Noninvasive Optical System for Real-Time Concentration Measurement in Optical Active Solution

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Noninvasive Optical System for Real-Time Concentration Measurement in Optical Active Solution

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ABSTRACT

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Over the last two decades, optical polarimetry method has been applied for concentration monitoring as a non-invasive alternative method. Commercial polarimeter is widely used to measure the concentration of chemical compounds with optical activity. However, the current device is based on off-line measurement method which involves sample extraction process to measure the concentration. The process does not reflect the real-time status of accurate sample concentration. Moreover, sample contamination may occur during the sample extraction process. In polarimetry method, the sensitivity of measurement can be controlled by varying optical path length. However, in current polarimeter, the sample cell should be exchanged to vary the optical path length. The process requires a considerable amount of time and may cause sample contamination problem. Therefore, it is necessary to develop a new polarimetry system which can measure exact concentrations without performing sample extraction in real-time. In this study, we introduced a new polarimetry probe system which may be utilized effectively to monitor concentration of solution. The new system was designed to have variable optical path lengths to control the optical rotation angle of polarized light. The

system and feasibility of concentration meter have been described in the following articles.

Key words: Polarimetry, Optical activity, On-line, Concentration meter, Probe

Chapter 1
Introduction

1.1 Background of research

Optical methods such as infrared and near-infrared spectroscopy, Raman spectroscopy, polarimetry, and fluorescence spectroscopy were continuously applied for sensing the concentration of solution for several years.[1-3] The advantages of optical methods are non-ionizing radiation to interrogate the sample, non-interference with the sample and the needlessness of consumable reagents.[1,3] Through the 1990s, optical polarimetry method was dramatically applied to glucose concentration monitoring in biological media such as aqueous humor and cell culture media as a non-invasive alternative method. The advantages of polarimetric sensing method are the use of readily available sources, the concomitant ability to use substantial path lengths in aqueous solutions, and the prospect of miniaturizing the optical components.[1-3,5-14]

Polarimeter is the optical device used for the concentration measurement of the optical active substance using the rotation angle of polarized light. The device was firstly conducted in sugar industry and has been commonly performed in the field of chemistry, biology and medical diagnostics.[1-3,5-16] The foundation of optical polarimetry for the detection of analyte concentrations is initially based on the first observation by Biot in the early 19th century. The observation discovered that when a linearly polarized light passes through solutions with chiral molecules such as glucose, the polarization plane was rotated in some degree. Characteristics of

molecules that exhibit this ability are called "optically active." The applicable equation for the interaction is given by:

$$[\alpha]_{\lambda}^{T} = \frac{100\alpha}{LC} \tag{1}$$

where $[\alpha]$ is the specific rotation of the optically active molecule at a given wavelength, λ, which is equal to the observed rotation, α, divided by the sample path length, L, and concentration, C. Therefore, the concentration of an optically active sample can be determined as the rotation of linear polarization is measured after passing through an optically active sample, with the given optical path length.[2,3,7]

In polarimetry method, the measurement sensitivity can be controlled by varying optical path length as shown in equation (1). The optical path length is proportional to the observed rotation angle. However, when applied to the current polarimeter, the sample cell should be exchanged to vary the optical path length. This process is time consuming and possibly cause sample contamination problem.

Typically, most commercial techniques for monitoring concentration of substance were based on off-line methods such as chemical sensor, chromatography. Off-line methods might have potential problems. It requires sample extraction process and additional reagents for compensation. Sample extraction from the solution can cause a contamination problem and takes time for analysis. Such process can not reflect the real-time status of sample due to discrete reading of concentration. An alternative method for on-line measurement is to place an electrochemical sensor within the sample. However this method may also initiate

problems such as difficulty of sterilization techniques, limited lifetime and lack of long-time stability.[4,5]

In this thesis, we introduce a new polarimetry probe system which can be utilized for measuring the concentration of optical active solution. It has a variable optical path length in order to manage the sensitivity of the optical rotation angle. Also, it can be immersed in sample so that it makes possible to on-line monitoring. Therefore, it can remove potential problems of off-line method. We describe the system and show the feasibility of the polarimetry probe system by measuring the glucose concentration in water-based glucose solution.

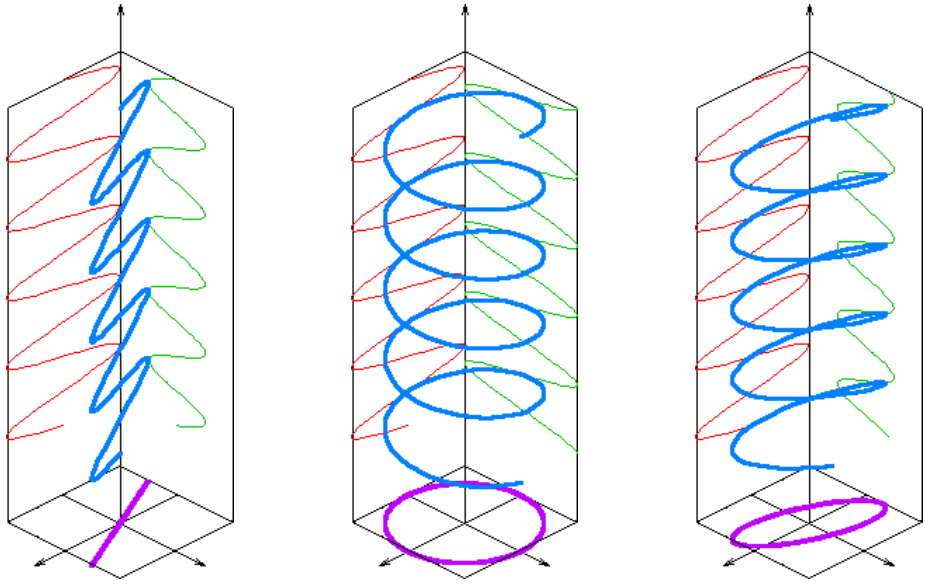
Chapter 2

Theory

2.1 Polarized light

The simplest manifestation of polarization to visualize is that of a plane wave, which is a good approximation of most light waves (a plane wave is a wave with infinitely long and wide wavefronts). All electromagnetic waves propagating in free space or in a uniform material of infinite extent have electric and magnetic fields perpendicular to the direction of propagation. Conventionally, when considering polarization, the electric field vector is described and the magnetic field is ignored since it is perpendicular to the electric field and proportional to it. The electric field vector may be arbitrarily divided into two perpendicular components labeled x and y (with z indicating the direction of travel). For a simple harmonic wave, where the amplitude of the electric vector varies in a sinusoidal manner, the two components have exactly the same frequency. However, these components have two other defining characteristics that can be dissimilar. First, the two components may not have the same amplitude. Second, the two components may not have the same phase, that is they may not reach their maxima and minima at the same time. The shape traced out in a fixed plane by the electric vector as a plane wave displays the current polarization state. The Figure 1 shows some examples of the evolution of the electric field vector (blue) with time (the vertical axes), along with its x and y

components (red/left and green/right), and the path traced by the tip of the vector in the plane (purple):



Linear Circular Elliptical

Figure 1 Three types of polarized light

In the leftmost Figure 1, the two orthogonal (perpendicular) components are in phase. In this case, the ratio of the strengths with the two components is constant. In addition, the direction of the electric vector (the vector sum of these two components) is specifically constant. Since the tip of the vector traces out a single line in the plane, this exceptional case is called "linear polarization". The direction of this line depends on the relative amplitudes of the two components. In the middle Figure 1, the two orthogonal components have exactly the same amplitude and are exactly ninety degrees out of phase. In this case, one component is zero

when the other component is at maximum or minimum amplitude. There are two possible phase relationships that satisfy this requirement: the x component can be ninety degrees ahead of the y component or it can be ninety degrees behind the y component. In this specific case, the electric vector traces out a circle in the plane. Thus, this specific case is called "circular polarization". The direction the field rotates in is determined by which of the two phase relationships exists. These cases are called right-hand circular polarization and left-hand circular polarization, depending on which way the electric vector rotates. All other cases, that is where the two components are not in phase and either do not have the same amplitude and/or are not ninety degrees out of phase are called "elliptical polarization" because the electric vector traces out an ellipse in the plane (the polarization ellipse).[18]

2.2 Optical activity and Specific rotation

Optical activity is the ability of a chiral molecule to rotate the plane of plane-polarized light. When polarized light passes through a substance containing chiral molecules (or nonchiral molecules arranged asymmetrically), the direction of polarization can be shifted (rotated). Figure 2 shows the rotation due to optical activity of chiral molecule.

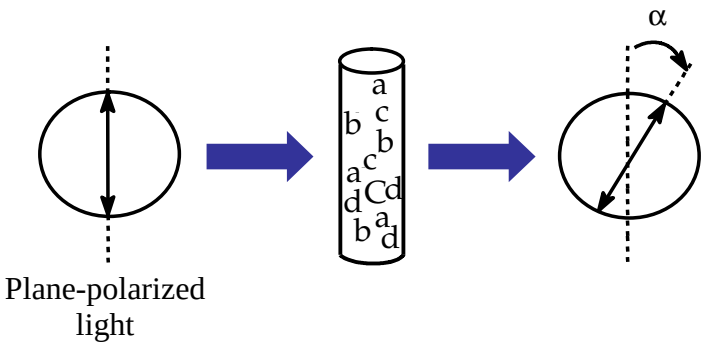


Figure 2 The characteristic of Chiral molecule: Optical activity

The plane of polarization could rotate clockwise or counter-clockwise due to the molecular conformation of an optically active compound. Molecules possessing the ability to rotate light to the left or counter-clockwise are denoted as levorotatory. Relatively, those rotate light angle to the right or clockwise are referred to as dextrorotatory as shown in Figure 3.[17,19]

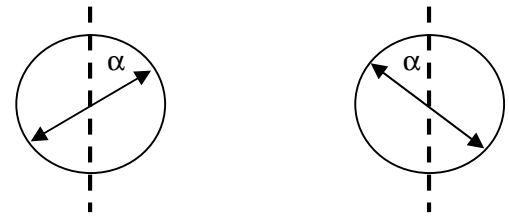


Figure 3 An example of optically active enantiomers

The specific rotation of a chemical compound is defined as the observed angle of optical rotation α when plane-polarized light is passed through a sample with a path length of 1 decimeter and a sample concentration of 1 gram per 1 deciliter. The specific rotation of a pure material is an intrinsic property of that material at a given wavelength and temperature. Values should always be accompanied by the temperature at which the measurement was performed and the solvent in which the material was dissolved. Often the temperature is not specified; in such cases it is assumed to be room temperature. Although the formal unit for specific rotation values is deg cm^2 g-1, scientific literatures practically apply the unit of degrees as the measurement. A negative value means levorotatory rotation and a positive value means dextrorotatory rotation.[20] The amount of rotation is proportional to the number of molecules encountered by a polarized light beam as it passes through a sample of an optically active compound.

In equation (1), it is assumed that the wavelength of monochromatic polarized light is constant. If the wavelength varies, the rotation also changes in

inverse proportion to the alterations. The variation in rotation with the wavelength of the light is called optical rotatory dispersion (ORD). The ORD values for glucose through the visible spectrum are shown in Table 1.[16] The plot of specific rotation as a function of wavelength is called ORD curve, shown in Figure 4.[1] Therefore, in equation (1), the use of single wavelength is sufficient to measure the concentration of sample and the change of wavelength can control the measurement sensitivity.

Wavelength(nm)	656	589	535	508	479	447
Specific Rotation ˚/(dm g/L)	41.9	52.8	65.4	73.6	83.9	96.6

Table 1 Specific rotation of glucose at various wavelengths of light

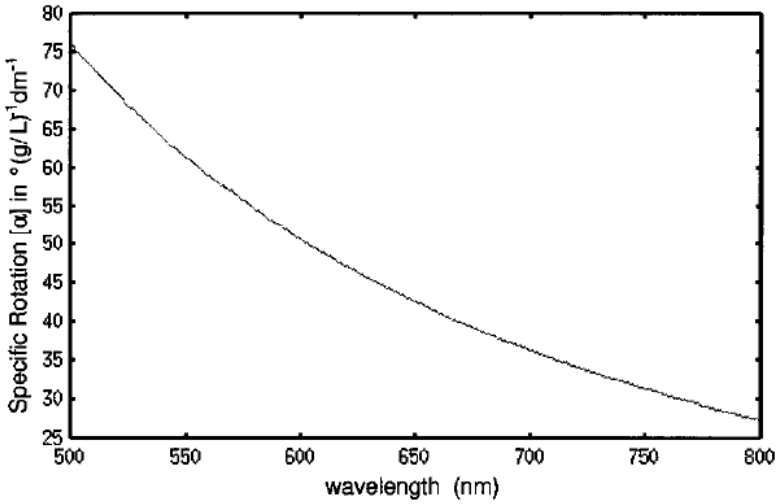


Figure 4 Glucose ORD curve in the visible spectrum

2.3 Polarimeter

Optical rotation is measured with an instrument called a polarimeter. There is a linear relationship between the observed rotation and the concentration of optically active compound in the sample. Polarimeter is based on the cross-polarization as shown in Figure 5. If the light is oscillated vertically, the light will be rotated by passing through the optical active sample. Subsequently, the light can be passed selectively according to the rotation angle which is proportion to the concentration of optical active sample.

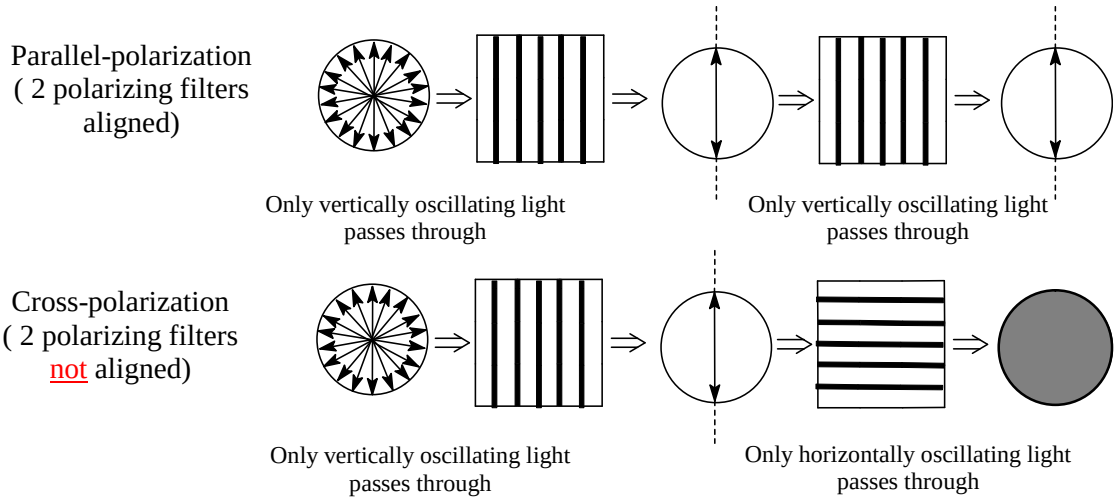


Figure 5 The parallel polarization and cross-polarization

Each optically active substance has its own specific rotation. Therefore, the amount of optical rotation is determined by the equation (1) as defined by Biot′s law.

$$[\alpha]_{\lambda}^{T} = \frac{100\alpha}{LC} \tag{1}$$

Where $[\alpha]$ is the specific rotation of the optically active molecule at a given wavelength, λ, which is equal to the observed rotation, α, divided by the sample path length, L, and concentration, C.[2,3,7]

Polarimeter is basically composed of 5 components: laser, linear polarizer, sample, analyzer (a second linear polarizer), and detector. The basic polarimeter is shown in Figure 6. A laser is used as light source. Two linear polarizers are placed in perpendicular direction. Due to cross-polarization, the analyzer (a second polarizer) determines the concentration by rotation angle of polarization axis. If no optical active substance is in sample, theoretically there would be no light from analyzer. If an optical active substance exists in sample, the light which is proportional to the rotation angle according to the concentration would be transmitted from the analyzer. Thus, the concentration of optical active substance can be calculated by polarimeter.

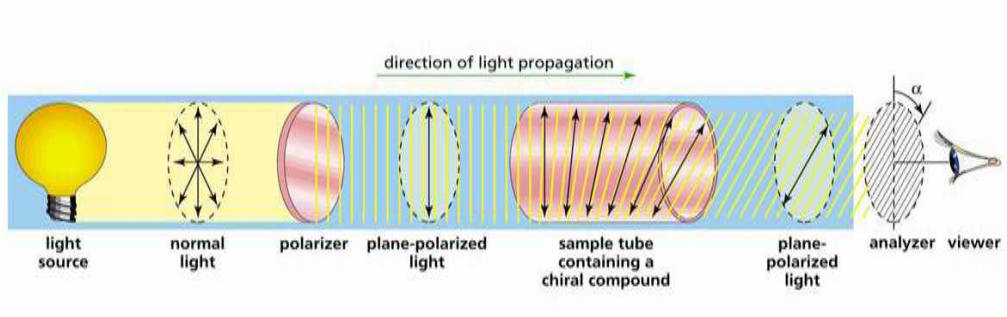


Figure 6 The block diagram of basic polarimeter

2.4 Modeling the polarimeter

Jones vector is used to represent polarization of light for optical components. Polarimeter can be expressed by the use of Jones matrices. Jones matrix for each optical component is indicated as shown in Table 2.

Optical component	Jones Matrix
Horizontal Linear Polarization	$\begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix}$
Vertical Linear Polarization	$\begin{pmatrix} 0 & 0 \\ 0 & 1 \end{pmatrix}$
Optically Active Sample (Clockwise rotation)	$\begin{pmatrix} cos(\phi_g) & -sin(\phi_g) \\ sin(\phi_g) & cos(\phi_g) \end{pmatrix}$
Compensation Faraday (counter-clockwise rotation)	$\begin{pmatrix} cos(\phi_f) & sin(\phi_f) \\ -sin(\phi_f) & cos(\phi_f) \end{pmatrix}$
Polarization Modulator	$\begin{pmatrix} cos(\theta_m sin(\omega_m t)) & sin((\theta_m sin(\omega_m t)) \\ -sin((\theta_m sin(\omega_m t)) & cos((\theta_m sin(\omega_m t)) \end{pmatrix}$

Table 2 Jones matrices for optical polarization components

(where θ_m is the depth of the Faraday modulation, ω_m is the modulation frequency, t is the time, ϕ_f and ϕ_g represent the rotation angle induced by the optical activity of compensation faraday rotator and glucose, respectively.)

In 2002, Qiujie et al proposed the modeling of polarimeter for monitoring glucose in the presence of varying the birefringence.[8] They used frequency modulation method to detect the phase difference. Faraday rotator was used for modulating frequency of initial condition in the system. Optical components of the system are a laser, polarizer, Faraday modulator, optically active sample, Faraday compensation, analyzer and detector.

Electric field vector can be written in the following form:

$$E = \begin{pmatrix} E_x(t) \\ E_y(t) \end{pmatrix}$$

where $E_x(t)$ and $E_y(t)$ represent the instantaneous scalar components of E.

Therfore, in Qiujie's system, the output Jones matrix can be derived by:

$$E = \begin{pmatrix} E_x(t) \\ E_y(t) \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix} * \begin{pmatrix} cos(\phi_f) & sin(\phi_f) \\ -sin(\phi_f) & cos(\phi_f) \end{pmatrix} * \begin{pmatrix} cos(\phi_g) & -sin(\phi_g) \\ sin(\phi_g) & cos(\phi_g) \end{pmatrix}$$

$$* \begin{pmatrix} cos(\theta_m sin(\omega_m t)) & sin(\theta_m sin(\omega_m t)) \\ -sin(\theta_m sin(\omega_m t)) & cos(\theta_m sin(\omega_m t)) \end{pmatrix} * \begin{pmatrix} 0 & 0 \\ 0 & 1 \end{pmatrix} * \begin{pmatrix} 0 \\ 1 \end{pmatrix}$$

$$= \begin{pmatrix} -sin(\theta_m sin(\omega_m t) + \phi_g - \phi_f) \\ 0 \end{pmatrix}$$

The output intensity I is proportional to the square of the electric field:

$$I \propto E^2 = sin^2(\theta_m sin(\omega_m t) + \phi) \qquad \text{where } \phi = \phi_g - \phi_f$$

Since the $(\theta_m Sin(\omega_m t)+\phi)$ is very small and the assumption is used:

$$I \propto E^2 = sin^2(\theta_m sin(\omega_m t)+\phi) \cong (\theta_m sin(\omega_m t)+\phi)^2$$

$$I \propto [\theta_m sin(\omega_m t)+\phi]^2 = \theta^2 sin^2(\omega_m t) + 2\phi\theta_m sin(\omega_m t) + \phi^2$$

$$= \frac{\theta^2{}_m}{2} - \frac{\theta^2{}_m}{2} cos(2\omega_m t) + 2\phi\theta_m sin(\omega_m t) + \phi^2$$

$$= (\frac{\theta^2{}_m}{2} + \phi^2) + 2\phi\theta_m sin(\omega_m t) - \frac{\theta^2{}_m}{2} cos(2\omega_m t) \quad (2)$$

As shown in equation (2), the detected output intensity consists of a DC term, a frequency-doubled term and a modulated frequency term. The modulation frequency term is used as the input of the compensation Faraday rotator. Locking the frequency at the modulated frequency in the null system, one can calibrate the concentration according to the input voltages of compensation Faraday rotator. [8]

In our polarimetry probe system, the optical train is composed of laser, polarizer, optically active sample, analyzer (polarizer) and detector. In our system, Faraday rotator was not used for minimizing optical components. The output intensity is depends on the rotation angle originated from optical activity of sample. Therefore, in our system, the output Jones matrix can be derived by:

$$E = \begin{pmatrix} E_x(t) \\ E_y(t) \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix} * \begin{pmatrix} cos(\phi) & -sin(\phi) \\ sin(\phi) & cos(\phi) \end{pmatrix} * \begin{pmatrix} 0 & 0 \\ 0 & 1 \end{pmatrix} * \begin{pmatrix} 0 \\ 1 \end{pmatrix}$$

$$= \begin{pmatrix} cos(\phi) & -sin(\phi) \\ 0 & 0 \end{pmatrix} * \begin{pmatrix} 0 & 0 \\ 0 & 1 \end{pmatrix} * \begin{pmatrix} 0 \\ 1 \end{pmatrix}$$

$$= \begin{pmatrix} 0 & -sin(\phi) \\ 0 & 0 \end{pmatrix} * \begin{pmatrix} 0 \\ 1 \end{pmatrix}$$

$$= \begin{pmatrix} -sin(\phi) \\ 0 \end{pmatrix}$$

The output intensity I is proportional to the square of the electric field:

$$I \cong E^2 = \begin{pmatrix} E_x(t) \\ E_y(t) \end{pmatrix}^2 = sin^2(\phi) \; ; \; \phi^2 \qquad (\text{since the } \phi \text{ is very small}) \qquad (3)$$

As shown in the equation (3), the detected output intensity is composed of DC term. In our polarimetry probe system, the output intensity can be detected according to the rotation angle originated from optical activity of sample.

Chapter 3

Pre-experiment

3.1 Experimental Set up

In order to examine the effect of the optical path length, *in vitro* experiments were performed. Figure 7 shows the schematic diagram of optical system designed to simulate the polarimetry probe system. A 5mW laser at a wavelength of 532nm (Stockeryale, Salem, NH) was used as a light source. The laser beam was modulated at 100Hz by an optical chopper (Thorlabs, Newton, NJ), then focused on an optical fiber (Thorlabs, Newton, NJ). After collimation, the laser beam was linearly polarized by a Glan-Thompson linear polarizer (Thorlabs, Newton, NJ). The linearly polarized laser beam propagates through the sample cell and the second polarizer which is aligned at perpendicular direction to the initial polarizer. The body of sample cell is composed of opaque plastic and the end of both sides of sample cell was applied with slides glass as shown in Figure 8. The laser beam was finally collected by a photodiode (Thorlabs, Newton, NJ). A digital lock-in amplifier (Standford Research Systems, Sunnyvale, CA) was operated to measure the signal at modulation frequency and obtain better SNR. The output signal of lock-in amplifier was sent to the computer to collect the data.

Glucose solution was arranged by dissolving D-glucose powder in distilled water. Glucose concentration ranges from 0 through 600 mg/dl in 100 mg/dl increment. Three sample cells were prepared in the length of 10cm, 15cm, and 20cm.

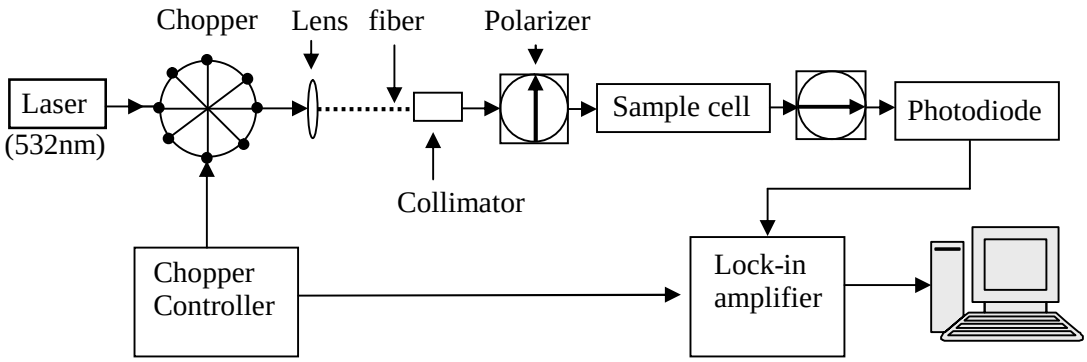


Figure 7 The schematic diagram of optical system to simulate the polarimetry probe system

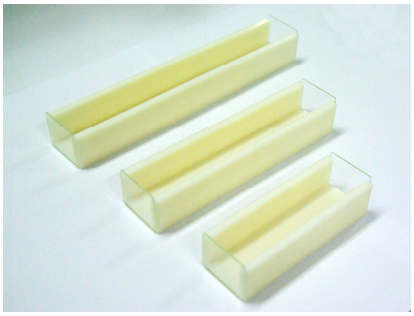


Figure 8 Three sample cells: 10cm, 15cm, 20cm

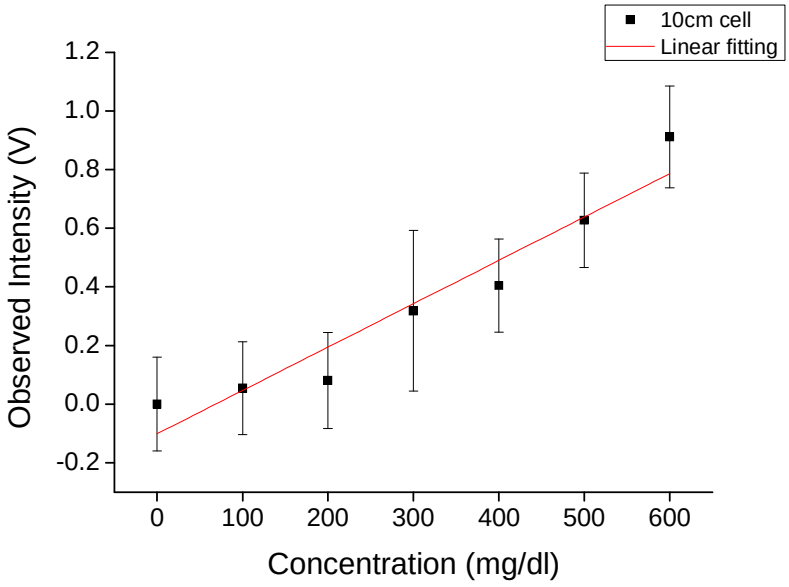
3.2 Results and Discussion

3.2.1 Validation of effect of variable path lengths

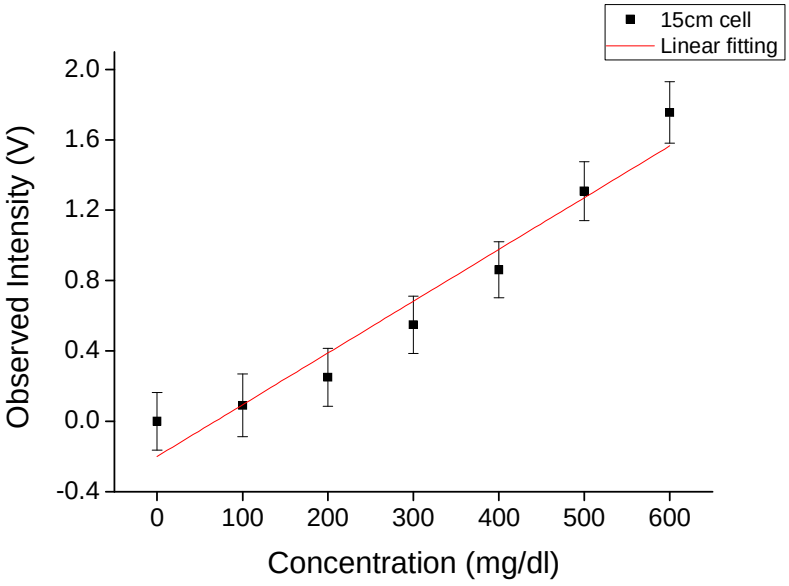
Three sets of *in vitro* experiments were performed to observe the effect of optical path length in polarimetry. The primary aim of the procedure is to show the effect of optical path length and the detected voltage alterations by glucose concentration. The sample cell was placed between two polarizers in the same position along the optical train. A concentration ranges from 0 mg/dl to 600 mg/dl in 100 mg/dl increment. Intensity of each concentration was measured during 20 seconds and the average value was recorded as shown in Figure 9. Figure 9 shows the observed intensity as a function of glucose concentrations for each sample cell: (a) 10cm, (b) 15cm, and (c) 20cm sample cell. Linear regression was performed for data analysis. Each case shows a linear trend of (a) R^2=0.91 (b) R^2 = 0.93 (c) R^2 = 0.93 (Origin 8.0 version). Figure 9 shows the observed intensity with the error bar which is standard deviation of measurement value during 20 seconds. The fluctuation of laser is within the purview of the standard deviation of measurement. In Figure 9, the result of 20cm sample cell is reportedly the most ideal to discriminate concentrations. For example, in 10cm sample cell the standard deviation of 100mg/dl can be an error in 200mg/dl measurement. It means that the range of laser fluctuation is involved in the difference of two concentrations intensities: 100 and 200mg/dl. In 20cm sample cell result, the laser fluctuation is not involved in the difference of intensities of each concentration in most cases. It makes possible to reduce the possibility of measurement error cause of laser fluctuation. In consideration of laser fluctuation, optical path length can be the key of discriminating

concentration. That is, in high concentrations, the short optical path length can be practicable to discriminate the concentrations. However, in low concentrations, the long optical path length is essential to discriminate the concentrations.

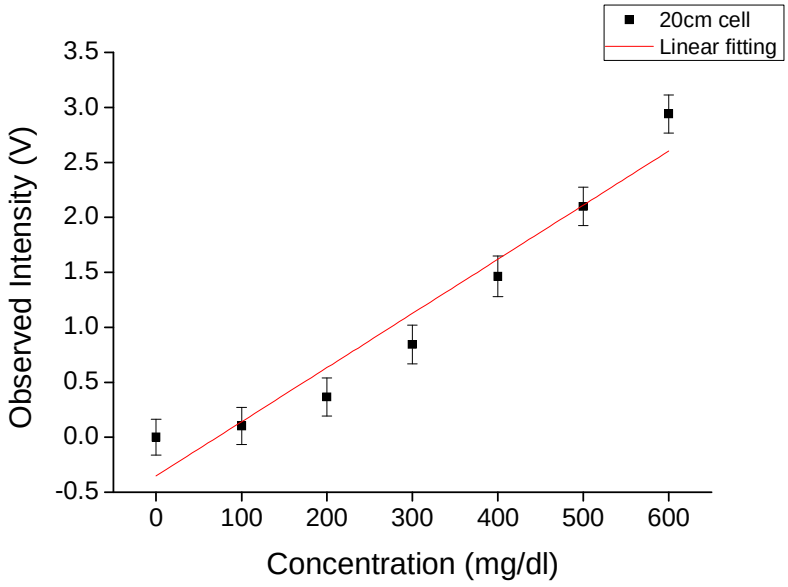
In addition, observed intensities of each case can be reflected in comparison. As theoretically expected, the observed intensity was increased as a function of optical path length. In fixed concentration, the intensity of 10cm sample cell was increased almost two times in 15cm sample cell and three times in 20cm sample cell. It means that the optical path length acts as a control factor in constant concentration of sample. In 10cm sample cell, low concentrations of 100mg/dl and 200mg/dl were experimentally difficult to discern. However, in terms of effectivity it was better to discriminate in 20cm sample cell. The result proves that the increment of optical path length increases the degree of rotation of polarized light. Thus, improvement of measurement sensitivity was reportedly observed. Based on the outcome, the polarimetry probe was designed to provide sufficient sensitivity and accuracy by varying the optical path length.



(a) 10cm sample cell



(b) 15cm sample cell



(c) 20cm sample cell

Figure 9 Observed intensity as a function of glucose concentration for each sample cell

Chapter 4

Design polarimetry probe system

4.1 First design of polarimetry probe

In polarimetry, the rotation degree of the polarized light is linearly proportional to the optical path length. The process indicates that longer optical path length results in greater measurement scale of detected signal. Based on the fundamental theory, an optical polarimetry probe was designed to control the optical path length and therefore, the measurement sensitivity.

The polarimetry probe was initially designed as shown in Figure 10. Figure 11 shows the prototype of polarimetry probe initially-designed. The diameter size of prototype probe is 2cm. Laser beam is guided to probe by optical fiber(Thorlabs, Newton, NJ) equipped with collimator. In the probe, laser beam is linearly polarized by a linear sheet polarizer (Thorlabs, Newton, NJ) and then, propagates to two right angle prism mirrors mounted at the bottom of the probe. After two right angle prism, laser beam passes through the open area to determine the concentration. In the following procedure, laser beam passes through the second linear sheet polarizer (Thorlabs, Newton, NJ) to determine the concentration by rotation angle. The laser beam passed through the second linear polarizer is collected at collimator with optical fiber which is forwarded to the detector. The detected signal at photo-detector is sent to the digital lock-in amplifier. The corresponding signal is amplified and is reported to the computer to be accurately recorded.

A considerable number of problems were discovered during experiment. First of all, the mid-level window of probe causes the light to be depolarized. Consequently, the depolarizing issue makes difficult to measure the signal difference according to each concentration. Thus, concentrations can not be discriminated. Secondly, in low concentration level, the maximum variable optical path length is approximately 9cm. The insufficient maximum length yields low signal output which makes difficult to detect the difference according to concentration. In Figure 9, the 10cm sample cell result shows the incapability of differentiating the low concentrations. Thirdly, the output collimator is problematic to properly mount on probe. The collimator demands to receive the light at the center of collimator lens. In the process of mounting right angle prism mirrors, the reflection angle can be tilted. Even a slight tilting angle allows complication to adjust the output position of the collimator.

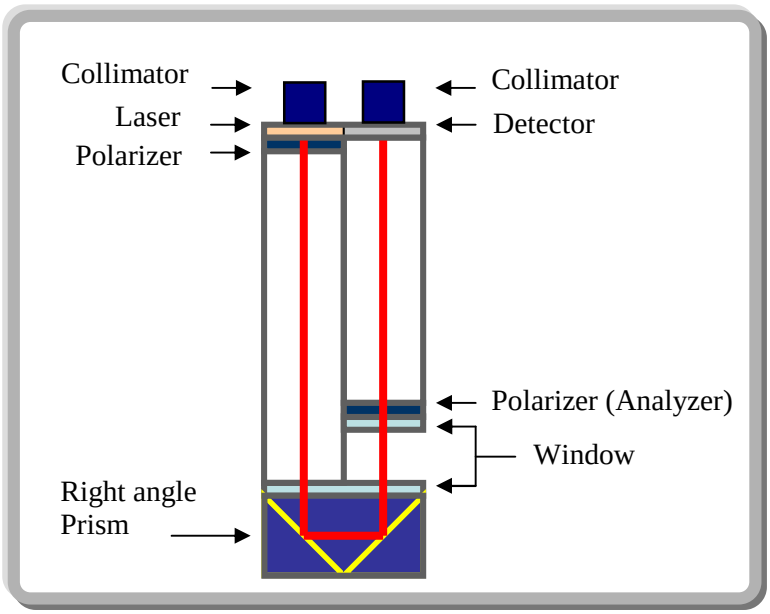


Figure 10 The first design of polarimetry probe

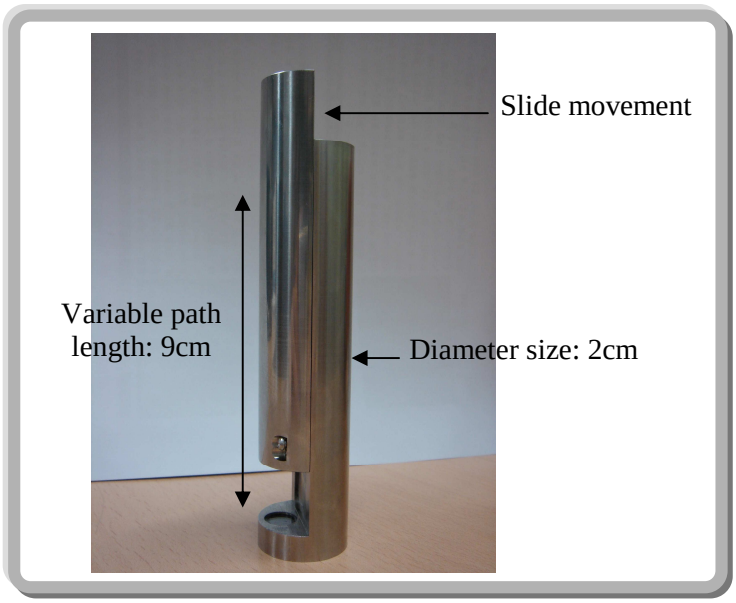


Figure 11 The prototype of polarimetry probe firstly designed

4.2 Second design of polarimetry probe

To solve the problems of initial design of polarimetry probe, the pre-experiment procedure is essential. As a result, we newly conducted the polarimetry probe to extend the variable optical path length. The new probe was designed to allow dual open sample area to minimize the probe length while doubling the optical path length. Figure 12 shows the new design of polarimetry probe. In terms of the structure, it has two tubes mounted in a guide rail. Laser beam is guided to the probe by optical fiber (Thorlabs, Newton, NJ) equipped with a collimator. In the probe, laser beam is linearly polarized by a Glan-Thompson linear polarizer (Thorlabs, Newton, NJ) and then, propagates to open sample area. With open sample area, laser beam passes through two right angle prism mirrors mounted at the bottom of the probe. After second open area, the laser beam passes through the second polarizer and is collected by photodiode. The digital lock-in amplifier presents the electrical signal detected by the photodiode and the electrical signal is forwarded to the computer in order to determine the concentration. Due to the limitation of laboratory technique, the probe has 4 x 4.5cm square in size.

However, the newly designed polarimetry probe also yielded a problem. Two right mirrors causes the polarized light depolarized. It also makes hard to differentiate the concentrations as the first polarimetry design.

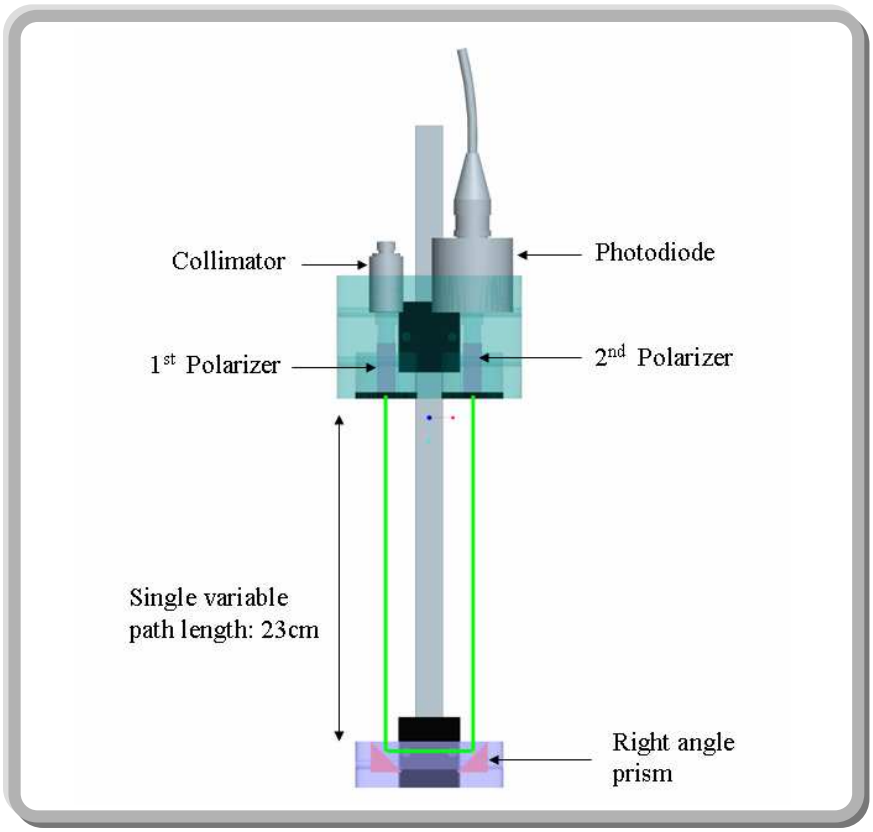


Figure 12 The second design of polarimetry probe

4.3 Final design of polarimetry probe

For the final conduct, we modify the previously designed polarimetry probe. Figure 13 displays the design of the modified polarimetry probe. In comparison with first-designed polarimetry probe, it relatively has a longer variable optical path length and employs the hosing-photodiode. It removes the difficulty of mounting optics and makes easy to receive the output light. Compared with the collimator of first-design, housing-photodiode increases the margin of detect area. Therefore, it allows the suitable mounting and provides the stable measurement. By using micro optics such as pin-photodiode, the polarimetry probe can be further downsized compactly. The compact size has a potential to be integrated into other instrument Compared with second-designed polarimetry probe, it relatively has single optical path. Sizewise, the probe can be closely decreased to 2 x 2 cm square. Moreover, the final design does not employ any window and mirrors which prohibits the possibility of depolarization. Figure 14 shows the final prototype of optical polarimetry probe.

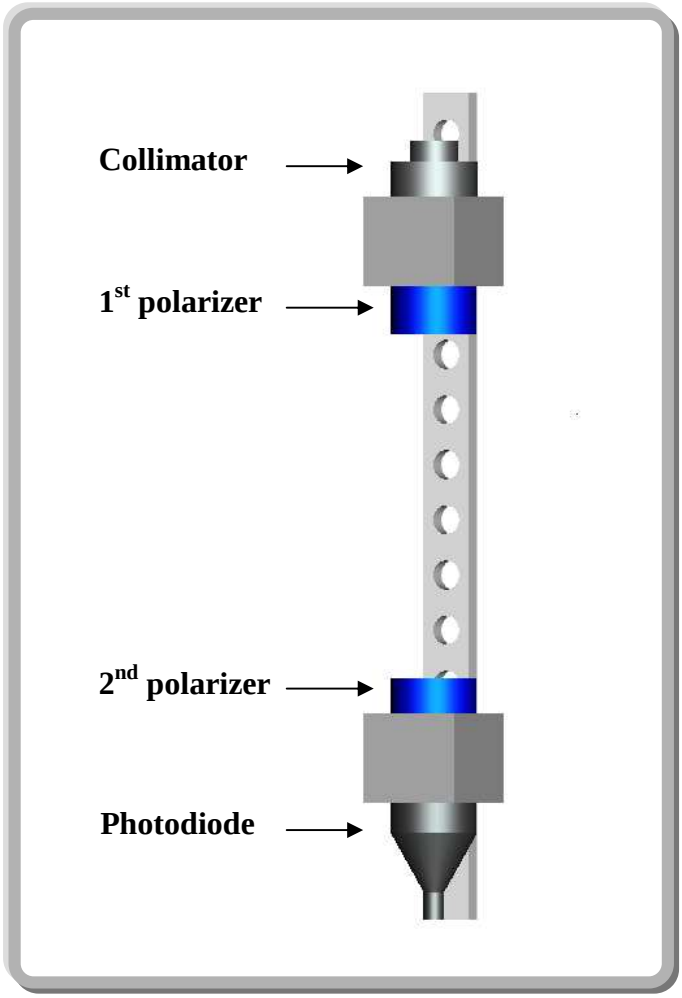
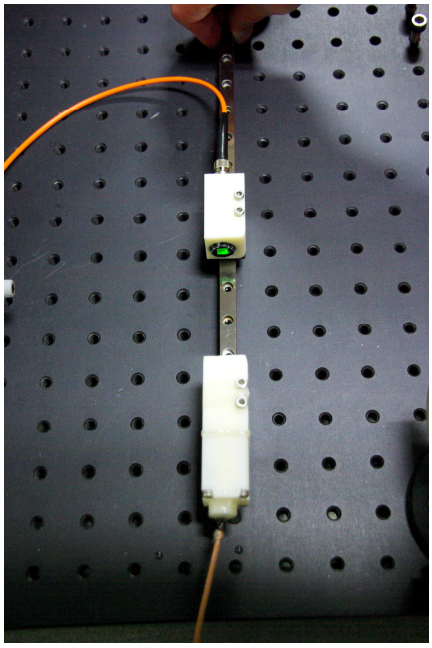
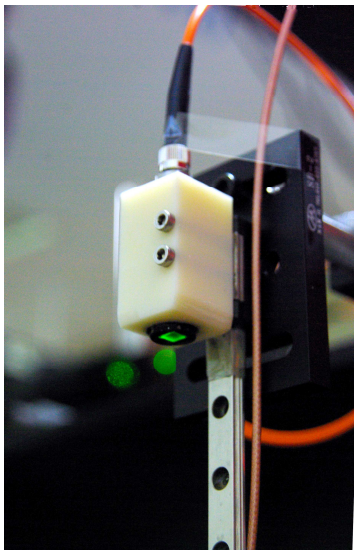
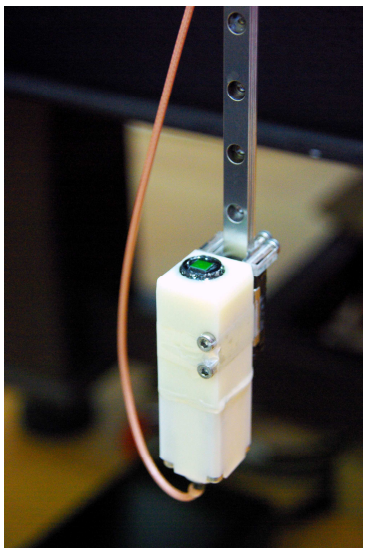


Figure 13 The final design of polarimetry probe



(a) Polarimetry probe





(b) The top of probe (c) The bottom of probe

Figure 14 The final prototype of polarimetry probe

Chapter 5

Polarimetry probe system experiment

The polarimetry probe system consists of two units: control part and probe part. The primary function of control part is to modulate the light and to process signals while that of probe part is to measure the signal. Figure 15 and Figure 16 shows the schematic diagram and the image of polarimetry probe system.

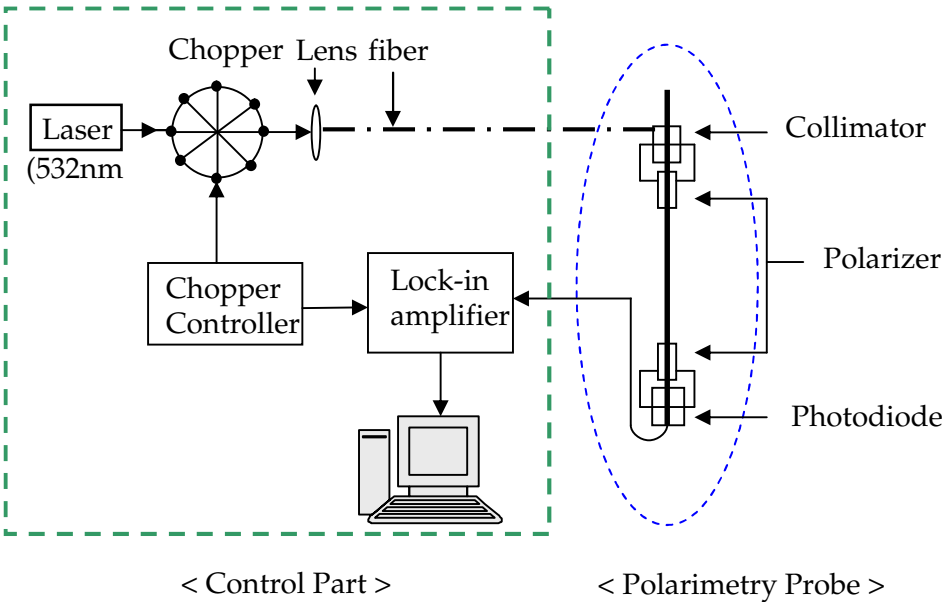


Figure 15 The schematic diagram of polarimetry probe system

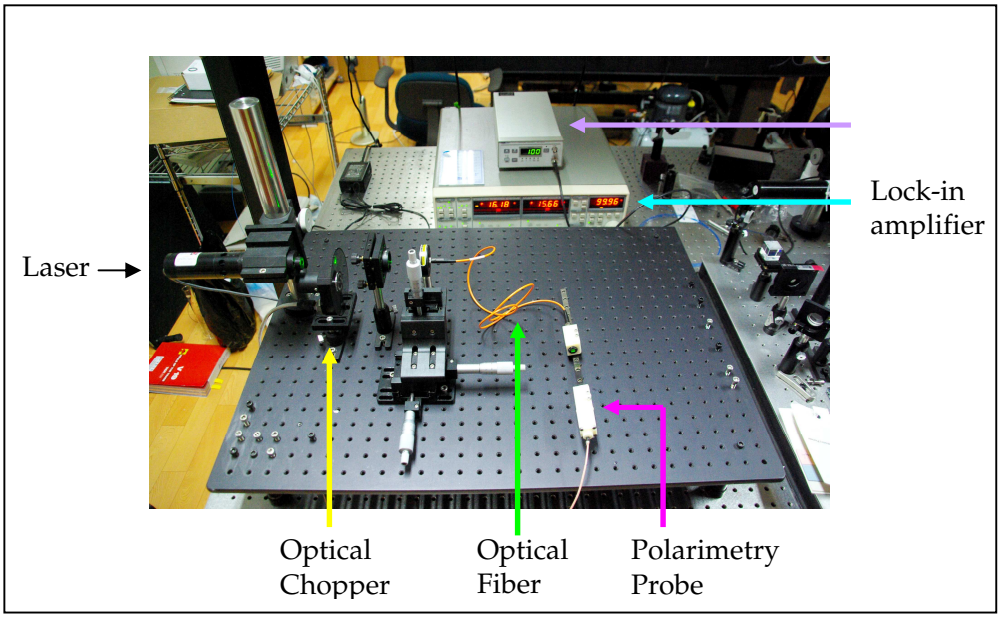


Figure 16 The image of polarimetry probe system

5.1 *In-vitro* experiment

To characterize the probe system, *in-vitro* experiment was performed. 20cm sample cell is used as optical path length of sample. Sample cell is placed on the guide rail during experiment. Measurement period is 20 seconds in each concentration.

5.1.1 Characterization of water based glucose concentration in probe system

Similarly to the preliminary experiments, the water based glucose solution was used to evaluate the performance of optical polarimetry probe system. Glucose concentration range of 0-1000mg/dl in 200mg/dl increment was used in this experiment.

Figure 17 shows the observed intensity resulted from the function of glucose concentration that demonstrates the same pattern with the pre-experiment. According to the linear regression analysis, the outcome shows the linearity with correlation coefficient of 0.988 (Origin 8.0 Version). Although observed in voltage scale to measure the intensity of experiment as shown in Figure 17(y axis), the differentiation of concentration would be shown much clearer when the scale of observation adjusts to milli voltage scale.

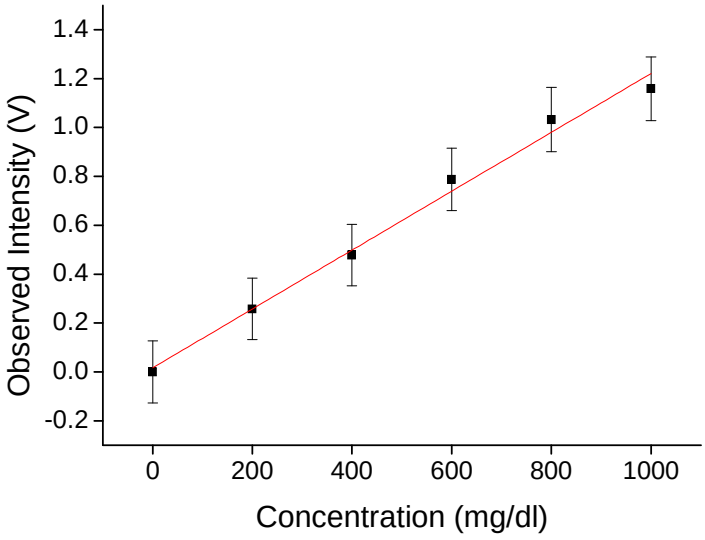


Figure 17 Observed intensity as a function of glucose concentration

5.1.2 Comparison with theoretical value

For research evaluation, the experiment data was compared with the theoretical value that linear regression analysis was performed in particular to confirm the correlation of theoretical and experiment values.

The predicted value can be calibrated using the equation (1) and Malus's law with the information of wavelength, specific rotation angle of glucose and glucose concentration. Given the specific rotation of glucose at 532nm, the rotation angle of glucose concentration can be calculated from the equation (1). Accordingly the output light intensity can be calculated by Malus'a law equation (4) with the input light intensity and the calculated rotation angle.

$$I = I_i \cos^2 \theta \tag{4}$$

Where I_i is the intensity of linearly polarized light before the analyzer and I is the intensity after passing the analyzer and θ is the angle between the plane of vibration and the axis of the analyzer. Table 3 indicates the theoretical values calculated by the equation (4), which implies linear relationship between theoretical value and concentration.

The predicted versus actual values were plotted in Figure 18. It shows the linearity with the correlation coefficient of 0.851. (Origin 8.0 Version)

Concentration (mg/dl)	Theoretical value (V)
0	0
200	$2.471 * 10^{-6}$
400	$9.884 * 10^{-6}$
600	$22.239 * 10^{-6}$
800	$39.536 * 10^{-6}$
1000	$61.776 * 10^{-6}$

Table 3 The theoretical value according to the glucose concentration

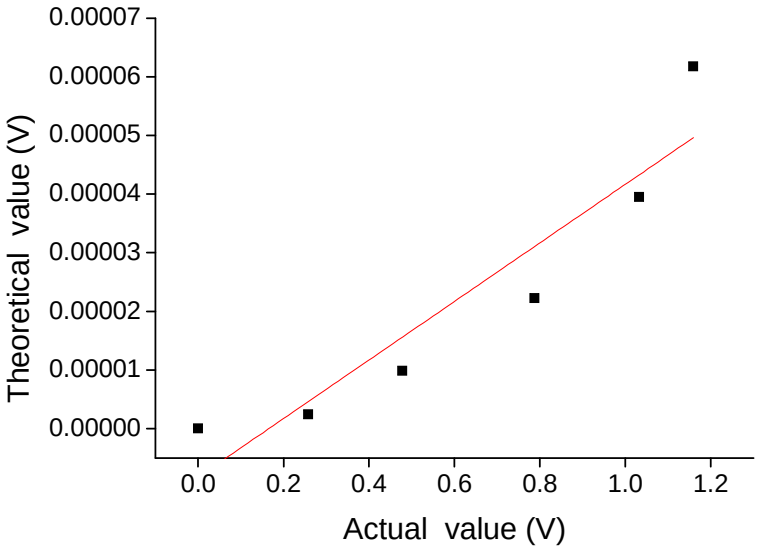


Figure 18 The theoretical versus actual value

5.1.3 Validation experiment

In order to validate the repeatability of experiment, validation experiment was performed. The result of Figure 17 can be used as a calibration function that resulted in equation (5).

$$y = 0.24081 * x - 0.22373 \quad (5)$$

Four random glucose concentrations were used in this experiment: 150, 350, 550, 750 mg/dl. Table 4 shows the comparison between calibration value from the equation (5) and measured value of concentration. The calibration versus measured values was plotted in Figure 19. It shows the high degree of linearity with the correlation coefficient of 0.989. (Origin 8.0 Version)

Concentration (mg/dl)	Calibration value (V)	Measured value (V)
150	-0.007	0.154446
350	0.281971	0.369764
550	0.570943	0.597032
750	0.859915	0.899798

Table 4 Calibration value versus measured value

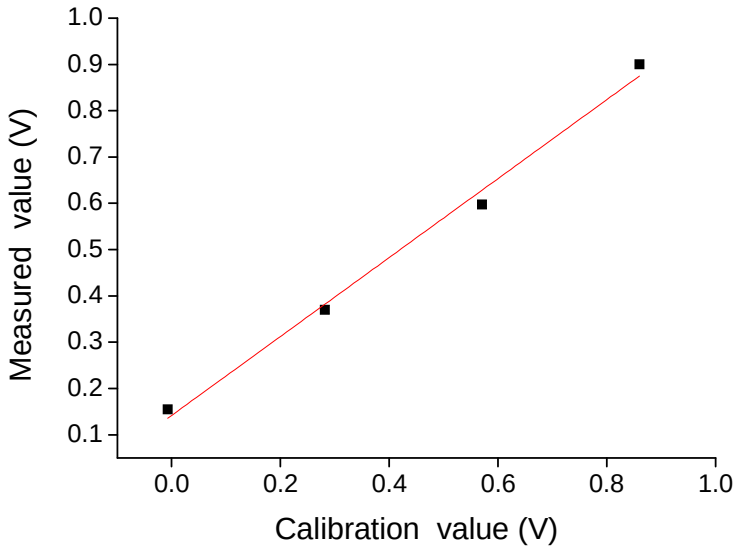


Figure 19 Calibration versus measured value

5.2 *In-situ* (Immersion) experiment

5.2.1 Characterization of water based glucose concentration in probe system

For the purpose of performance evaluation, *in-situ* experiment was performed. During experiment, polarimetry probe is placed in the solution as immersion state. Glucose concentration range of 0-3000mg/dl in 500mg/dl increment was used in this experiment. Measurement period is 20 seconds in each concentration. Figure 20 shows the observed intensity resulted from the glucose concentration. It shows the linearity with the correlation coefficient of 0.938. (Origin 8.0 Version)

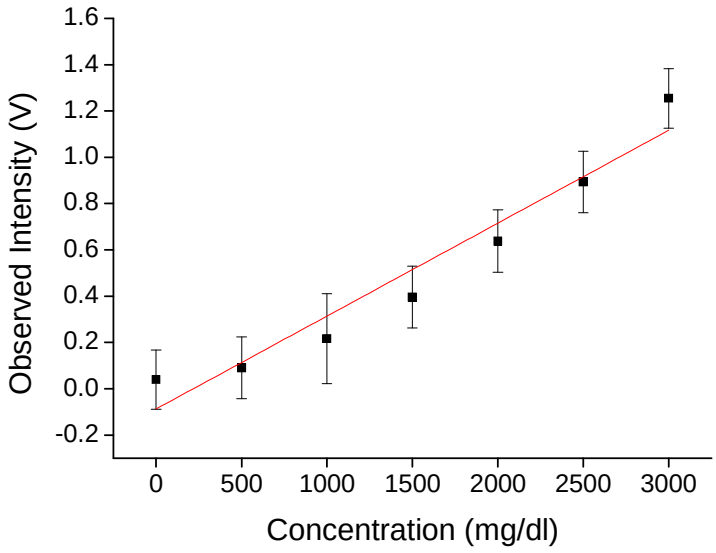


Figure 20 Observed intensity as a function of glucose concentration

Chapter 6

Conclusion and Future work

The aim of this study is to develop an optical path length variable polarimetry probe for optical active sample measurement. The polarimetry probe is designed to vary the optical path length thus the feasibility of concentration meter is confirmed.

Commercial polarimetry system is based on off-line measurement which causes potential problems to measurement such as sample contamination, unstability and time delay. However, the polarimetry probe system in this research has adopted on-line measurement for several advantages: noninvasive method, variable optical path length, needless of sample extraction and compensation reagent, and compactness and simplicity.

In 1997, Cote' et al developed the closed-loop polarimetry system using phase measurement technique for measurement of glucose concentration. Compared with the phase measurement system, optical polarimetry probe system is simple and easy to measure. Cote' et al employed a faraday rotator to verify the measuring phase difference to decide compensating value for initial condition setting.[6,7] However, the optical polarimetry probe that is developed in this research is rather simple due to its dependence on light transmittance originated from optical rotation.

The future research will focus on two aspects. One is to make a complete product. The system that developed to prove our concept could have been realized

by the inspiration from the prototype that served as a guide rail. Made with round shape aluminum material, the final product will look similar to Figure 11 that is easily applicable in solution.

Some problems have also found during experiment. Firstly, compared with the result of pre-experiment and *in-vitro* experiment, the observed intensity of *in-situ* experiment was decreased. Accordingly, the range of concentrations was increased to detect the signal in *in-situ* experiment. The difference between *in-vitro* and *in-situ* experiment was the state of sample. In *in-vitro* experiment, the solution was contained in sample cell while the solution was in contact with polarizer in *in-situ* experiment. The difference between three experiments is suspected to the result from unknown interaction between polarizer and solution. Secondly, reflection light was depolarized proving the inappropriateness of second design polarimetry probe regardless of the polarization-maintain right angle prism. The improvement of this problem could shorten the length of probe that would optimize the appearance of the final design polarimetry probe.

Another aspect is related to application field. Polarimetry probe can be applied in many fields. For example, in cell culture process, glucose concentration can be defined by the variation of cell culture where the time of exchanging medium is crucial. Polarimetry probe can detect the glucose concentration of medium without the extraction of sample nor the chasm in observation time. Also, it can control the measurement sensitivity by variable optical path length in low concentration. Consequently, the polarimetry probe system can be used as an indicator of culture medium and warning system which informs the time of medium exchange.

Current system is verified in water-based glucose solution. It has only single optical active substance easily detectable. In a mixture such as cell culture medium, one optical active substance will affect another optical active substance's rotation angle in detected signal, which causes an measurement error. Therefore, if the application field is to required to detect two or more optical active substances, the method to differentiate rotation angle independently in mixture needs to be further studied.

Polarimetry probe system might have less accuracy but it has an advantage of variable optical path length. In addition, on-line measurement is possible in polarimetry probe system. The prototype of polarimetry probe system still has some problems. However, the polarimetry probe system promises good performance with few complements.

Chapter 7

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국 문 초 록

광 측정 방식은 비침습적 측정방식으로, 의학 및 생물학 분야에서 그 응용이 증가하고 있다. 현재 주로 응용되고 있는 농도 측정 방식은 일반적으로 샘플을 밖으로 추출 후, 분석기를 통한 오프라인 검출 방식과 배양기 내부에 전극형 측정장치를 장착하여 측정하는 온라인 검출 방식이 응용되고 있다. 오프라인 측정 방식의 경우, 과도한 수작업에 의한 사용자 측정 에러, 샘플채취과정에서의 감염위험, 보정을 위한 표준시약의 필요성과 같은 단점이 있고, 온라인 측정 방식의 경우, 고가 및 안정성 그리고 반응기 내부 조건에 따른 오차 발생들의 단점이 야기되고 있다.

편광계 측정방식(Polarimetry)은 샘플에 비 침습적인 광 측정 방식으로, 빛의 투과광량 변화로부터 샘플의 농도를 검출할 수 있다. 본 연구에서는 편광계 측정방식을 응용, 비침습적이며 온라인 측정이 가능한 광학 시스템에 대하여 논하고자 한다. 특히 기존의 측정방식에 비해, 배양액의 별도의 샘플 없이, 직접 응용이 가능한 것을 특징으로 하며, 또한 편광 측정방식에 있어서, 광 경로의 가변을 통한 측정감도 향상이 가능한 것을 특징으로 한다.

핵심 되는 말: 편광계, 광활성도, 온라인, 농도측정장치, 프로브