin standard was calculated by:

$$R_{HHS} = R_{air} \times \frac{I_{\perp HHS} - I_{\perp water}}{I_{\perp air} - I_{\perp d}} \tag{4}$$

Where $I_{\perp d}$ is the dark current of the fiber and was determined by blocking the light source.
The Fresnel reflection for hemoglobin tested solution was calculated as:

$$R_{\perp} = R_{HHS} \times \frac{I_{\perp HGB} - I_{\perp d}}{I_{\perp HHS} - I_{\perp water}} \quad (5)$$

In the same manner, the measured absolute reflectance of the backscattering from the hemoglobin solution was calculated by:

$$R_{\perp} = R_{HHS} \times \frac{I_{\angle HGB} - I_{\angle d}}{I_{\angle HHS} - I_{\angle water}} \quad (6)$$

Hence the Fresnel reflection, R, could be calculated by:

$$R = R_{\perp} - R_{\angle} \quad (7)$$

Fresnel equation of reflection is given by:

$$R = \frac{(n_f - n_s)^2}{(n_f + n_s)^2} \quad (8)$$

$$n_s = n_f \times \frac{1 - \sqrt{R}}{1 + \sqrt{R}} \quad (9)$$

n_f for fiber was calculated from the measured reflected intensity of water and air as in [26]. Two solutions for n_s from equation 9 were obtained. The solution led to the lower value of n_s was chosen.

2.3. Statistical analysis

$n_s$was represented as mean ± SD. The Student's t-test was used to analyze the significance of the differences between anemic hemoglobin concentration and control; $p < 0.05$ was regarded as significant. Pearson's correlation analysis was done to test the strength of association between n_s and hemoglobin concentration.

3. Results and discussion

The blood refractive index curve decreased significantly, as shown in Figure 1, as the wavelength increased for control and anemic blood. The measured n_s values for control were significantly higher than anemic blood for all wavelengths. The differences between the means of n_s for control and anemic blood were significant at all wavelengths used ($p < 0.0001$). Correlation coefficients for the relationships between $n_s$and wavelength was $r^2 =$ 0.9 and 0.87 for control and anemic blood, respectively. Sardar et al. studied the n_s of whole blood with different concentrations using a laser refractometer with hollow prism. They found that for 60% blood solution, the refractive index was 1.37 at the wavelength of 632.8 nm [27]. Cheng et al. indicated that the refractive index of

whole blood was in the range of 1.373 to 1.341 by utilizing the total internal reflection [28]. Bolin et al. used a fiber optical laser refractometer to determine the refractive index of whole blood at 632.8 nm. They got n_s equal to 1.4 for blood samples [29]. The values of n_s obtained in this study, as well as its variation with the wavelengths used, are compatible with previous studies conducted on the optical properties of blood [17,20,30,31]. As indicated in the present study n_s decreases obviously as wavelength increases, which is shown in Figure 1. The finding of the present study was in accordance with the previous studies in reporting the relationship between n_s and wavelengths.

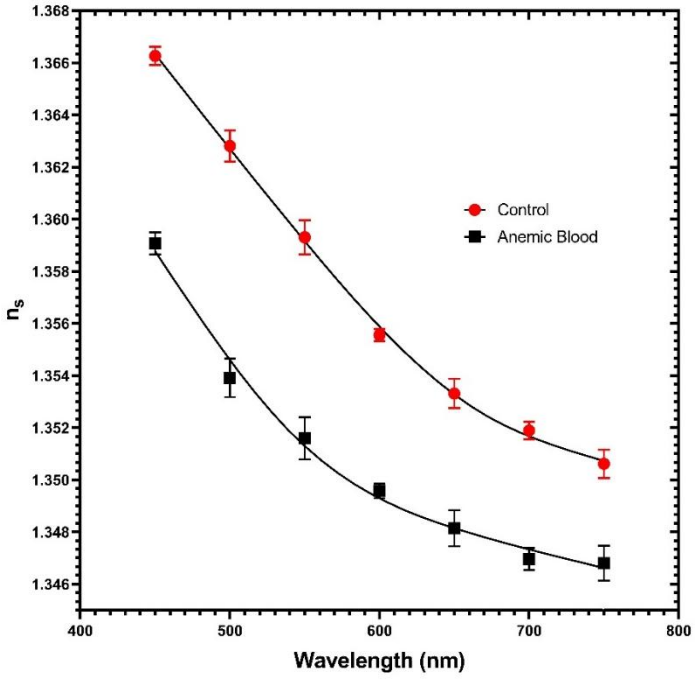

Figure 1. Refractive index decrease with an increase in wavelength and a marked difference in its value between control and anemic blood. Hemoglobin concentrations were 159.3 g/l and 99.6 g/l for control and anemic blood, respectively. The difference between the refractive index of anemic blood and control is statistically significant ($p << 0.0001$).

In this study, the relationship between refractive index and hemoglobin concentration showed a direct relationship for control and anemic blood. Figure 2 displays the heat map of n_s of different hemoglobin concentration solutions obtained from healthy volunteers at different wavelengths. There was variation in n_s due to the change in the hemoglobin concentration. The same results obtained for the hemoglobin solution obtained from volunteers suffering from iron deficiency anemia, as shown in Figure 3. Indeed the values of n_s for the hemoglobin solution, obtained from healthy volunteers were higher than that obtained from anemic volunteers. This could be explained as the hemoglobin concentration in the case of healthy volunteers is higher than the anemic one. Strong and positive correlations were obtained for the relationship between n_s and HGB control and anemic blood at the investigated wavelengths.

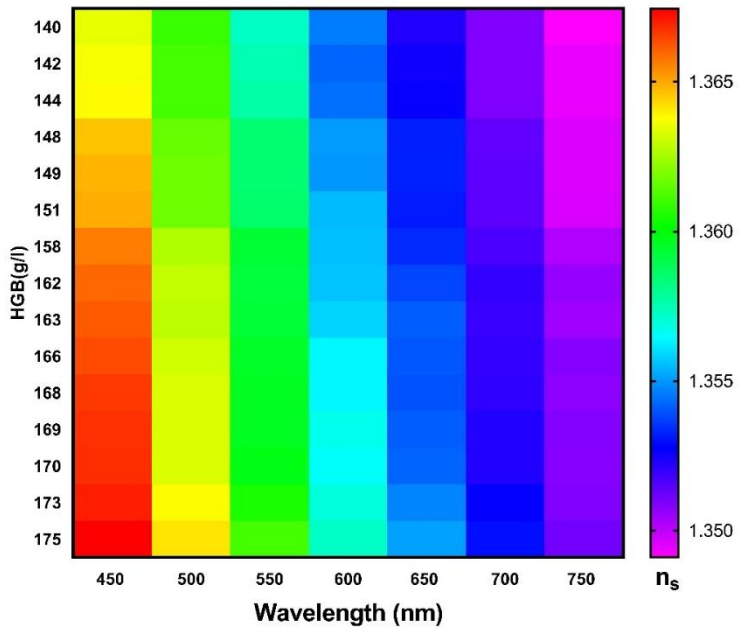

Figure 2. Heat map for the refractive index of 15 healthy subjects with different hemoglobin concentrations at different wavelengths.

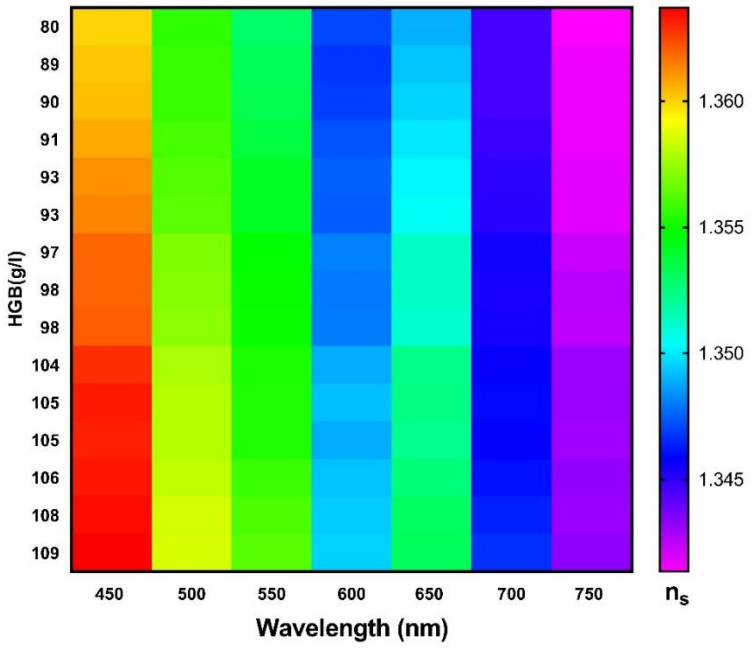

Figure 3. Heat map for the refractive index of 15 anemic subjects with different hemoglobin concentrations at different wavelengths.

The correlations coefficients and their significance are given in Tables 1 and 2 for control and anemic blood, respectively. Zhernovaya, O. et al. measured the refractive index of oxygenated and deoxygenated hemoglobin at wavelengths between 400 and 700 nm for the hemoglobin concentrations up to 140 g/l. They indicated that the measured values of the refractive index

depended on hemoglobin concentration linearly in the range of wavelengths used [16]. Friebel and Meinke reported the linear dependence between the refractive index, and the concentration is valid for higher concentrations [32]. Jin et al. studied the optical properties of the hemoglobin solution of 64.5 g/l at 25 ˚C by total internal reflection at the wavelengths of 532 and 632.8 nm. They reported that the refractive index of the hemoglobin solution was 1.335 at 632.8 nm [33]. Consistent with previous studies, the present study demonstrated the linear relationship between the hemoglobin concentration and n_s for all wavelengths used. This finding is in accordance with the previous studies, which proved the dependency of n_s on hemoglobin concentration at lower and higher ranges over the wavelengths of the visible spectral region.

Table 1. The correlations between the refractive index and hemoglobin concentration of healthy subjects at different wavelengths.

		Refractive index, n_s						
		450 nm	500 nm	550 nm	600 nm	650 nm	700 nm	750 nm
HGB (g/l)	Pearson correlation	0.9945***	0.9933***	0.9680***	0.9740***	0.9677***	0.9782***	0.9886***
	Sig. 2-tailed	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	N	15	15	15	15	15	15	15

Table 2. The correlations between the refractive index and hemoglobin concentration of anemic subjects at different wavelengths.

		Refractive index, n_s						
		450 nm	500 nm	550 nm	600 nm	650 nm	700 nm	750 nm
HGB (g/l)	Pearson correlation	0.9933***	0.9966***	0.9769***	0.9902***	0.9933***	0.9782***	0.9882***
	Sig. 2-tailed	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	N	15	15	15	15	15	15	15

Conclusion

In this study, the refractive index of blood in the visible spectral range was measured. A significant difference between the refractive index of hemoglobin solution of healthy individuals and anemic ones was indicated. The established setup and the assumption can be used in measuring the refractive index for a wide range of hemoglobin concentrations under normal and pathogenic conditions, which shows the potential of the established setup to be used for diagnostic applications in hematology.

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