2024, 21(2): 0465-0472
https://doi.org/10.21123/bsj.2023.7775
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Investigate Spectroscopic Experimental and Theoretical Model for Hemoglobin Nanoscale Solution

Hanan Auda Naif*, ***Asaad M. Abbas***, ***Mahasin Fadhil Hadi Al-Kadhemy***

Physics Department, College of Science, Mustansiriyah University, Baghdad, Iraq
*Corresponding Author.

Received 15/09/2022, Revised 20/01/2023, Accepted 22/01/2023, Published Online First 20/07/2023, Published 01/02/2024

Abstract

In the current study, haemoglobin analytes dissolved in a special buffer (KH_2PO_4(1M), K_2HPO_4(1M)) with pH of 7.4 were used to record absorption spectra measurements with a range of concentrations from (10^{-8} to 10^{-9}) M and an absorption peak of 440nm using Broadband Cavity Enhanced Absorption Spectroscopy (BBCEAS) which is considered a simple, low cost, and robust setup. The principle work of this technique depends on the multiple reflections between the light source, which is represented by the Light Emitting Diode 3 W, and the detector, which is represented by the Avantes spectrophotomer. The optical cavity includes two high reflectivity $\geq 99\%$ dielectric mirrors (diameter 25mm, radius of curvature 100mm) and a quartz cuvette 1 cm to put the samples in the system. This system is also composed of some lenses, aires, and optical fibres to transfer the light from the light source to the optical cavity and after that to the detector. This setup is considered ~3-fold more sensitive when it is compared with another spectroscopic technique as it reduces the effect of noise due to fluctuations in the light intensity. Additionally, the theoretical study estimated the absorption spectra of the haemoglobin concentrations using Table Curve 2D software. The absorption spectra curve was fitted using a suitable curve-fitting equation for these spectra, which was represented by the Gaussian function. The similarity of the theoretical and practical spectra demonstrated that the estimated models can replace the experimental measurements, which leads to a reduction in the cost and time required for the absorption spectroscopy measurements.

Keywords: Absorption spectroscopy, BBCEAS, Haemoglobin, Nanomolar concentrations, Theoretical model.

Introduction

Haemoglobin is an important molecule that is responsible for delivering oxygen through the arteries to the respiratory tissues; besides, it helps to transfer carbon dioxide from the veins to the lungs[1, 2]. It is considered the main heme protein in the red blood cells, and it represents up to 95% of the protein content in erythrocytes[3,4].This heme contains one iron atom in the centre of the protoporphyrin ring, which binds with four nitrogen atoms to form a metallic core [5,6]. There are certain devices and methods applied to measure the haemoglobin in the blood, such as the light scattering technique, the haemoglobin cyanide method, and the absorption difference method. Concerning the cyanide methemoglobin method, there is a problem with the toxic materials that are used. On the other hand, the light scattering method needs a sophisticated model of a RBC as a spheroid; consequently, its measurements depend on the accuracy of this model [7, 8] The absorption spectroscopy method is important in medicine for blood analysis because it can record differences between some forms of hemoglobin; additionally, it can provide information on the energy levels in the molecules and expose variations in the molecular confirmation[1, 9]. However, this method has its own drawbacks: it requires a large blood volume ≥ 10μL, a long time for measurements (several minutes), it is more costly compared to the others[10]. To address these shortcomings, spectroscopic absorption techniques such as the CRDS, CEAS, and BBCEAS, which use an optical cavity to improve light interaction with matter inside a cavity, can be used to address very low concentrations of 10 nL; the method is less expensive [11, 12]. Because most analytes are in the liquid phase [13-16], it has a wide spectral range of wavelengths (190 nm to 10 m), high spatial resolution, and reliability, and it is considered very important in many medical fields, such as health monitoring. To provide theoretical support for the experimental method, to determine whether the method can be applied to other compounds, and to prove the ability of the system before running it, software is applied to ensure the similarity between the theoretical and practical spectra; a fitting equation is applied for these samples using model software. Numerous research studies are carried out in this field, assessing the theoretical model and experimental results[17, 18]. This study depended on the Logistic Power Peak (LPP) function, and they recorded high matching between theoretical and experimental measurements. Moreover, Al-Arab *et al*. [18] studied a theoretical model to evaluate some photo-physical processes in titanium dioxide nanoparticles that were mixed with fluorescein dye and evaluated depending on the experimental fluorescence spectra measurements. They stated that the model matched the experimental measurements because it was based on curve fitting using the Logistic Power Peak function.

The current study presents a method for detecting haemoglobin based on the Broadband Cavity Enhanced Absorption Spectroscopy technique applied to select the powerful wavelengths of 400–500 nm for determining the absorbance spectra of the haemoglobin concentrations. The chosen wavelengths and corresponding haemoglobin concentrations were used to create a Gaussian function model with Table Curve 2D software to see if there is any similarity between the theoretical and practical spectra in order to demonstrate an estimated model that leads to a reduction in the cost and time required for absorption spectroscopy measurements.

Materials and Methods

Samples preparation:

Five concentrations of the analyte were prepared with these values: 1.86 E-8, 1.67 E-8, 1.48 E-8, 1.1 E-8, and 5.58 E-9 (M).The analyte used in this study was haemoglobin from Sigma Aldrich, U.K, with an absorption peak of 440 nm. It was dissolved in a buffer consisting of 1 M of each of these inorganic components: potassium phosphate dibasic K_2HPO_4 and potassium phosphate monobasic KH_2PO_4, with a pH of 7.4.

The experimental setup of the BBCEAS measurements:

Fig. 1, shows the experimental setup of the absorption cavity technique. It consists of a 3W white Light Emitting Diode (Lumileds SR-12) as a light source, an optical cavity containing two high reflectivity mirrors R ≥ 0.99 with a 25mm diameter and 100mm radius of curvature (Layertec, Germany), a quartz cuvette (Hellma, UK) used as a container for the haemoglobin, and a detection system represented by a single channel Avantes spectrometer.

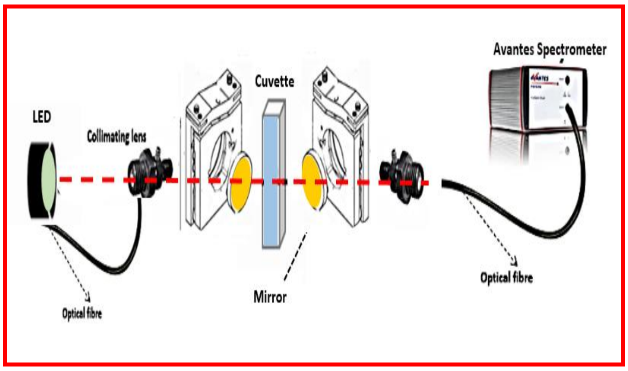

Figure 1. A schematic diagram of the set up for BBCEAS

Results and Discussion

The absorption spectra for the haemoglobin at different low concentrations (1.86 E-8, 1.67 E-8, 1.48 E-8, -1.1 E-8, and 5.58 E-9 M) are presented in Fig. 2. The highest peak of the absorption is at $\lambda_{abs.}$= 440 nm. From Table. 1, it is evident that the increase in the concentration of haemoglobin does not affect the wavelength of the absorption spectrum, where it remains constant, but rather the intensity of the spectrum, which increased with the rise in the concentration due to the increase in the number of absorbed particles. This result agreed with the Bajuszova *et al*. and Naif *et al.* studies [10, 11], *as* these studies used the BBCEAS technique to record the absorption spectra.

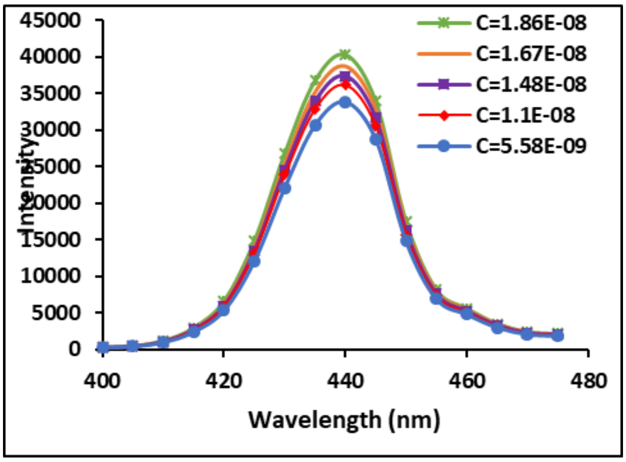

Figure 2. The Experimental absorption spectra of the range of concentrations of Haemoglobin

Theoretical calculations:

Table Curve 2D, version 5.01 software was used to obtain the best fitting curve and fit equation for all of the absorption spectra in order to evalute a theoretical model.

Table 1. Absorption information for Haemoglobin

Concentration(M)	$\lambda_{abs.}$(nm)	Intensity
1.86E-8	440	40338.85
1.67E-8	440	38769.17
1.48E-8	440	37441.44
1.1E-8	440	36202.52
5.58E-9	440	33878.48

A theoretical estimation of the absorption spectra of the range of concentrations of haemoglobin is obtained using the Table Curve 2D software. A curve-fitting process is applied to the curves in Fig. 2; the results are presented in Fig. 3. This drives researchers to draw a suitable one-curve fitting equation that can be used for these spectrums. The adopted curve-fitting equation in this study is the Gaussian function. The graph of the Gaussian function has the symmetric characteristic of a "bell curve" shape [19]. The mathematical description of this function is given by [20]:

$$Y = a\, exp(-0.5\left(\frac{x-b}{c}\right)^2) \qquad 1$$

Where x and y prove the intensity and wavelength of the absorption spectrum. The parameter *a* acts as the height of the curve's peak, *b* is the centre position of the peak that denotes the maximum wavelength of the absorption spectrum, and *c* (the standard deviation, sometimes referred to as the Gaussian RMS width) controls the width of the bell. Parameter *c* is related to the full width at the half maximum (FWHM) of the peak. These parameters varied with the concentration of hemoglobin, as illustrated in Table. 2. The correlation factor between the theoretical and experimental curves can be indicated by r2. Each parameter was schemed in

https://doi.org/10.21123/bsj.2023.7775
P-ISSN: 2078-8665 - E-ISSN: 2411-7986

contrast to the concentration; these relationships are shown in Figs. 4–6. The best-fit equations are displayed in the curves.

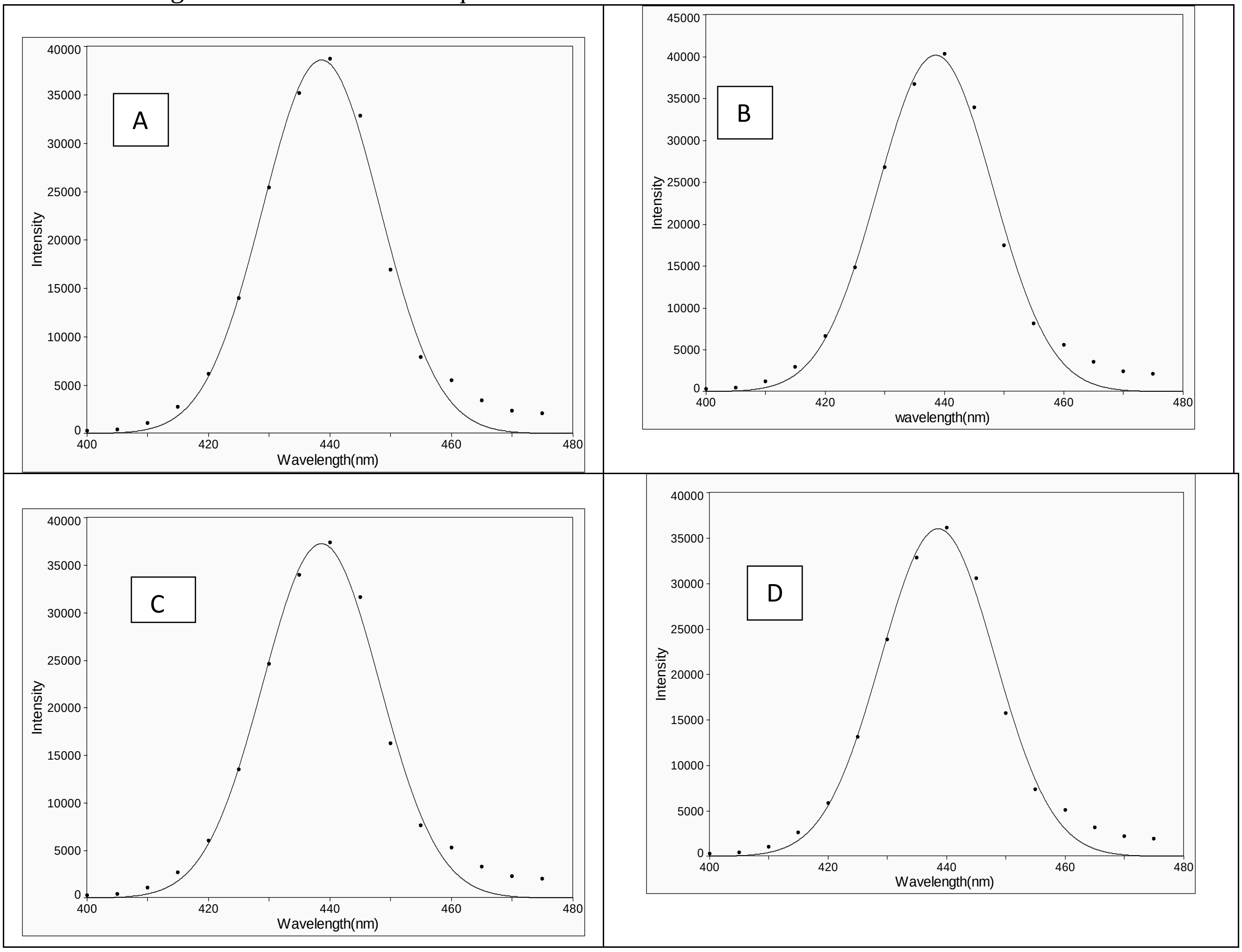

Figure 3.Fitting curves for experimental absorption spectra of different concentration of Haemoglobin A- 1.86E-8M B- 1.67E-8M C- 1.48E-8M D-1.1E-8M E- 5.58E-9M

Table 2.Parameters of estimation theoretical equation with different concentration of Haemoglobin

Concentration (M)	**a- Parameter**	**b- Parameter**	**c- Parameter**	r^2
1.86E-8	40206.159	438.56165	9.6324636	0.98822433
1.67E-8	38627.662	438.66476	9.6087471	0.98786755
1.48E-8	37288.015	438.64479	9.607427	0.98775205
1.1E-8	36056.687	438.62332	9.6223232	0.98787585
5.58E-9	33754.186	438.649854	9.6099506	0.98766947

The estimated equation for the theoretical model of the absorption spectrum was calculated for the two test concentrations of haemoglobin; it is exhibited in Table. 3.

Fig. 4 indicates the relation between a-parameter and concentration and the fitting equation, which is represented by the Tyloer expansion, for this curve as follow[21]:

$$y = 120727.21 - 0.0015461974\frac{\ln x}{x} - \frac{0.028688284}{x} + 3.467899e^{-13}\frac{\ln x}{x^2} \quad (2)$$

Fig. 5, acts the relation between b-parameter and concentration. The fitting equation of this curve is

2024, 21(2): 0465-0472
https://doi.org/10.21123/bsj.2023.7775
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

$$y = 438.86333 - 3.4158382e^{+16}x^2 + 5.1323382e^{+20}x^{2.5} - 1.9727349e^{+24}x^3 \quad 3$$

Also, Fig. 6, demonstrates the c-parameter related with concentration and fitting equation denoted by

$$y = 9.538209 + 1.1312865e^{+16}x^2 - 1.6865012e^{+20}x^{2.5} + 6.4298616e^{+23}x^3 \quad 4$$

The benefit of these fitting Eqs. 2 to 4 of (a,b and c) parameters is to find the theoretical parameters for test thoeretical concentrations.

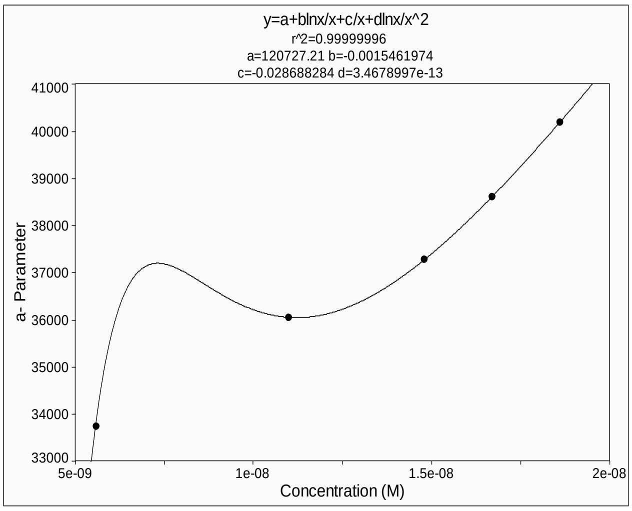

Figure 4. The relationship between the concentration and a-parameter

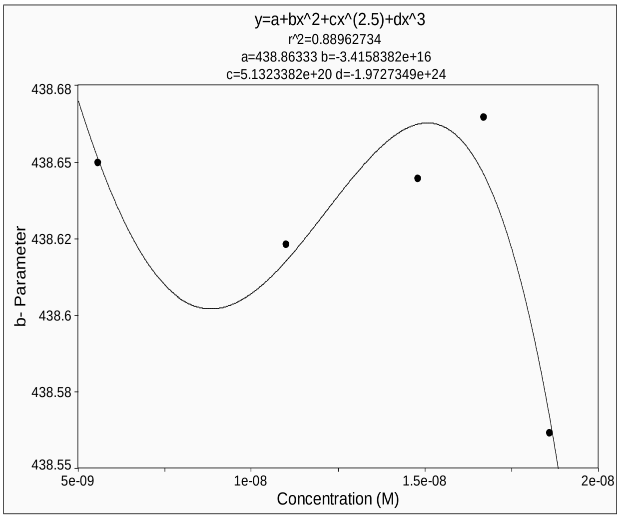

Figure 5. The relationship between the concentration and b-parameter

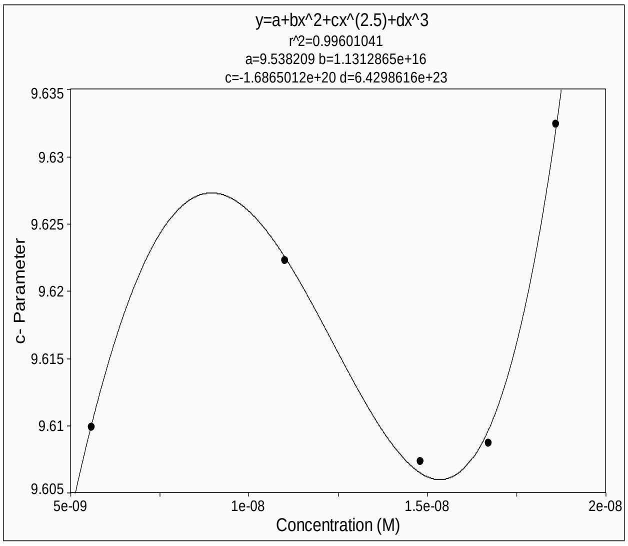

Figure 6. The relationship between the concentration and c-parameter

Table 3. Parameters of estimation theoretical equation for test concentrations of Haemoglobin

Test Concentrations (M)	a-Parameter	b-Parameter	c-Parameter
9E-9	36598.949	438.613	9.6217184
7E-9	37158.185	438.64966	9.6053399

The estimated theoretical and experimental absorption spectra are illustrated in Fig.7. It can be concluded that the behavior of the theoretical spectrum is similar to that of the experimental spectra, and that the maximum theoretical absorption wavelength is about 440 nm. This model permits the researchers to scheme the relationship between the absorption spectrum with any concentration ratio of the haemoglobin that has not been experimentally investigated.

2024, 21(2): 0465-0472
https://doi.org/10.21123/bsj.2023.7775
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

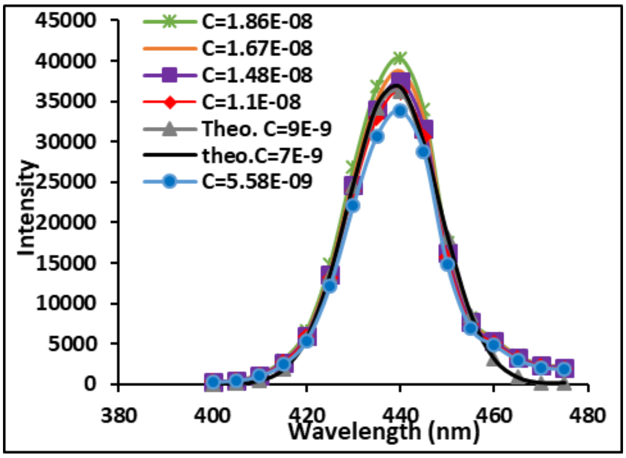

Figure 7. The experimental and Estimated theoretical absorption spectra for Haemoglobin

The results of the practical absorption spectra were compared with the theoretical results for the same value of the concentrations of haemoglobin and were considered for the three concentrations 1.1E-8, 7.44E-9 and 9.3E-9M, as shown in Figs. 8 to 10. The theoretical equations for each concentration are indicated in Eqs. 5 to 7. The results revealed a very large match between the theoretical and practical curves in terms of the maximum wavelength and intensity.

Estimated theoretical equations are[20]:

For C= 1.1E-8

$$Y = 36060.194EXP(-0.5(\frac{(X-438.61707)}{9.6226753})^2) \quad 5$$

For C=7.44E-9

$$Y = 37208.718EXP(-0.5(\frac{(X-438.6375)}{9.610451})^2) \quad 6$$

For C= 9.3E-9

$$Y = 36468.431EXP(-0.5(\frac{(X-438.61137)}{9.6227319})^2) \quad 7$$

By depending on these equations, the estaimed model can be adopted to the low concentrations of the heamoglobin measurements. Regarding the limtation, the BBCEAS system has drawback and it represents by the fluctaion in the light source which lead to increase the noise in the signal and this affects on the recoreded measureents.

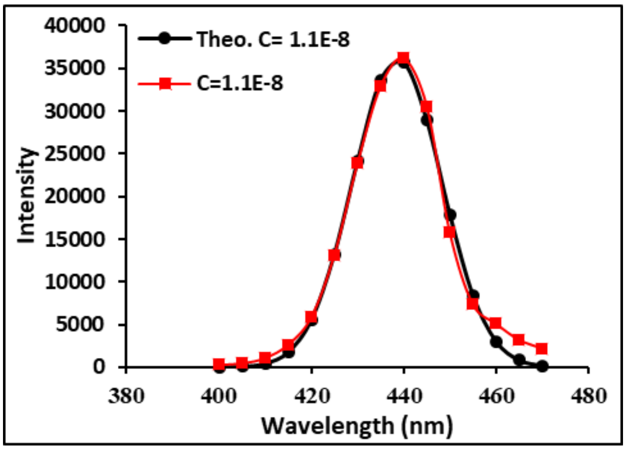

Figure 8. The experimental and Estimated theoretical absorption spectrum for Haemoglobin with C=1.1E-8

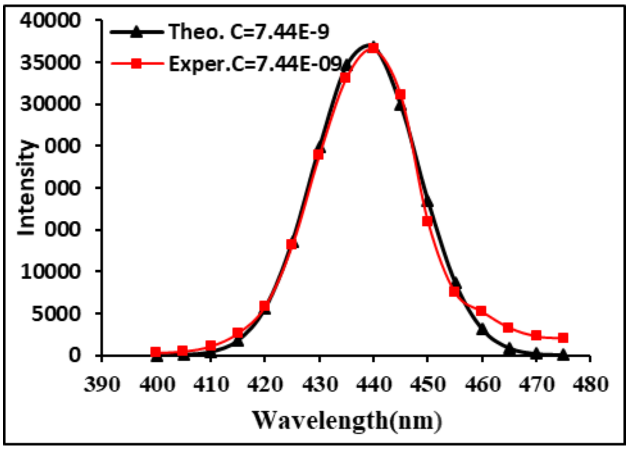

Figure 9. The experimental and Estimated theoretical absorption spectrum for Haemoglobin with C=7.44E-9

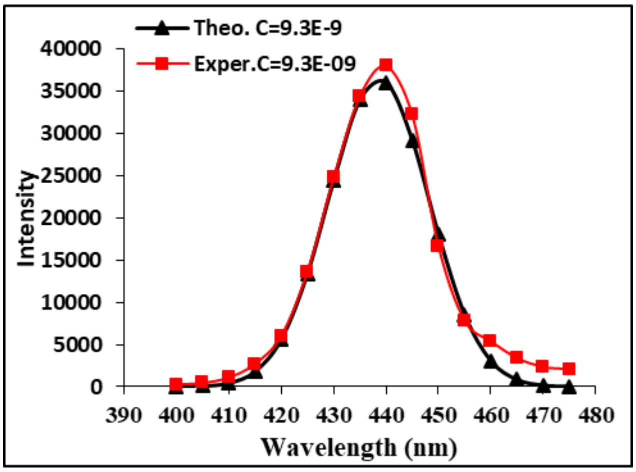

Figure 10. The Experimental and Estimated Theoretical Absorption Spectrum for Haemoglobin with C=9.3E-9

Conclusion

The BBCEAS technique was used to generate nanoscale absorption spectra for haemoglobin at concentrations ranging from 10^{-8} to 10^{-9}M. A theoretical estimation for nanoscale concentrations of these absorption experimental spectra for haemoglobin has been made, and this is considered a novel study as it deals with very low concentrations, especially with haemoglobin which is considered very necessary in many fields of the monitoring of human health, so this method can be adopted with this range of concentrations. The best-fitting equation for these samples was the Gaussian equation, which becomes apparent from the similarity of the theoretical and practical spectra. For that reason, the estimated models can replace the experimental measurements, which saves time, costs, and efforts.

Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in Mustansiriyah University.

Author's Contribution Statement

H. A. N. executed the practical part, including the acquisition of data and its interpretation, design, and analysis, and participated in the manuscript. A.M. A. helped with the analysis. M. F. A.K. helped with manuscript conceptualization, acquisition of data, and interpretation. All authors participated in the reading, revision, and proofreading of the final manuscript.

References

1. Xiuling L, Guanghui M, Zhiguo S. Hemoglobin-Based Blood Substitutes – Progress and Challenges.Comprehensive Biotechnology (Third Edition). Reference Module in Biomedical Sciences. 2019; 5:709-722. https://doi.org/10.1016/B978-0-444-64046-8.00317-7 .
2. Charbe NB, Castillo F, Tambuwala MM, Prasher P, Chellappan DK, Carreño A. *et al.*, A new era in oxygen therapeutics? From perfluorocarbon systems to haemoglobin-based oxygen carriers, Blood Rev. 2022; 54: 100927. https://doi.org/10.1016/j.blre.2022.100927.
3. Atkins CG, Buckley K, Blades MW, Turner RFB. Raman spectroscopy of blood and blood components. Appl Spectrosc. 2017; 71: 767–793. https://doi.org/10. 1177/0003702816686593.
4. Chung EH, Bhagavan NV. Chapter 25-Hemoglobin and metabolism of iron and heme. Essentials of Medical Biochemistry (Third Edition). Academic Press. 2023; 573-611.https://doi.org/10.1016/B978-0-323-88541-6.00028-4.
5. Sawicki KT, Chang HC, Ardehali H. Role of heme in cardiovascular physiology and disease. J Am Heart Assoc. 2015; 5: 4. https://doi.org/10.1161/JAHA.114.001138.
6. Nkrumah B, Nguah SB, Sarpong N, Dekker D, Idriss A, May J, *et al*. Hemoglobin estimation by the HemoCue(R) portable hemoglobin photometer in a resource poor setting. BMC Clin Pathol. 2011; 21(11): 5 https://doi.org/10.1186/1472-6890-11-5 .
7. Kim U, Song J, Ryu S, Kim S, Joo C. A Rapid and Chemical-free Hemoglobin Assay with Photothermal Angular Light Scattering. J Vis Exp. 2016; 7(118): 55006. https://doi.org/10.3791/55006 .
8. Dybas J, Bokamper MJ, Marzec KM, Mak PJ. Probing the structure-function relationship of hemoglobin in living human red blood cells. Spectrochim Acta A Mol Biomol Spectrosc. 2020; 239: 118530. https://doi.org/10.1016/j.saa.2020.118530 .
9. Hsieh M, Wu T, Su C, Cheng W, Ozbek N, Tsai K, *et al*. Comparison of an electrochemical biosensor with optical devices for hemoglobin measurement in human whole blood samples. Clinica Chimica Acta. 2011; 412: 2150–2156. https://doi.org/10.1016/j.cca.2011.07.026 .
10. Bajuszova Z, Naif H, Ali Z, McGinnis J, Islam M. Cavity enhanced liquid-phase stopped-flow kinetics. Analyst. 2018; 143(2): 493-502. http://dx.doi.org/10.1039/C7AN01823A .
11. Naif HA, Saeed AA, Al-Kadhemy MFH. Spectral Behaviour of the low concentrations of Coumarin 334 with Broadband Cavity Enhanced Absorption Spectroscopy. Baghdad Sci J. 2022; 19(2): 0438 https://bsj.uobaghdad.edu.iq/index.php/BSJ/article/view/6190.

12. Haodong Z, Jing L, Saimei H, Zhanpeng X, Julian E, Sailing H. Incoherent broadband cavity-enhanced absorption spectroscopy for sensitive measurement of nutrients and microalgae. Appl Opt. 2022; 61: 3400-3408. https://doi.org/10.1364/AO.449467 .
13. Anang N, Hamid MSA, Muda WMW. Simulation and Modelling of Electricity Usage Control and Monitoring System using ThingSpeak. Baghdad Sci J. 2021; 18(2): 0907. https://doi.org/10.21123/bsj.
14. Zheng K, Zheng C, Zhang Y, Wang Y, Tittel FK. Review of Incoherent Broadband Cavity-Enhanced Absorption Spectroscopy (IBBCEAS) for Gas Sensing. Sens. 2018; 18: 3646. https://doi.org/10.3390/s18113646
15. Nakashima Y, Sadanaga Y. Validation of in situ Measurements of Atmospheric Nitrous Acid Using Incoherent Broadband Cavity-enhanced Absorption Spectroscopy. Anal Sci. 2017; 33(4): 519-524. https://doi.org/10.2116/analsci.33.519.
16. Pakkattil A, Saseendran A, Thomas AP, Raj AS, Mohan A, Viswanath D, *et al*. A dual-channel incoherent broadband cavity-enhanced absorption spectrometer for sensitive atmospheric NOx measurements. Analyst. 2021; 146(8): 2542-2549. https://doi.org/10.1039/D1AN00132A
17. Al-Arab H, Al-Kadhemy M, Saeed A. Estimation of Theoretical Models of Photophysical Processes for Fluorescein Laser Dye with Ag Nanoparticles. Gazi Univ J Sci. 2021; 34(2): 550-560. https://doi.org/10.35378/gujs.666716
18. Al-Arab HS, Al-Kadhemy MFH, Saeed AA. The Establishment of a Theoretical Model for the Estimation of Some Photo-Physical Processes in Laser Dyes. Iraqi J Sci. 2020; 61(4): 780-790. DOI: https://doi.org/10.24996/ijs.2020.61.4.10
19. Lambros A, Dimitrios F, Lampros Mi. Atherosclerotic Plaque Characterization Methods Based on Coronary Imaging. 5-Plaque Characterization Methods Using Optical Coherence Tomography. Academic Press. 2017; 95-113. https://doi.org/10.1016/B978-0-12-804734-7.00005-1
20. Dyson N. Chromatographic integration methods. RSC–-chromatography monographs. Royal Society of Chemistry. Phytochemical Analysis. London. 1991;15. https://doi.org/10.1002/pca.2800020608
21. Hardy G, Körner T. Derivatives And Integrals. In A Course of Pure Mathematics-Cambridge Mathematical Library. Cambridge. University Press. 2008; 210-284. https://doi.org/10.1017/CBO9780511989469.008 .

دراسة النموذج العملي والنظري الطيفي لمحلول الهيموكلوبين النانوي

حنان عوده نايف، اسعد مجبل عباس ، محاسن فاضل هادي الكاظمي

قسم الفيزياء، كلية العلوم، الجامعة المستنصرية، بغداد، العراق.

الخلاصة

في الدراسة الحالية، تم استخدام محلول الهيموكلوبين المذاب بمذيب خاص يحتوي على 1مولاري من كل من K_2HPO_4 و KH_2PO_4 مع ضبط الاس الهيدروجيني على 7.4لتسجيل قياسات الامتصاص الطيفية لمدى من التراكيز تتراوح بين 10^{-9} الى 10^{-8} مولاري عند الطول الموجي 440 نانومتر باستخدام تقنية التجويف البصري لتحسين قياسات الامتصاص الطيفيةBBCEAS، والتي تعتبر تقنية بسيطة وذات كلفة غير عالية وايضا تمتاز بصلابة النظام المكون لها. تعتبر تقنية التجويف البصري من التقنيات الحديثة التي تعتمد على توليد انعكاسات متعددة بين المصدر الضوئي والمتمثل بالثنائي الباعث للضوء بقدرة تساوي 3W والكاشف المتمثل بجهاز Avantes spectrometer، حيث يؤدي الى زيادة طول المسار البصري اي ان العلاقة طردية بين مقدار الامتصاصية وطول المسار البصري للضوء وتكون هذه التقنية من مرآتين من مادة عازلة ذات انعكاسية عالية تصل الى 99% ≥ مع قطر يساوي 25mm ونصف قطر تكوريساوي 100mm ، تتوسطها حامل مصنوع من مادة الكوارتزوبعرض 1cmلوضع العينات فيها مع مجموعة من العدسات مختلفة الانواع ومجمعات الاشعة بالاضافة الى استخدام الالياف البصرية في عملية انتقال الضوء من المصدر الضوئي الى داخل التجويف البصري ومن ثم اخراج الاشعة وايصالها الى الكاشف. هذه التقنية تعتبر اكثرحساسية من ناحية تسجيل القياسات بثلاث مرات عن باقي تقنيات الامتصاص الطيفية المعروفة وذلك لانها تقلل من كمية الضوضاء المصاحب للقياسات.اضافة الى ذلك، تم تقدير دراسة نظرية لطيف الامتصاص لتراكيز الهيموكلوبين باستخدام برنامج Table curve 2D. تم تطبيق معادلة منحني كاوس المناسبة لهذه الاطياف ووجد ان التشابه الذي تم تسجيله بين الدراسة النظرية والعملية لاطياف الامتصاص يوضح صحة النموذج المفترض وبالامكان اعتماده بدل الدراسة العملية والتي تساهم في تخفيض التكلفة والوقت اللازم لتسجيل هذه القياسات.

الكلمات المفتاحية: اطياف الامتصاص،تقنية BBCEAS ، هيموكلوبين ، تراكيز نانوية، نموذج نظري.