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박막광도파로 센서를 이용한 산화 및 환원 혈색소의 새로운 흡광계수 측정법

강 신 원

=Abstract=

A New Method for Determining the Absorption Coefficient of Oxy- and Deoxyhemoglobin by use of a Thin-film Optical Waveguide Sensor

Shin-Won Kang

A simple method for determining the absorption coefficient of oxyhemoglobin and deoxyhemoglobin in human blood is proposed as an application of the complex propagation constant of a guided wave in a thin-film optical waveguide. A serial multichannel sample chamber is constructed on the waveguide to vary the interaction length between the evanescent field and the sample, and the dependence of the sensor response on the interaction length is investigated for the various concentration of two hemoglobins. The sensor response is linearly proportional to the interaction length and the concentration of two hemoglobins. The attenuation constant due to the evanescent field absorption between the samples is experimentally obtained with the designed sensor, and then the absorption coefficient is determined by the proposed method. The absorption coefficients determined by the proposed method fairly well coincided with those obtained by the conventional transmission measurement.

Key words : absorption coefficient, hemoglobin derivatives, thin-film optical waveguide, evanescent field, interaction ratio

INTRODUCTION

The absorbance spectra and the absorption coefficient of biochemicals at wavelength are very important parameters that uniquely characterize particular molecules and can be used for both identification and quantification. The absorption coefficients of oxyhemoglobin(HbO_2) and deoxyhemoglobin(HHb) a primary concerns in the design of blood oxygen instrumentations[1]. One of the most common methods of hemoglobin derivatives analysis is the spectrophotometric method based on the Beer-Lambert's law. However, most samples are turbid, colored or have scatterers such as erythrocyte. Therefore, as most of other studies done in measurements of these hemoglobin derivatives[2~9], samples must be diluted and purified to make the measurement in 1cm path-length cuvette possible, otherwise contained in special cuvettes with path-length of extremely less than 1cm.

In this paper, I proposed a simple method of determining the absorption coefficient of HbO_2 and HHb by use of the evanescent field absorption in the thin-film planar waveguide

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sensor. In order to analyze the properties of the sample through energy absorption from the evanescent field, the complex propagation constant of a guided wave in thin-film planar waveguide should be considered. The physical parameters of waveguide which are used in the experiment and the dependence of guided wave attenuation constant due to evanescent field absorption on the absorption coefficient of the light absorbing samples are previously investigated. A serial multichannel sample chamber is constructed on the thin-film waveguide to vary the interaction length between the evanescent field and samples. The dependence of the guided wave attenuation in the waveguide on the interaction length is investigated for the various concentration of two fully oxy- and deoxygenated hemoglobins. And also sensor response to the various total hemoglobin concentration of erythrocyte is observed. The results obtained through multichannel waveguide sensor analysis are compared with those obtained by the conventional spectrophotometric method.

THEORY AND METHOD

Schematic cross-section of the proposed waveguide sensor model with the prism coupled is depicted in Fig. 1. When the refractive indices of top layer (n_3) and substrate (n_1) are lower than those of waveguide (n_2), the energy of coupled lights only at incoupling angle θ_{in} is confined to the waveguide and propagated in the Z-axis. In this case, exponentially-decaying (evanescent) field is generated in the substrate and the top layer. If the absorbing biochemical species are presented in these medium, absorption of energy from the evanescent field is occurred by those when the light wavelength corresponds to absorption band of the biochemical species, and the power of propagating light is decreased.

In order to analyze this phenomenon, the complex propagation constant of a guided wave in a thin-film planar waveguide is considered. If the refractive index of sample (n_3) is lower than that of waveguide (n_2), the analysis model of the waveguide practically coincides with that of a three-layer structure waveguide.

The complex dispersion relation is derived from the given boundary conditions, and the eigenvalue equation of three-layers structure waveguide at TE mode[10] is

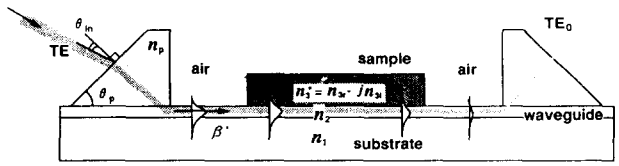

Fig. 1. Schematic cross-section of the prism coupled waveguide sensor based on the evanescent field absorption.

$$q_2T=\tan^{-1}(p_1/q_2)+\tan^{-1}(p_3/q_2)+m\pi, \quad (m=\text{mode order}=0, 1, 2, 3\cdots.) \tag{1}$$

and

$$p_1 = k_0\{(\beta^*/k_0)^2 - n_1^2\}^{1/2} = k_0(N^{*2}_e - n_1^2)^{1/2},$$
$$q_2 = k_0\{n_2^2 - (\beta^*/k_0)^2\}^{1/2} = k_0(n_2^2 - N^{*2}_e)^{1/2},$$
$$p_3 = k_0\{(\beta^*/k_0)^2 - n^{*2}_3\}^{1/2} = k_0(N^{*2}_e - n^{*2}_3)^{1/2},$$
$$n^*_3 = n_{3r} - jn_{3i} = n_{3r} - j(\alpha/2k_0),$$
$$N_e^* = N_r - jN_i(\beta''/k_0),$$

Where

$\beta^* = \beta' - j\beta''$: complex propagation constant in the waveguide,

k_0=the wave number in vacuum ($=2\pi/\lambda_0$),

n_3^*=complex refractive index of the top medium,

α=bulk absorption coefficient of the top medium,

N_e^*=complex effective refractive index in the waveguide,

T=the waveguide thickness.

The substrate, the waveguide and the top layer are represented by subscripts 1, 2 and 3, respectively. And also, real and imaginary part of a complex are represented by subscripts r and i. Real and imaginary part in Eq. (1) correspond to the mode dispersion and the transmission loss in the waveguide, respectively. When losses are occurred (absorbing samples are present), and if $n_{3r} \gg n_{3i} (=\alpha/2k_0)$ and $\beta' \gg \beta''$, the interaction ratio $(R)^{[11\sim13]}$, which is the absorption rate of an evanescent field (N_i) relative to the bulk absorption rate of unguided beam propagating through sample (n_{3i}), can be approximately calculated from the imaginary part of Eq. (1) by

$$R=N_i/n_{3i}$$
$$=\{n_{3i}(n_2^2-N_r^2)(N_r^2-n_1^2)^{1/2}\}/[N_r(n_2^2-n_{3r}^2)\{N_r^2-n_{3r}^2)^{1/2}$$
$$+(N_r^2-n_1^2)^{1/2}(N_r^2-n_{3r}^2)^{1/2}\}]. \quad (2)$$

If N_r and T are known, R can be theoretically ascertained. The effective index N_r can be determined by measuring the coupling angle with a prism coupling method[14]. The value of N_r for the given mode is related to the incident angle θ_{in} and the vertical angle θ_p between the base and the incident face of the prism in Fig. 1.

Therefore,

$$N_r=\sin\theta_{in}\cdot\cos\theta_p+(n_p^2-\sin^2\theta_{in})^{1/2}\sin\theta_p \quad (3)$$

where n_p is the refractive index of the prism. In addition, the thickness and/or the refractive index of the waveguide can be computed from N_r through the Eq. (1).

Since $N_r=\beta''/k_0$ and $n_{3i}=\alpha/2k_0$ as given in Eq. (1), the dependency of β'' on the absorption coefficient of the absorbing species is obtained from Eq. (2) and expressed as follows;

$$\alpha=2\beta''/R. \quad (4)$$

This equation indicates that α is proportional to the imaginary part of the complex propagation constant ($=\beta''$) of the waveguide. β'' can be determined by the dependence of the guided wave on the waveguide length because the guided wave in the Z axis is proportional to $\exp(-2\beta''Z)$[12,15].

Thus, in this study, when the evanescent field of the waveguide is linearly absorbed by the absorbing samples at the channel n as shown in Fig. 2, the transmitted power (P_{CHn}) of the waveguide can be expressed by

$$P_{CHn}=P_0\exp(-2\beta''L_n), \quad (5)$$

where P_0, P_{CHn} are the transmitted power for the reference solution (Fig. 2(a)) and for the presence of the absorbing sample at channel n (interaction length L_n, Fig. 2(b)), respectively.

Therefore, sensor response (A) at the channel 1 and 2 are given by

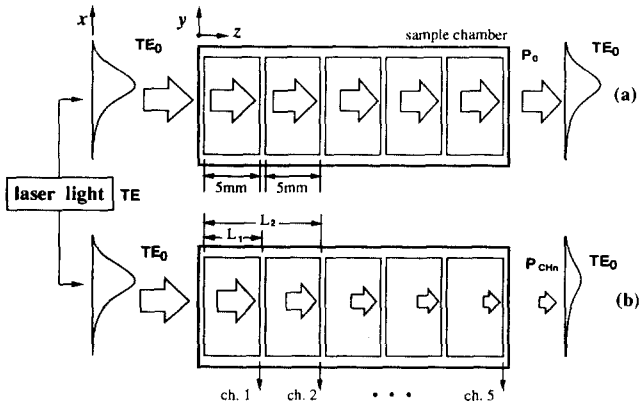

Fig. 2. A configuration of sensing part; **(a)** for reference solution **(b)** for presence of the absorbing samples.

$$A_{CH1}=-\log(P_{CH1}/P_0)=2\beta''L_1, \quad (6a)$$
$$A_{CH2}=-\log(P_{CH2}/P_0)=2\beta''L_2, \quad (6b)$$

Therefore, β'' an be determined by

$$\beta''=(A_{CH2}-A_{CH1})/2(L_1-L_2). \quad (7)$$

If more than two channels are allowed, the method becomes self checking and greater accuracy can be obtained by a least-square fitting method.

From Eq. (4) and (6), sensor response, namely absorbance (A), can be reexpressed as stated in the Beer-Lambert's law relationship as follows;

$$A=2\beta''L=R\alpha L=R\alpha_s CL, \quad (8)$$

where α_s is the specific absorption coefficient, which is the absorbance divided by the product of interaction length (L) and the concentration of substance (C). Hence, the absorption coefficient of samples can be obtained by experimentally given β''.

EXPERIMENTS

1. Waveguide Preparation and parameters Evaluation

Pyrex glass substrates (26mm (w) × 76mm (l) × 1mm (d),

Table 1. Determined waveguide parameters, and absorption coefficients at 488nm of wavelength for various concentration of oxy and deoxyhemoglobin together with the value obtained by the Beer-Lambert's law.

Parameters:

Waveguide thickness : T=1.167μm , *Effective Refractive index at* TE_0 : N_r=1.581, *Interaction Ratio* [a] : R=2.17 x 10^{-3}

[a]Calculated for n_1=1.487, n_2=1.590 and n_{3r}=1.34.

Conc.	Oxyhemoglobin					Deoxyhemoglobin				
	2β" slope	Determinant coefficient	Absorption coefficient α	α_x[mm]$^{-1}$[g/dl]$^{-1}$		2β" slope	Determinant coefficient	Absorption coefficient α	α_x[mm]$^{-1}$[g/dl]$^{-1}$	
[g/dl]	[mm]$^{-1}$ x10^{-3}	rr	[mm]$^{-1}$	sensor	[b]Beer's law	[mm]$^{-1}$ x10^{-3}	rr	[mm]$^{-1}$	sensor	[b] Beer's law
1.45	1.348	0.9880	0.621	0.428		0.890	0.9890	0.410	0.283	
2.90	2.607	0.9962	1.201	0.414		1.778	0.9938	0.819	0.283	
4.35	3.918	0.9976	1.806	0.415	0.427	2.656	0.9978	1.224	0.281	0.279
5.80	5.407	0.9991	2.491	0.429		3.516	0.9966	1.620	0.279	
7.25	6.551	0.9945	3.019	0.416		4.337	0.9968	1.999	0.277	

[b]obtained with UV-visible spectrophotometer

refractive index n_1=1.487 at λ=488nm) were immersed into 99.5% toluene solution and boiled for 15 minutes at 110℃. Substrates were also immersed into 99% acetone solution and 99% ethyl alcohol and cleaned with ultrasonic cleaner for 15 minutes, respectively, followed by drying for 30 minutes at 60℃. Thin films were deposited by RF sputtering of Corning-7059 glass (n_2=1.590 at λ=488nm) on pyrex glass substrates.

The incident angles for given modes were measured by a prism coupler (45-45-90 prism : Dense Flint : n_p=1.806 at λ =488nm), and N_r were determined by Eq. (4). In addition, the thickness of waveguide T was determined by Eq. (1) and interaction ratio R at the given modes were obtained by Eq. (3). Evaluated physical parameters for waveguide sensor design are listed in Table 1.

2. Sample Chamber Preparation

A sample chamber, which can dynamically vary the interaction length between the evanescent field and samples, was fabricated from vinyl chloride (refractive index$<n_2$), adhered to the surface of the waveguide, and baked for 30 minutes at 60℃. Fig. 2 illustrates the shape of a sample chamber which consisted of five serial droplet cells of 5mm (l)×8mm (w)× 0.2mm (d) volume.

3. Samples Preparation

Whole blood was drawn by venipuncture from healthy adults. Heparin was used as anticoagulant. Whole blood was centrifuged at 4℃ at 1000×g for 20minutes until the erythrocytes were packed. The supernatant was discarded, erythrocytes were washed twice in an equal volume of cold 0.154M NaCl solution and obtained by recentrifuging. The packed and washed cells were hemolyzed by adding minutes a volume of 10% Triton-X solution. The mixture was stirred for 30 min at 4℃, then red cell ghost were precipitated by ultracentrifugation (Hitachi ; SCP55H2) for 1 hour at 72000×g. Obtained hemoglobin solutions were microscopically examined to verify complete hemolysis. The hemoglobin solutions were classified with phosphate buffered saline (PBS, pH7.2) solutions. In order to obtain the fully oxidized HbO_2, classified samples were stirred for 30 minutes at room temperature. HHb was obtained by adding 2mg/cc of sodium dithionite ($Na_2S_2O_4$) a few minutes before the measurement because the HHb is the only form capable of reversibly binding to molecular oxygen. The total hemoglobin (THb), %HbO_2 and %HHb of samples were measured by CO-oximeter (Corning 2500) to verify the complete oxy- and deoxygenation of hemoglobins. The typical ranges of values for each hemoglobin were HbO_2>95% and HHb>95%, respectively.

4. Absorbance Spectra Determination

The absorption spectra of HHb and HbO_2 were measured by a spectrophotometer (Hitachi ; U-2000) using the conven-

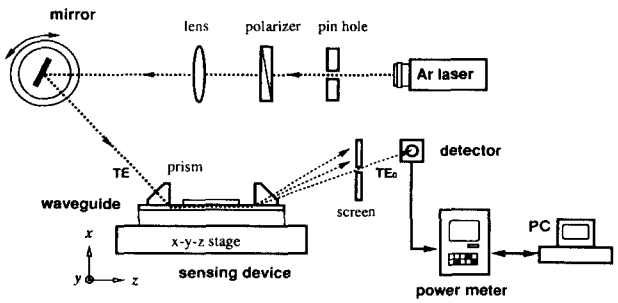

Fig. 3. The schematic diagram of the experimental setup.

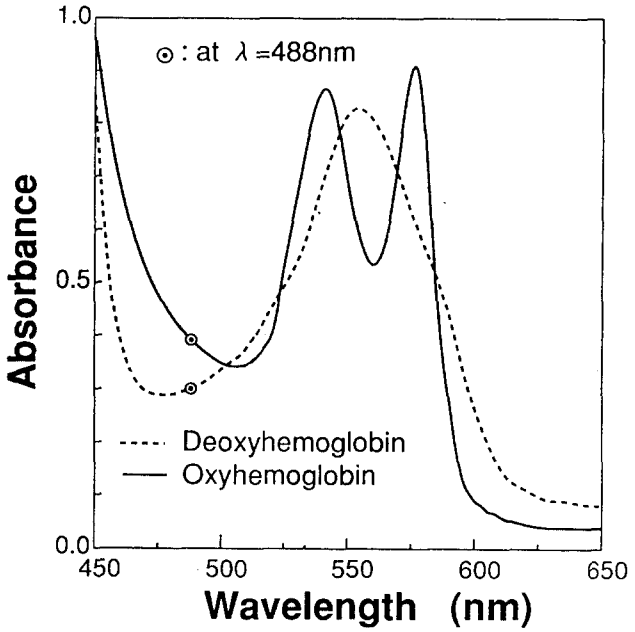

Fig. 4. Absorbance spectra of two hemoglobin derivatives, which is measured with a UV-vis spectrophotometer (Concentration for two hemoglobin spectra were 8×10^{-2}g/dl)

tional light path-length cuvettes (see Fig.4). To get absorbance spectrum of HHb, 6mg of sodium dithionite was added to HbO_2. From the absorbance curves as shown in Fig. 4 and using the Beer-Lambert's law, the specific absorption coefficient (α_s) at 488nm wavelength of light, which is absorbance divided by the product of the sample path length and the concentration of the absorbing substance, was determined.

5. Measurement Procedure

The schematic diagram of the experimental setup is shown in Fig. 3. Fabricated waveguide with a sample chamber is placed on the x-y-z stage. Incoupling and outcoupling prisms were fixed to waveguide surface with rubber-lined brass chucks. A TE polarized 488nm (Ar laser, NEC, GLG3050A) line was coupled into and out of the waveguide by using a pair of 45-135-90-90 prisms (Dense Flint: n_p=1.806 at λ =488nm). The lowest optical mode (m=0) was selected by adjusting the coupling angle until all the additional modes could be eliminated in the pin holed screen.

Decoupling output was detected by an optical power-me, ter (Advantest®, TQ8210). PBS solution was used as reference solution to evaluate the absorption of absorbing samples and to normalize any input-coupling variations and surface conditions.

4μl of PBS solution was deposited in each channel using pipettes (Labsystems, Finnpipette Digital, vol. 0.5~10μl) and transmitted power of reference solution P_0 was measured (see Fig. 2). After each 4μl of HbO_2 was supplementarily deposited in the multicells and each transmitted power of sample P_{CHn} was measured in series (see Fig. 2). To get sensor response for HHb, 2mg of sodium dithionite was added to HbO_2 and repeated the same procedure as that of HbO_2. Thereafter, sensor response, namely absorbance $A_{CHn}=-\log(P_{CHn}/P_0)$ was calculated and displayed by the PC.

RESULTS AND DISCUSSION

Visible absorbance spectra to 0.08g/dl of both HbO_2 and HHb, which is measured with a conventional transmission measurement (a UV-vis spectrophotometer) using a 1cm path length cuvette, were shown in Fig. 4. It showed that the absorption spectra of HbO_2 and HHb are very different, and absorbance of HbO_2 at 488nm of wavelength was higher than that of HHb.

The sensor response versus various concentration of samples for TE_0 polarized 488nm line of Ar laser at the ch. 5 (L=25mm) were shown in Fig. 5. The sensor response for HHb and HbO_2 was increased according to increment in their concentration. Two hemoglobins in the chamber interact with the evanescent portion of the guided wave attenuating those in proportion to concentration. It means that energy of the evanescent field is absorbed by samples when the light wavelength corresponds to the absorption band of two

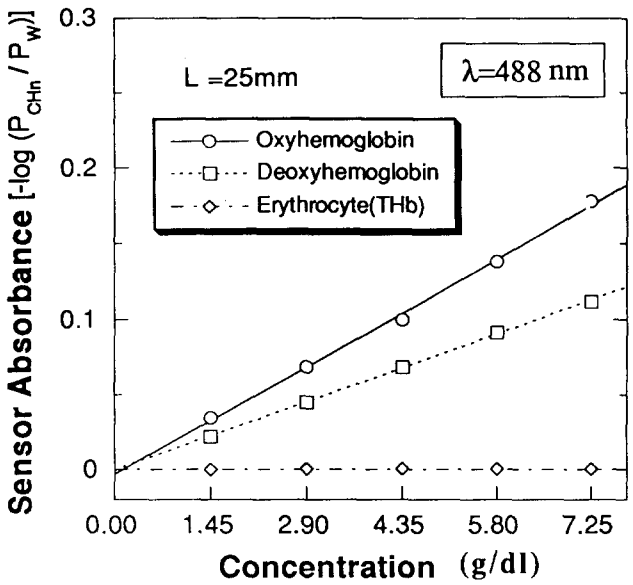

Fig. 5. The sensor response $[-\log(P_{CHn}/P_0)]$ versus concentration of samples.

samples. The magnitudes of absorbances for two hemoglobin derivatives corresponded to the results as shown in Fig. 4. However, the sensor response of erythrocyte maintained nearly constant state, while the THb concentration increased. It could be expected from the fact that the penetration depth of evanescent wave ($d_p < 0.1\mu m$) was too small to be approached to the hemoglobins in the erythrocyte ($7 \sim 8\mu m$ across, $1 \sim 2\mu m$ thick).

The sensor response to various concentration versus the interaction length was plotted in Fig. 6. It indicates that sensor absorbance is proportional to concentration of samples and their deposited length interacting with evanescent field of the waveguide. From the slopes in Fig. 6 and using Eq. (8), α and α_s were determined, and are listed in Table 1 together with the values given by Beer-Lambert's law response obtained with a UV-vis spectrophotometer. The slopes were obtained by the least square regressions. In the table, we can observe that although α and β' are linearly proportional to sample concentration, α_s is not changed at the given wavelength of light. Replicate measurement, if α_s are known, the concentration of species can be determined. And also the magnitude of α_s for HbO_2 at is larger than that of HHb. This result corresponds to the result from the absorbance spectra

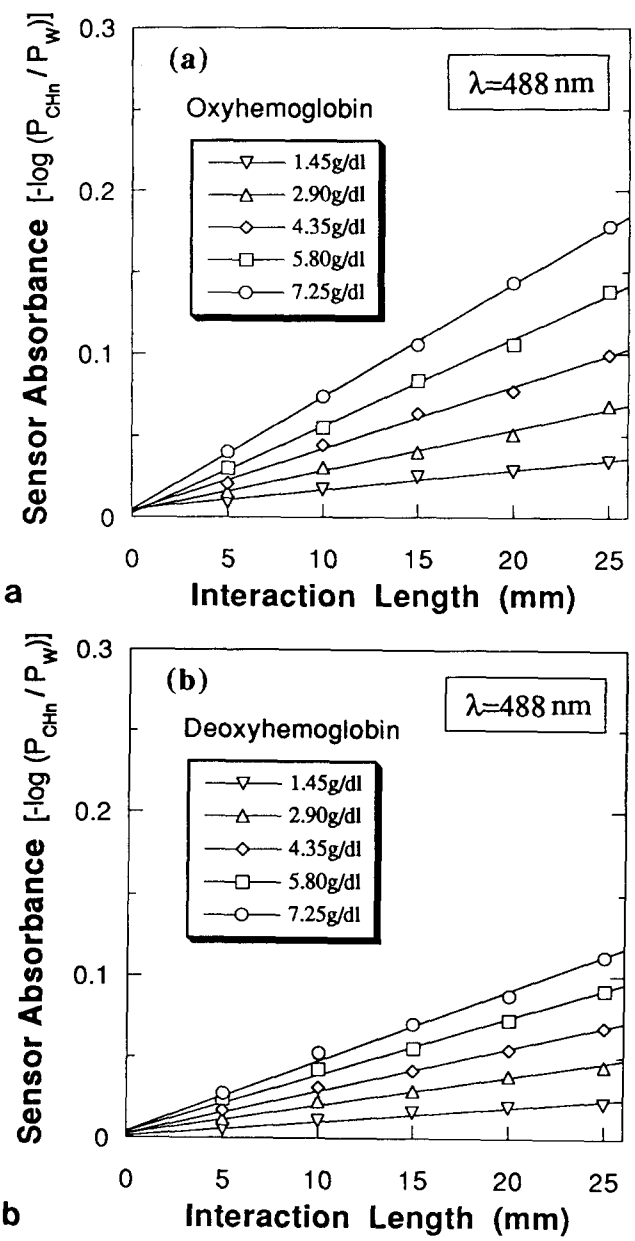

Fig. 6. The dependence of sensor response to various concentrations of oxy- and deoxyhemoglobin on the interaction length at TE_0, 488nm wavelength of Ar lase ; **(a)** for oxyhemoglobin **(b)** for deoxyhemoglobin.

α_s depicted in Fig. 4., and also the as for two hemoglobin derivatives are fairly well agreed with those obtained with a UV-vis spectrophotometer.

It was also previously[12, 13] shown that the sensitivity and the dynamic range of the proposed sensor were improved compared to those of the conventional transmission meas-

urement which is based on the Beer-Lambert's law.

In this proposed method, quick absorbance change due to evanescent field absorption make it possible to instantaneously detect the concentration variation of the sample. The effect of the oxidation of the deoxyhemoglobin during the measurement can, therefore, be neglected and accurate measurement is possible, However, the reproducibility and the long term satability of the sensor appear to be limited by the surface contamination and the memory effect, which are the only major drawbacks of the sensor. In this work, surface contamination problem was removed by depositing the reference solution prior to the sample solutions and memory effect was also overcomed by the pretreatment with a low concentrated sample solution.

A further research on the improvement of the sensor geometry must be carried out to more perfectly solve the problems mentioned above. Choosing the grating coupler instead of the prism coupler and/or using the flow cell instead of the droplet cell are candidates for the furture work.

CONCLUSIONS

A new method for determining the absorption coefficient of HbO_2 and HHb has been described using a multichannel thin-film waveguide sensor. In order to demonstrate the efficiency of this method, I designed the multichannel waveeguide sensor and compared the absorption coefficients obtained by the proposed method through one with those obtained by the conventional spectrophotometric method. As a result, it is shown that the former well coincides with the latter. It could also be observed that the sensing device used in the evanescent field sensing principle of integrated optic waveguide has strong immunity to the scatterer (erythrocyte), and the advantage of small volume of samples as comparing to the conventional spectrophotometer measurement. Besides, the dependence of the guided wave attenuation in the waveguide on the interaction length was investigated for the various concentration of two fully oxy- and deoxygenated hemoglobins.

The proposed method proved to be easy, fast and accurate in determining of HbO_2 and HHb concentration and the absorption coefficients. Therefore, it is expected that the proposed method is able to be applied to the determination of the absorption coefficient of the various turbid, aqueous biochemicals.

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=국문초록=

박막광도파로 센서의 복소전파상수를 이용하여 인간혈액내의 산화 및 환원 혈색소의 흡광계수를 쉽고 빠르게 측정할 수 있는 방법을 제안한다.

제작된 광도파로위에 에바네센트 필드와 시료간의 상호작용길이를 변화시킬 수 있도록 직렬의 다채널 시료 충전셀을 만들고 여러 가지 농도의 두가지 혈색소 시료들에 대한 센서응답을 조사하여 상호작용길이에 대한 의존성을 살펴보았다. 센서응답은 상호작용길이와 시료의 농도에 선형적으로 비례한다. 시료들과 에바네센트 필드 흡수에 따른 제작된 센서의 감쇄정수를 실험적으로 구하고 제안된 방법으로 흡광계수를 결정한다. 제안된 방법으로 구한 두가지 혈색소에 대한 흡광계수는 종래의 투과광 측정법으로 구한 값과 잘 일치한다.