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Broadband Cavity Enhanced Absorption Spectroscopy of Thin Films of Haemoglobin

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Abstract

In this study, a sensitive technique (Broadband Cavity Enhanced Absorption Spectroscopy (BBCEAS)) is employed for measuring the absorption of thin films deposited onto a glass substrate. A thin film of the biological solution such as Lyophilied Bovine haemoglobin is deposited on glass microscope coverslips. Drop coating method was used to deposit a thin film over the microscope coverslips. The number of passes is calculated: 612 passes for the high reflectivity mirror were obtained. The best measurements are made with the mirror set of reflectivity of ($R \geq 0.99$) which produced an α_{min} value of 0.0043 cm^{-1} and *LOD* of 1.9×10^{-7}M.

Keywords: Thin films; Drop coating method; Absorption spectroscopy.

الخلاصة

بينت الدراسة الاتية ان منظومة محلل الامتصاص الطيفي المحسن ذات المدى العريض (BBCEAS) استخدمت كتقنية ذات حساسية عالية لدراسة طيف الامتصاص لغشاء رقيق مترسب على شريحة زجاجية.غشاء رقيق من محلول بايولوجي من الهيموكلوبين البقري المجفف بالتجميد رسب على شريحة المجهر الزجاجية الرقيقة. طريقة التقطير استخدمت لترسيب الغشاء على شريحة المجهر الزجاجية. عامل التحسن لهذه التقنية (CEF) او عدد الانعكاسات للضوء في المنظومة حسبت والتي تساوي 612 انعكاس. الحساسية للمنظومة المختبرية قيست بواسطة حساب اقل تغير في معامل الامتصاص α_{min} وكانت تساوي $0.0043\ cm^{-1}$. حدود الكشف للمنظومة (LOD) للهيموكلوبين البقري تساوي 1.9×10^{-7}M.

Introduction

Haemoglobin is an important component for all complex animal life. It is an iron-rich protein which transferred by red cells. It absorbs oxygen from the lungs and delivers it to the tissues in the body in order to maintain cell viability. It then returns carbon dioxide from tissues to the lungs. Haemoglobin in an adult person contains two similar proteins with alpha and beta chains. In fetuses and infants, the haemoglobin particles contain two gamma and two alpha chains. Later, the gamma chains convert gradually to beta chains [1]. The figure (1) shows the structure of haemoglobin.

The Beer-Lambert law is applied in absorption spectroscopy analytical techniques to determine the absorption spectrum of a sample.

The spectrum of haemoglobin is measured by the cavity enhanced absorption spectroscopy method, which is one of a number of approaches to absorption spectroscopy. The haemoglobin spectrum changes depending upon what it is bound to; consequently the spectrum of oxyhaemoglobin (bound to oxygen) will differ from carboxy-haemoglobin (bound to carbon dioxide) and both will again differ from the spectrum of deoxy-haemoglobin (bound to nothing).

Numerous reports on the absorption spectra of haemoglobin and its derivatives were found. The absorption spectra of oxyhaemoglobin, carbonnylhaemoglobin, reduced haemoglobin, methaemoglobin, and metcyanhaemoglobin were determined in the visible and infrared region [2]. The comparison between four clinical derivatives of foetal and adult human haemoglobin was made in the visible and near infrared spectral range (450-1000nm) to determine millimolar absorptivities [3]. A tiny differences were determined in the absorption spectra be-

tween bovine and human haemoglobin especially in the oxyhaemoglobin, dexyhaemoglobin, deoxyhaemoglobin, and carbonxyhaemoglobin spectra [4].

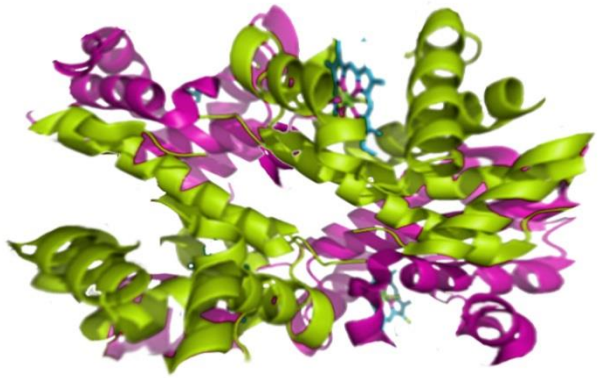

Figure (1): Structure of human haemoglobin. The green parts refer to the protein's α subunits while the pink parts refer to the protein's β subunits, and the iron-containing heme groups in green [1].

For more than two decades, the poor sensitivity of conventional absorption spectroscopy has been enhanced by using an optical cavity[5]**Error! Reference source not found.**. A successful absorption measurements were made by applying CRDS and CEAS on gas phase species [6-10], because the absorption and scattering losses were very lower compared to the measurements for liquid-phase and solid-phase species; consequently, a larger number of passes through the gas sample can be achieved. Many studies have verified CRDS and CEAS for the liquid phase by employing creative methods to minimize the losses due to scattering from the optical surface [11-14]. Relatively few studies have applied CRDS and CEAS to the solid phase [15-19]. In the present paper, the broadband cavity enhanced absorption spectroscopy (BBCEAS) setup is used to measure the absorption spectrum of a thin film of Lyophilized bovine haemoglobin solution deposited on a borosilicate coverslip slide by using the drop coating method at visible wavelengths.

Experimental setup

The setup of Broadband Cavity Enhanced Absorption Spectroscopy for the thin film is shown in figure (2). It is largely the same as that used in our previous study [20] and consequently only a brief description is given and is not repeated here. In BBCEAS, the output light from the white LED is collimated by a series of lenses and irises and inserted into an optical cavity which formed by two high reflectivity mirrors (R). The output from the cavity is focused into an Andor's cooled CCD spectrometer. The measurements of transmission spec rum of the cavity in a blank uncoated glass slide (I_o) and in a sample spectrum with the thin film coated glass slide (I) are measured respectively.

Drop coating is probably the simplest method for depositing a uniform thin film of desired molecule on a glass slide. The Haemoglobin solution used to create the thin film is prepared from distilled water. As the same suggests the solution is simply dropped onto the substrate and the thin film is created following the evaporation of the solution. The drop coating method has two main advantages: it is simple to use, and it can be used to create the sample directly in-situ inside the cavity without disturbing the alignment of the coverslip within the cavity.

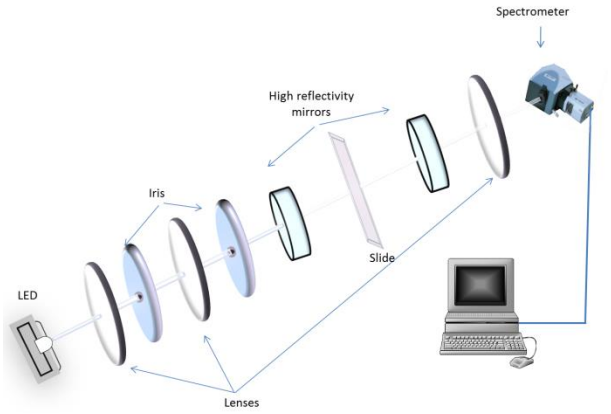

Figure (2): The setup of Broadband Cavity Enhanced Absorption Spectroscopy for the thin film.

Experimental Methodology

The experimental methodology to calculate absorption spectra, CEF values, α_{min} values and LODs are the same as described in our previous study [20] and consequently is not repeated here.

Result and Discussion

A borosilicate coverslip with 0.14 mm thickness was coated with a thin layer of haemoglobin dye molecules by the drop coating method as described in previous study [20]. The boro-

silicate coverslip was used directly from the packet without being cleaned. It was inserted between two pieces of optical quality lens tissue and a few drops of solution were spread on the surface of the slide. Distilled water was used as a solvent to dissolve the haemoglobin dye, which resisted evaporation due to need to break the hydrogen bonds. Consequently, it would require either a lot of heat or a long time to evaporate. For this reason, the thin film of haemoglobin was put in oven at 37^{o}C for 10 min. to protect it from ambient humidity until the solvent had evaporated. A problem was detected when the water had evaporated completely. The lens tissue did not fall away naturally and instead stuck to the borosilicate coverslip due to the large protein unit that was available in the haemoglobin molecules. All measurements are summarized in table (1). The Table lists important figures of merit obtained from these data such as the CEF or the number of passes obtained the wavelength of measurements, the calculated α_{min} values for each measurement and the LOD of the analyte.

Table (1): The outline measurements made with the thin film of haemoglobin analyte.

R ≥	0.99	0.999
Analyte	Haemoglobin	Haemoglobin
CEF	53	612
λ/nm	556	567
l µm	4.43	3.59
α_{min}/cm^{-1}	0.0128	0.0043
LOD/M	5.7×10^{-7}	1.9×10^{-7}

BBCEAS results by using the drop coating method for haemoglobin and with R ≥ 0.99 mirrors

The experiment setup was used with R ≥ 0.99 mirrors set and a cooled Andor spectrometer to measure the absorption spectrum of a thin film of haemoglobin deposited on a borosilicate coverslip by using the drop coating method. Figure (3) shows representative absorption (I_o-I/I) spectra of a thin film of haemoglobin at 556 nm, recorded with white LED and 0.99 mirrors set and an Andor spectrometer at range of low concentrations from 1×10^{-5} M to 1×10^{-4} M.

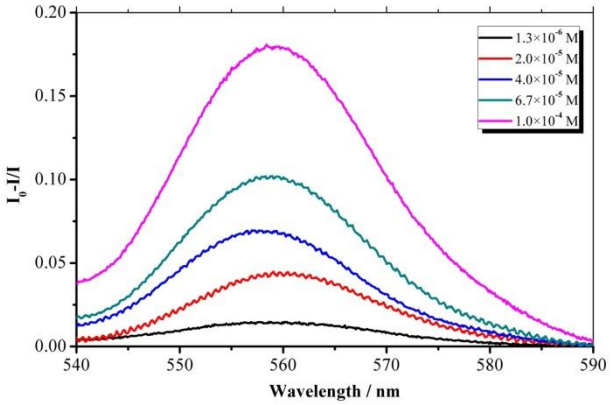

Figure (3): Absorption (I_o-I/I) spectra of a thin film of haemoglobin, for a series of concentrations from smallest to highest (1×10^{-5}- 1×10^{-4}) M.

Figure (4) shows the plot of absorption (I_o-I/I) of a thin film of a thin film of haemoglobin deposits on a borosilicate coverslip recorded with the white LED and mirror set of reflectivity of (R ≥ 0.99).

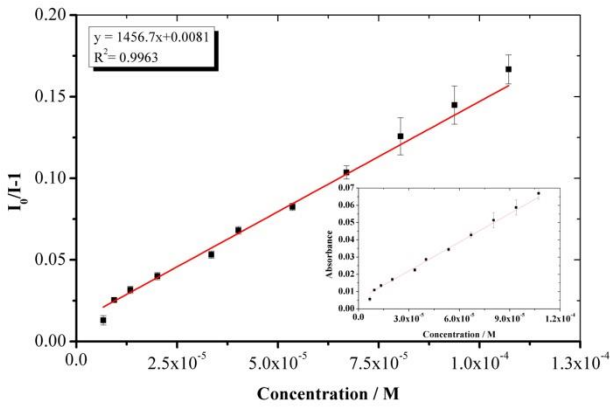

Figure (4): Absorption (I_o-I/I) versus concentrations plot of a thin film of haemoglobin deposited on a borosilicate coverslip , in the range (6×10^{-6} M to 1×10^{-4} M), obtained using the white LED, a cooled Andor spectrometer, and the reflectivity of mirror set of (R ≥ 0.99). The inset shows the linear relation between the absorbance value and concentration of a thin film of haemoglobin. The fitting between linear least squares and the measurements illustrates in the absorption plot referred by the equation on the diagram.

In the inset figure, the relation between the absorbance value and concentration of a thin film of haemoglobin was plotted. For each concentration, three measurements of a thin film of haemoglobin were made and the error

bars of each data shows the standard deviation of the measurements. A linear least squares regression through the linear plot of absorption versus concentration yields a straight line (equation of the line is given in figure 4) with the correlation coefficient $R^2 = 0.996$.

BBCEAS results by using the drop coating method for haemoglobin and with R ≥ 0.999 mirrors

The same experiment setup was used with R ≥ 0.999 mirrors set and a cooled Andor spectrometer to measure the absorption spectrum of a thin film of haemoglobin deposited on a borosilicate coverslip by using the drop coating method. Figure (5) shows representative absorption (I_o-I/I) spectra of a thin film of haemoglobin at 567 nm, recorded with white LED and 0.999 mirrors set and an Andor spectrometer at range of low concentrations from ~2×10^{-5} M to ~3×10^{-4} M.

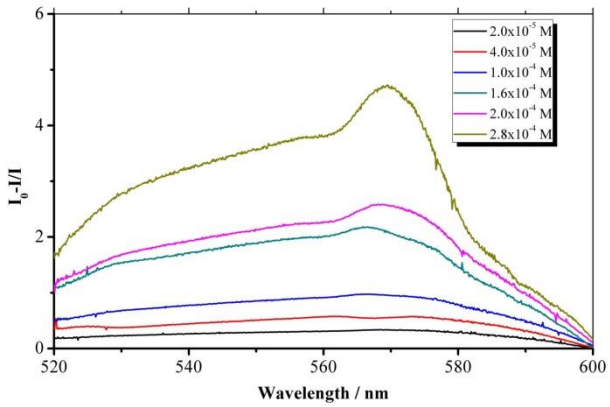

Figure (5): Absorption (I_o-I/I) spectra of a thin film of haemoglobin, for a series of low concentrations from ~2×10^{-5} M to ~3×10^{-4} M.

Figure (6) shows the plot of absorption (I_o-I/I) of a thin film of haemoglobin deposits on a borosilicate coverslip recorded with the white LED and mirror set of reflectivity of (R≥0.999). The inset figure shows the relation between the absorbance value and concentration of a thin film of haemoglobin of a thin film of haemoglobin. For each concentration, three measurements of a thin film of haemoglobin were made and the error bars of each data shows the standard deviation of the measurements. A linear least squares regression through the linear plot of absorption versus concentration yields a straight line (equation of the line is given in figure 6) with the correlation coefficient $R^2 = 0.962$.

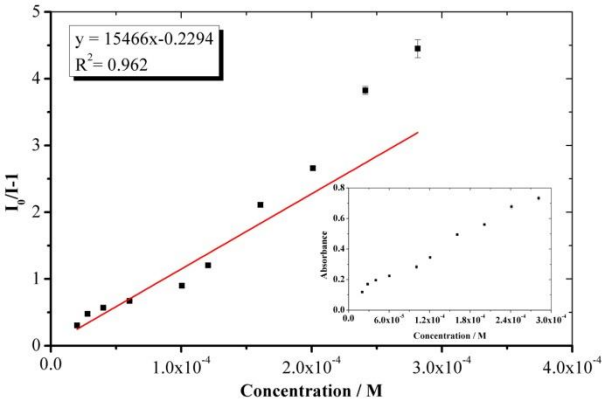

Figure (6): Absorption (I_o-I/I) versus concentrations plot of a thin film deposited on a borosilicate coverslip, in the range from ~2×10^{-5} M to ~3×10^{-4} M, obtained using the white LED, a cooled Andor spectrometer, and the R ≥ 0.999 mirror set. The inset shows the linear relation between the absorbance value and concentration of a thin film of haemoglobin. The error bars represent the 1 σ error limit of three replicate measurements at each concentration. The fitting between linear least square and the measurements illustrates in the absorption plot referred by the equation on the diagram.

This study has attempted to make sensitive absorption measurements on a condense phase by using the BBCEAS relative to conventional absorption spectroscopy. Below some remarks on the difficulty of the experiment will be made. Such difficulties concerned measuring the absorption spectrum for very low concentrations, especially with the R ≥ 0.999 mirror set due to the base line shift. This base line shift arose from the difficulty of making an appropriate holder for a borosilicate coverslip. Another problem was the adhesion of the lens tissue to the glass slide when coated with a thin film of haemoglobin due to fact that water has a poor volatility and needs a considerable time to evaporate.

The creation of a thin film is affected by the type of solvent that is used to dissolve the compound. The CEF values for a biological compound (haemoglobin) were inaccurate due to the use of water that evaporates slower than other solvents. Moreover, the lens tissue used should fall away naturally after evaporating the solvent from the thin film. For a haemoglobin thin film, the lens tissue in fact sticks on the

surface of the glass slide, which compromises the homogeneity of the coating.

The general trend as shown previously for the liquid phase studies was of increasing CEF values with increasing mirror reflectivity. The sensitivity of the measurements was calculated using the minimum detectable change in the absorption coefficient αmin. Comparing between mirror reflectivities the R ≥ 0.999 mirrors appeared to produce a more sensitive result due to the large increase in CEF value. Table 1 also lists the LODs of the haemoglobin thin film for both reflectivity mirror sets. These values depend on the sensitivity of the measurement.

Conclusions

BBCEAS has been used to measure the visible wavelength spectra of thin films of haemoglobin deposited on glass substrates and placed at zero degrees angle of incidence between the cavity mirrors. Thin films were created by drop coating onto thin glass slides. Measurements were made with R ≥ 0.99 and 0.999 mirrors for the drop coated thin films. The best results were obtained for the drop coated thin films on the thin glass substrates with the R ≥ 0.999 mirrors.

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