The 25th Iranian Conference on
Optics and Photonics (ICOP 2019),
and the 11th Iranian Conference on
Photonics Engineering and
Technology (ICPET 2019).
University of Shiraz,
Shiraz, Iran,
Jan. 29-31, 2019

Shiraz University

رفتار خطی هموگلوبین انسانی در حلال‌های آب، اتانول و متانول

بهروز جلیلیان[۱]، محمد صادق ذاکرحمیدی[۲]، رضا خردمند[۳]

۱- پردیس بین المللی ارس، دانشگاه تبریز، ایران. bjalilian445@gmail.com

۲- پژوهشکده فیزیک کاربردی و ستاره‌شناسی، دانشگاه تبریز، ایران. zakerhamidi@tabrizu.ac.ir

۳- پژوهشکده فیزیک کاربردی و ستاره‌شناسی، دانشگاه تبریز، ایران.
r_kheradmand@tabrizu.ac.ir

چکیده-در این پژوهش رفتار خطی هموگلوبین انسانی در حلال‌های آب، اتانول و متانول با غلظت ۱×۱۰^{-۵} مولار بعد از آماده سازی و نگهداری در دمای ۴ درجه سانتیگراد برای مدت ۲۴ ساعت با استفاده از طیف‌سنجی مرئی- فرا بنفش و فلوئورسانس مورد بررسی قرار گرفت. نتایج طیف جذبی و فلوئورسانس نشان می‌دهد گذار الکترونی و تغییر در ماهیت اولیه هموگلوبین به دلیل حلال است. همچنین طیف فلوئورسانس نمونه هموگلوبین انسانی در متانول، جابجایی قرمز بیشتری نسبت به دو حلال دیگر از خود نشان می-دهد.

کلید واژه- جذب، حلال، خطیت، فلوئورسانس، هموگلوبین

The Linear Behavior of the Human Hemoglobin in Water, Ethanol and Methanol solvents

B. Jalilian[1, 2], M. Zakerhamidi[2], R. Kheradmand[3]

1- Aras International Campus, University of Tabriz, Tabriz, Iran. bjalilian445@gmail.com
2- Associate Professor, Photonics department, Research Institute for Applied physics & Astronomy (RIAPA), University of Tabriz, Tabriz, Iran. zakerhamidi@tabrizu.ac.ir
2- Associate Professor, Photonics department, Research Institute for Applied physics & Astronomy (RIAPA), University of Tabriz, Tabriz, Iran. r_kheradmand@tabrizu.ac.ir

Abstract

In this work has investigated the linear behavior of the Human hemoglobin with using UV-V is spectroscopy and fluorescence in several solvents (1.0×10^{-5}M) where prepared in 5ml from water, ethanol and methanol solvents and After storing at 4°C for at least 24hr. The absorption spectra and Fluorescence results showed that electron transition and change in the initial nature of hemoglobin is due to solvent. Also the fluorescence spectrum of human hemoglobin sample in methanol shows a higher redshift than two other solvents.

Keywords: absorption, Fluorescence, hemoglobin, linear, solvent

[Downloaded from www.opsi.ir on 2026-11-05]

This paper is authentic if it can be found in www.opsi.ir.

1. Introduction

Hemoglobin is one of the most important proteins in vivo of the human. It carries oxygen from lungs to peripheral tissues and hydrogen ions from capillaries to lungs in the vascular system. It can also transport carbon dioxide and adjust the pH, which is closely connected with the health of the organism. Hb is composed of two same α-chains and two same β-chains. Four heme groups locate in the crevices near the surface of the hemoglobin, each attached to one polypeptide chain [1].The quaternary structure of hemoglobin consists of a globular structure with four α-β sub-units. Each sub-unithas α- or β-polypeptide chain attached to a heme component. Heme consists of an iron ion held in heterocyclic porphyrinring ring [2]. The number of amino acids varies slightly among species. In human Hb, the α chain has 141 amino acids, and the β chain has 146 amino acids. In carp, the numbers are 142 and 147, respectively [3]. Hb is a type of allosteric protein that can regulate its biological activity through interactions between subunits, making binding easier when oxygen molecules have already combined [4]. In this current work, we investigated the linear behavior of hemoglobin in several solvents using UV-Vis spectroscopy.

2. Experimental

2.1. Materials

Human hemoglobin (HHb) was purchased from Sigma Chemical Company. HHb solutions (1.0×10^{-5}M) were prepared in 5ml from water, ethanol and methanol solvents and after storing at 4°C for at least 24hr.

2.2. Apparatus and methods

The UV–vis absorption spectra were collected at room temperature on a UV-vis-2450 spectrophotometer (Shimadzu, Japan) equipped with 10 mm quartz cells in the wavelength range from 200 to 900 nm. The fluorescence spectra was recorded on an F-4600 fluorescence spectrophotometer (Hitachi, Japan). The excitation and emission slit widths were set at 5.0 nm. The scanning speed was 8000 nm/min.

3. Results and discussion

3.1. UV–visible absorption spectra

UV–vis absorption spectroscopy technique can be used to explore the structural changes of protein [5]. From Fig. 1, it can be seen that HHb has five absorption peaks in water solvent. The absorption peak at 272 nm was related to Trp, Tyr and Phe and absorption peak at 405 nm correspond to porphyrin-Soret band and weak absorption peaks at 535, 575 and 630 were related to Q band [6], which is due to the $\pi \rightarrow \pi^*$ electron transition [7]. Because heme belongs to the group of porphyrin complexes, the absorption spectrum of a typical porphyrin complex consists of a strong transition to the second singlet-excited state (S0 to S2) at about 400 nm (the Soret band) and a weak transition to the first singlet-excited state (S0 to S1) at about 550 nm (the Q-band).The Soret- and Q-band absorptions both arise from $\pi \rightarrow \pi^*$ transitions [8]. The Soret band of HHb can provide rich information of the change of HHb conformation [6].

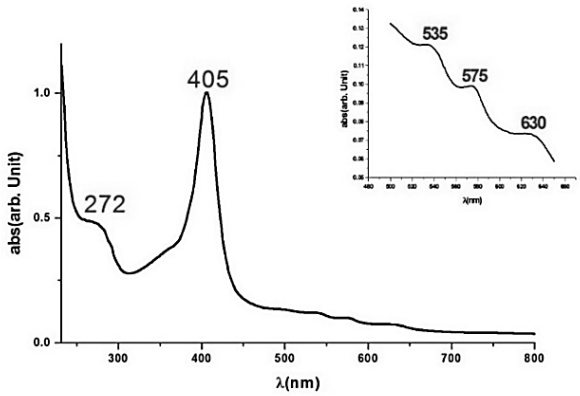


Figure 1: absorption spectrum of HHb in water solvent.

From Figures 2 and 3 it can be seen that HHb has three and four absorption peaks in ethanol and methanol respectively. The experimental results showed that methanol and ethanol have toxic effects on HHb. The effects increased along with the increasing concentration of alcohols. The results of UV–vis spectra revealed that alcohols led to conformational changes of HHb, including the loosening of the skeleton structure and the decreasing of α - helix in the second structure [5]. The UV–vis absorption spectra reflect the conformational changes of proteins. Moreover, the peak shape and peak position of protein could indicate the important changes of structure such as

[Downloaded from www.opsi.ir on 2026-11-05]

This paper is authentic if it can be found in www.opsi.ir.

unfolding and denaturation [9]. The strong absorption peaks of HHb at 206 and 208 nm in methanol and ethanol reflects the framework conformation of protein respectively. It also corresponds to the peptide bond, which is due to the $\pi \rightarrow \pi^*$ electron transition [10]. The absorption peak at 222 nm was related to α – helix structure [7]. From Figures. 2 and 3, it can be seen that HHb in methanol and ethanol solvents has absorption peaks at 274 nm and 400 nm, which correspond to the aromatic ring amino acid, and porphyrin-Soret band, respectively [6].

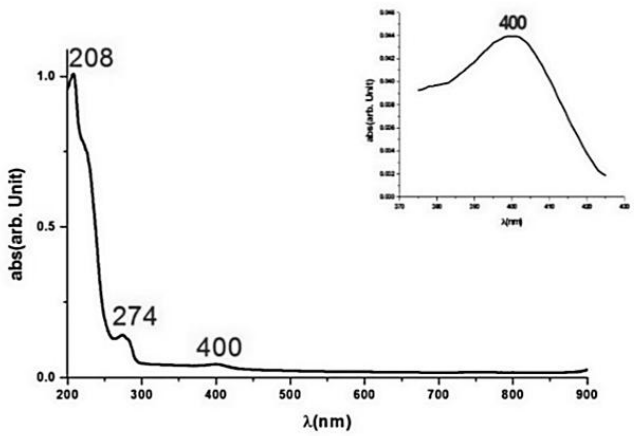


Figure 2: absorption spectrum of HHb in ethanol solvent.

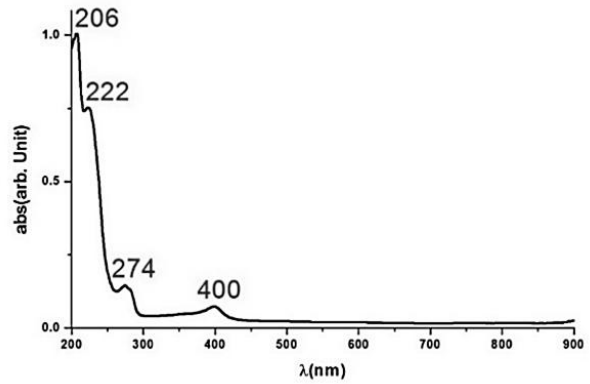


Figure 3: absorption spectrum of HHb in methanol solvent.

3.2. Fluorescence investigation

The fluorescence intensity and the location of fluorescence peak reflect the microenvironment of the chromophore group of protein [5]. Considering the absorption wavelength for human hemoglobin in water is 272 and 405 nm, therefore the excitation wavelength for human hemoglobin Considered 260 and 385 nm, which is clearly seen from the following diagrams, The emission wavelength for human hemoglobin is 310 And 462 nm. Therefore, the emission wavelength at 310 nm in this sample is due to intrinsic hemoglobin Fluorescence due to tryptophan and the emission wavelength at 462 nm is related to heme. Therefore Hemoglobin mainly emitted intrinsic fluorescence due to residue tryptophan and tyrosine [10].

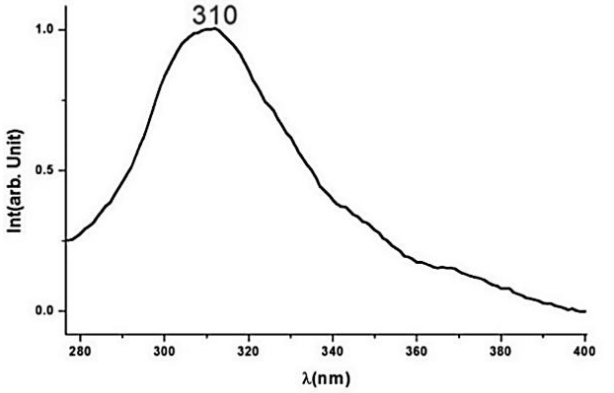


Figure 4: Fluorescence spectrum of HHb in water solvent with excitation wavelength 260nm.

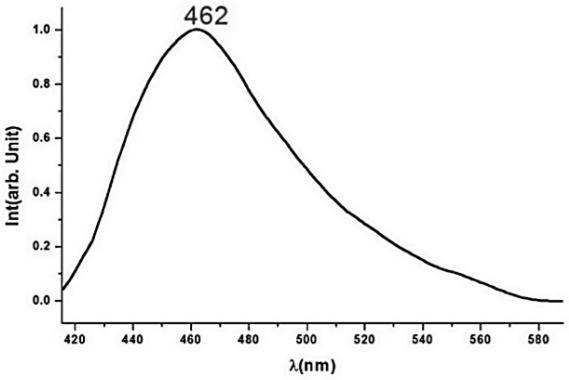


Figure 5: Fluorescence spectrum of HHb in water solvent with excitation wavelength 385nm.

Because the absorption wavelength of human hemoglobin in ethanol is 274 and 400 nm, the excitation wavelength was 270 and 370 nm for this sample, which is visible from the below figures, and according to the excitation wavelengths Selected, the emission wavelength for human hemoglobin is 336 and 468 nm. Also, due to the absorption wavelength of human hemoglobin in methanol is 274 and 400 nm, the excitation wavelength was 390 nm for human hemoglobin and based on this excitation wavelength, the emission wavelength for human hemoglobin is 477 nm, which As previously mentioned, It is mentioned that the emission wavelength is related to the fluorescence of porphyrin-heme (sourt

[Downloaded from www.opsi.ir on 2026-11-05]

This paper is authentic if it can be found in www.opsi.ir.

bond). Therefore, the intrinsic fluorescence of proteins due to the amino acids tryptophan, tyrosine and phenylalanine, changes in response to structural changes due to the small ligand / molecule binding [4]. Also, the fluorescence intensity and peak position can reflect the polarity surrounding the fluorophore molecule and the small medium of amino acid residues [10].

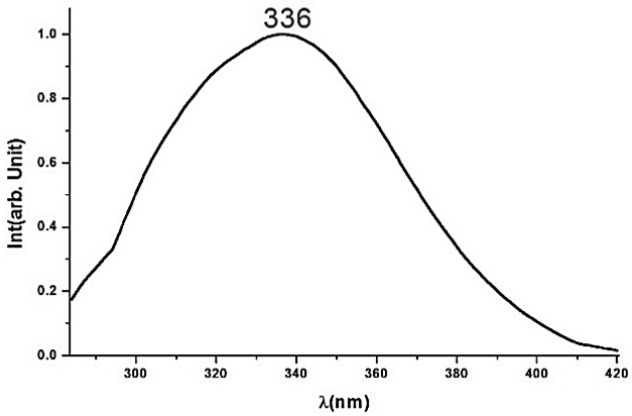


Figure 6: Fluorescence spectrum of HHb in ethanol solvent with excitation wavelength 270nm.

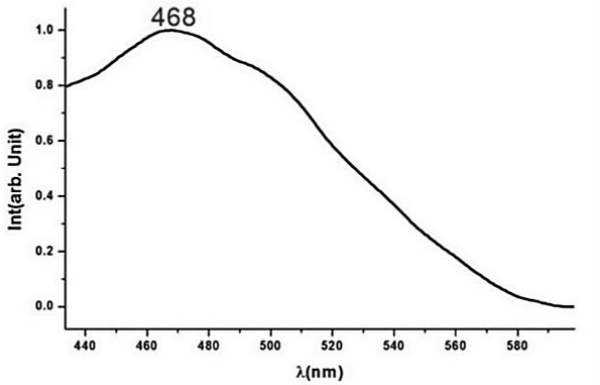


Figure 7: Fluorescence spectrum of HHb in ethanol solvent with excitation wavelength 370nm.

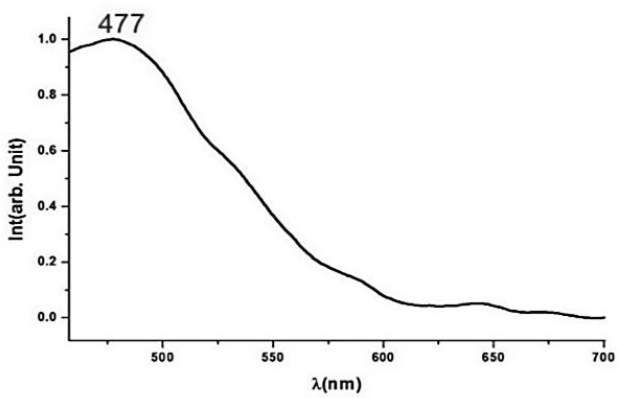


Figure 7: Fluorescence spectrum of HHb in Methanol solvent with excitation wavelength 390nm.

4. Conclusion

The figure clearly indicates that hemoglobin in methanol has a redshift relative to the two other solvents, indicating that the molecular levels of hemoglobin are closer to each other in this case. Also, the broadening caused by the influence of ethanol and methanol alcohols on hemoglobin represents a change in the initial nature of hemoglobin.

References

[1] J. Albani, "A fluorescence decay time study of tryptophan in isolated hemoglobin subunits", Elsevrer Science Publishers B. V. (Biomedical Division), FEBS 2376, Vol. 182, number 2, 1985.

[2] D.S. Chung, "A study of the Interaction of Bovine Hemoglobin with Synthetic Dyes Using Spectroscopic Techniques and Molecular docking", Frontiers in Chemistry, 2017.

[3] F.B. Jensen, et al., "Hemoglobin Structure and Function", Fish Physiology, Vol. 17, Fish Respiration /I, pp. 1-40, 1998.

[4] P. Liu, et al., "Specific absorption spectra of hemoglobin at different PO2 levels: potential noninvasive method to detect PO2 in tissues", Journal of Biomedical Optics, Vol. 17(12), 125002, 2012.

[5] C. Jun, et al., "Study on the toxic interaction of methanol, ethanol and propanol against the bovine hemoglobin (BHb) on molecular level", Spectrochimica Acta Part A, Vol. 79, pp. 1406– 1410, 2011.

[6] L. Wang, et al., "Spectroscopic investigation on the toxic interactions of Ni^{2+} with bovine hemoglobin ', Spectrochimica Acta Part A, Vol. 76, pp. 155–160, 2010.

[7] H.M. Zhang, "A fluorimetric study of the interaction of C.I. Solvent Red 24 with haemoglobin", Dyes and Pigments, Vol. 82, pp. 156–163, 2009.

[8] Q. Sun, "Mechanism of two-photon excited hemoglobin fluorescence emission", Journal of Biomedical Optics, Vol. 20(10), 105014, 2015.

[9] Y. Wu, et al., "the binding behavior of itraconazole with hemoglobin: Studies from multi-spectroscopic techniques", Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, Vol. 131, pp. 407–412, 2014.

[10] M. Sheik Bahae, et al., "Sensitive Measurement of Optical Nonlinearities Using a Single Beam", Quantum Electron, Vol. 26, pp. 760-769, 1990.

[Downloaded from www.opsi.ir on 2026-11-05]