JOURNAL OF BIOMEDICAL OPTICS 2(3), 275–281 (JULY 1997)

NONINVASIVE POLARIMETRIC MEASUREMENT OF GLUCOSE IN CELL CULTURE MEDIA

Gerard L. Coté and Brent D. Cameron
Texas A&M University, Bioengineering Program, 233 Zachry Engineering Center, College Station, Texas 77843-3120

(Paper JBO-119 received Nov. 10, 1996; revised manuscript received Apr. 9, 1997; accepted for publication Apr. 19, 1997.)

ABSTRACT

In order to enhance cell culture growth in bioreactors, biosensors such as those used for glucose detection must be developed that are capable of monitoring cell culture processes continuously and preferably noninvasively. The development of a unique noninvasive, optically based polarimetric glucose sensor is reported. The data were collected using a highly sensitive, lab-built polarimeter with digital feedback and a red laser diode source. A range of glucose concentrations was evaluated using both glucose-doped double-distilled water and a bovine serum-based medium. The serum-based medium is the nutritional environment in which the cell cultures are grown. Both media were examined across two glucose concentration ranges—a lower range of 100 mg/dl in 10-mg/dl increments and a higher range up to 600 mg/dl in 50-mg/dl increments. The linear regression in all experiments yielded standard errors of prediction of less than 8.5 mg/dl across both ranges. *© 1997 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(97)00403-6]

Keywords bioreactor; biosensor; polarimetry; glucose.

1 INTRODUCTION

Cell culture models provide *in vitro* systems for investigating specific mechanisms during drug discovery and development,[1] viral research,[2] research on cell aging,[3] and tissue engineering[4–7] in precisely controlled environments. This work focuses on developing a novel glucose sensor to aid in growing cell cultures, primarily for tissue engineering.

The number of patients suffering from tissue loss or organ failure each year is in the millions.[8] In order to accommodate the shortage of replacement tissue and organs, a substantial research effort has been focused on the production of implantable *in vitro* tissues. The culturing of a variety of tissues under clinostatic suspension in rotating vessel bioreactors has increased cell viability, adhesion, tissue formation, and differentiation. However, the growth rate could be optimized by automated control of the cell culture environment and processes.[4–7] In order to do this, on-line and preferably noninvasive sensors must be implemented to monitor various parameters of the cell culture medium and feed that information back for regulation of the environment. One vital parameter of interest is the concentration of glucose in the cell culture medium. The basis for cell culture media is an isotonic solution of nutrients and cofactors.[7] In a typical medium, glucose is the major carbohydrate energy source. Cell proliferation can cause rapid consumption of glucose, which in turn can limit growth.[6,7] Thus, the relative change in concentration of glucose is one factor contributing to the rate of cell culture growth. In the long term, by permitting closed-loop control of cell culture processes, including glucose concentration, optimization of the growth rate may be obtained.[4–7] Independent of the closed-loop control, the current primary advantage of this approach is that it would be noninvasive, thereby not requiring the removal of the samples for analysis.

In addition to standard bioreactor studies, researchers at the National Aeronautics and Space Administration at the Johnson Space Center (NASA-JSC) are particularly interested in an on-line and noninvasive miniature glucose sensor for use with their integrated rotating wall vessel (IRWV) bioreactor. This sensor would be used for feedback control of the glucose concentration present in the cell culture medium to maintain an optimal growth concentration between 60 and 200 mg/dl. The current technique used to monitor the glucose concentration of the medium is to withdraw a sample from the bioreactor and analyze it externally with a tabletop glucose enzyme electrode sensor such as the Beckman Glucose Analyzer-2,[4] which has an accuracy of ±10%. However, this method poses major problems for researchers. It allows possible contamination of the cell cultures during extraction of the medium. Continuous glucose readings are not available for well-regulated control of the glucose concentration using this technique, and the periodic, off-line readings require personnel to perform the analysis manually. A particular problem for

Address all correspondence to Gerard L. Coté. Tel: (409) 845-4196; Fax: (409) 847-9005; E-mail: cote@acs.tamu.edu

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NASA-JSC is that the tabletop analysis machine is only adequate for ground-based operation and is not suitable for space flight due to its large size and weight. Therefore, the complete automation and control of the cell culture processes within the bioreactor are inhibited until a new, continuous, and preferably noninvasive method for estimating glucose concentration is developed.

An alternative to withdrawing a sample of the medium is to place an electrochemical sensor within the bioreactor. The general form of such a sensor is a transducer element containing a catalyst and additional chemistry that provides an electrical signal proportional to the analyte concentration of interest.[9] The problems associated with this type of sensor include difficult sterilization techniques, limited lifetime, lack of long-term stability of the glucose oxidase reaction, and fouling of the membranes.[9] Thus, these types of devices are not the optimal choice for monitoring glucose within a bioreactor.

Recently, there has also been a considerable effort toward the development of near-infrared (NIR) and midinfrared spectroscopic glucose sensors.[10–20] These sensing approaches have been developed for use in agricultural food analysis, particularly the fruit industry, and as a potential means of monitoring blood glucose levels in diabetic patients. As a blood or cell culture glucose sensor, the midinfrared range above 2.5 μm is problematic due to the high absorption from water. However, the recent combination of NIR spectroscopy with powerful multivariate statistical techniques, if carefully applied, offers the advantage of latent variables that produce a more accurate model for the prediction of glucose. In addition, the NIR approach offers the possibility of simultaneously monitoring multiple chemicals within the bioreactor. However, the NIR absorption bands up to 2.5 microns can overlap significantly and are influenced by temperature as well as hydrogen bonding effects. To date, a key problem of the NIR approach, particularly in the 2 to 2.5-micron region, is the lack of low heat, high-powered, multiple-wavelength radiation sources. Inexpensive, reliable, multiwavelength lasers may prove to be the key to successful monitoring for this application, but are currently not commercially available in this NIR region. Finally, care must be taken when using multivariate statistics to produce a model that is not specific to a single data set on a given day.

In this research, a polarimetric system utilizing closed-loop digital feedback control has been developed and implemented as a novel means to monitor the required glucose measurements of cell culture medium on-line and noninvasively without potential contamination of the cell culture medium. Polarimetry is based on the principle that if linearly polarized light is transmitted through a chiral substance such as glucose, a rotation of the light vector will occur in proportion to the concentration of that substance.[21,22] The amount of polarized light rotated as the result of an optically active substance also depends on the wavelength of the light used for the measurement, the thickness of the layer traversed by the light or rather path length, and the temperature of the optically active material.[21,22]

The rotation of linearly polarized light has been used for many years to quantify the concentration of a chiral substance in solution, in particular sugars.[21] Recently, several investigators have suggested detection of glucose *in vivo* using polarimetry as a noninvasive alternative for diabetic glucose monitoring.[22–26] In addition, bulk commercially available ellipsometers are used to measure rotation and birefringence of various substances.[27] However, to date, we are unaware of any other group that has tried using the polarimetric method for the measurement of glucose in cell culture medium. A key problem with using the polarimetric approach *in vivo* is that skin possesses high scattering, which depolarizes most of the light. Alternative sites such as the eye have been suggested for the indirect detection of blood glucose concentrations; however, this too has problems, including corneal birefringence and eye motion artifacts. In order for polarimetry to be used as a noninvasive technique for monitoring cell culture glucose levels, the signal must be able to pass from the source through the medium and to the detector without total depolarization of the beam. Unlike the *in vivo* application of this approach, a windowed cell with a fixed path length can be used in the bioreactor in place of complex biological tissue such as the skin or the cornea of the eye. The problem is thus simplified to one of monitoring changes in glucose concentration in the presence of slightly depolarizing, optically rotatory, confounding chemicals found in this medium.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SYSTEM

The block diagram of the experimental setup designed and implemented in this research is illustrated in Figure 1. The light source is provided by a 5-mW, 670-nm (red) laser diode. The use of a constant current feedback driver (Merideth Instruments, Glendale, Arizona) ensures output power stability of the laser diode. Laser diodes at this wavelength are compact, inexpensive, have low power consumption, provide enough penetration into this relatively clear sample medium, and allow for more optical rotation due to glucose compared with the longer wavelengths in the NIR range. A Glan-Thompson 100,000:1 polarizer (Newport Corp., Irvine, California) then linearly polarizes the light beam before modulation. This polarizer is oriented in the preferred polarization state of the laser in order to maximize transmitted power. After the initial polarizer, the polarization vector is modulated via a Faraday rotator (Deltronic Crystal Inc.,

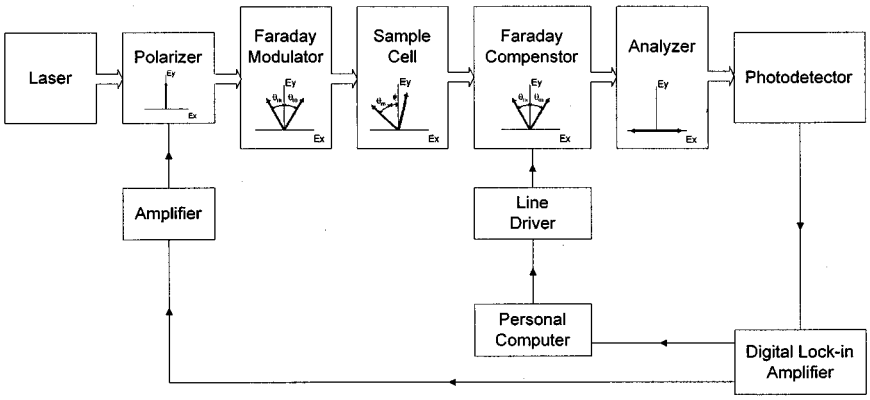

Fig. 1 Benchtop polarimeter system used to sense glucose. The vector diagrams illustrate the state of polarization throughout the optical train. The laser generates a light beam that passes through the input polarizer. The polarization of the light is sinusoidally modulated at an angle θ_m using the first Faraday rotator and is passed through the optically rotatory glucose sample, which causes an additional static rotation (ϕ) in the polarization of light. The second Faraday rotator is used to null this rotation in the closed-loop system. The analyzer is then used to convert the modulation of the polarization vector into an intensity modulation that can be sensed by the light detector. The light impinging on the detector after total compensation produces a pure sinusoid at twice the modulation frequency, and the DC voltage applied to the second Faraday rotator to eliminate the sample rotation (ϕ) is thus proportional to the glucose concentration.

Dover, New Jersey) at a frequency of 1.09 kHz. This frequency was chosen to be well above common light noise, yet still provide a reasonable modulation depth of slightly less than ±1 deg, given the constraints of the Faraday coil used. The AC modulation signal is provided by the internal function generator of the digital lock-in amplifier (Stanford Research Systems SR830, Sunnyvale, California), which is then amplified to provide enough power to drive the Faraday rotator. In order to achieve resonance of the 200-mH Faraday rotator, a 0.1-μF capacitor is placed in series with the coil. After the initial Faraday rotator, the light beam passes through a 1-cm sample cell constructed of optical-grade glass. A second Faraday rotator then provides the feedback in the system in order to null the rotation of the polarization vector due to the glucose sample. The next component in the optical train is another Glan-Thompson polarizer that is known as the analyzer. This is oriented 90 deg with respect to the initial polarizer in order to achieve cross-polarization. The final component in the optical train is a silicon-based detector (Thorlabs Inc., Newton, New Jersey), which outputs a voltage proportional to the detected light intensity or the square of the electric field. This electrical signal is the input into the digital lock-in amplifier.

The lock-in determines the relative amplitude of the signal present at the modulation frequency. This information is then sent to the personal computer as an input into the digital compensator. The digital compensator then eliminates the rotation of the polarization vector due to the optically active sample by transmitting a voltage from the personal computer to the lock-in's D/A port. A line driver then amplifies the current of this signal in order to drive the compensation Faraday rotator. The computer records the compensation voltage, which is proportional to the concentration of the sample. In an open-loop system, without feedback to the second Faraday rotator, if the sample were not optically active, a symmetric sinusoidal wave form at twice the fundamental frequency would be depicted at the detector output. This yields effectively zero volts from the lock-in since it is referenced to the fundamental modulation frequency. If the sample were optically active, in the open loop system, an asymmetric signal would be seen at the detector. This signal now has fundamental modulation frequency components causing the output of the lock-in to register a signal off null. In the closed-loop system, this voltage is put into the second Faraday rotator to again force the signal to its symmetric state.

2.2 SAMPLE PREPARATION AND DATA COLLECTION

For water-based glucose studies, a stock solution of glucose and water was prepared from 2 g of D-glucose and 200 ml of double-distilled water. Six hours were allowed for mutarotation to occur in the solution. This step is needed when D-glucose is dissolved in solution since an equilibrium-specific rotation occurs between the D- and L- conformations of glucose. Concentrations from 0 to 600 mg/dl in increments of 50 mg/dl and 0 to 100 mg/dl in increments of 10 mg/dl were obtained by diluting the stock solution with an appropriate addition of double-distilled water.

For the cell culture medium studies, a sterile base solution of cell culture medium (GTSF-2) was supplied by NASA-JSC and manufactured by KRUG Life Sciences (Houston, Texas) with a baseline concentration of 100 mg/dl of glucose. A 600 mg/dl stock solution of glucose-doped cell culture medium was prepared by adding 500 mg of D-glucose powder to 100 ml of the base solution. Six hours

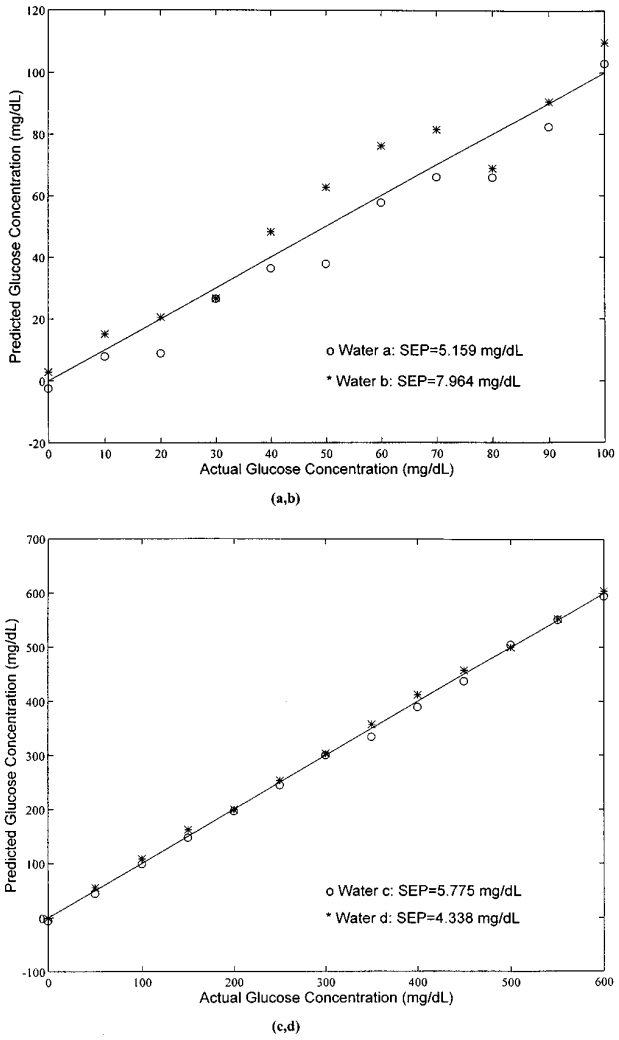

Fig. 2 Glucose-doped water data showing true prediction of the glucose concentration of one data set based on a linear regression model using the other data set for both (a,b) the low concentration and (c,d) high concentration ranges. The data sets were taken with the digitally controlled benchtop polarimeter system using a 1 cm test cell. The line is shown for reference as the theoretical perfect fit.

was allowed for mutarotation to occur in this stock solution. Individual samples were then prepared by diluting down the high-concentration glucose-doped stock solution with an appropriate amount of the 100 mg/dl glucose base solution. This provided for two concentration ranges between 100 and 600 mg/dl with a minimum of 50 mg/dl increments and a 100 to 200 mg/dl range with 10 mg/dl increments. The lower range starts at 100 mg/dl because of the glucose concentration provided in the stock solution. This is not problematic since the relationship between glucose concentration and the rotation of polarized light for a given path length is linear across the entire range of physiologic concentrations.

A total of eight experiments were conducted in which the data for each experiment were collected by randomly placing each concentration in the sample cell. In these experiments, the temperature of the sample was not regulated; however, the changes in the specific rotation of glucose due to small temperature variations are negligible. In addition, in the final bioreactor system, the temperature is tightly maintained at 36 ± 1 °C. Four sets each of glucose-doped water and cell culture medium were collected at two replicate concentration ranges of 100 and 600 mg/dl in 10 and 50 mg/dl increments, respectively. Finally, before the results were analyzed, the signal of the ''blank'' or lowest concentration was subtracted from each of the raw signal values.

3 RESULTS

After data collection, least-squares linear regression analysis was performed to determine the ''best-fit'' line in order to minimize the sum of the squares of the residuals for the voltage versus concentration data. Once the linear model was computed for each data set, the calibration model was validated by independently predicting glucose concentrations for the remaining similar data set. For instance, the linear regression model derived for the first water data set in the 0 to 600 mg/dl range was used to independently predict the glucose concentrations for the second remaining similar data set. The model of the second water data set was then used to predict the concentrations of the first data set. The validation plots of the glucose-doped water and cell culture medium results are shown in Figures 2 and 3, respectively. These plots are shown with predicted concentration values that deviate from a theoretical line passing through 0 with a slope of 1, which represents the error-free estimation. Table 1 summarizes the calibration and validation statistics for each data set, including the correlation coefficient and the standard error of prediction (SEP).

As can be seen in Table 1, each glucose-doped water data set possesses a high degree of linearity, with correlation coefficients exceeding 0.9754 and 0.9996 for the lower and upper concentration ranges, respectively. The mean SEPs between all water-based calibration and validation data sets are 5.69 mg/dl and 5.81 mg/dl, respectively. The main sources of this error include the feedback control before and after the A/D and D/A converters, the line driver used to amplify the current driving the compensation Faraday rotator, and slight differences in doping the stock solution across days. Evaluation of the linear regression model indicated that the slope coefficient varied less than 8.1% between data sets. In addition, the computed 95% confidence interval contains zero, which indicates a high probability that no fixed bias is present in the model. This is to be expected in all the glucose-doped water cases since glucose is the only optically active component present in the medium.

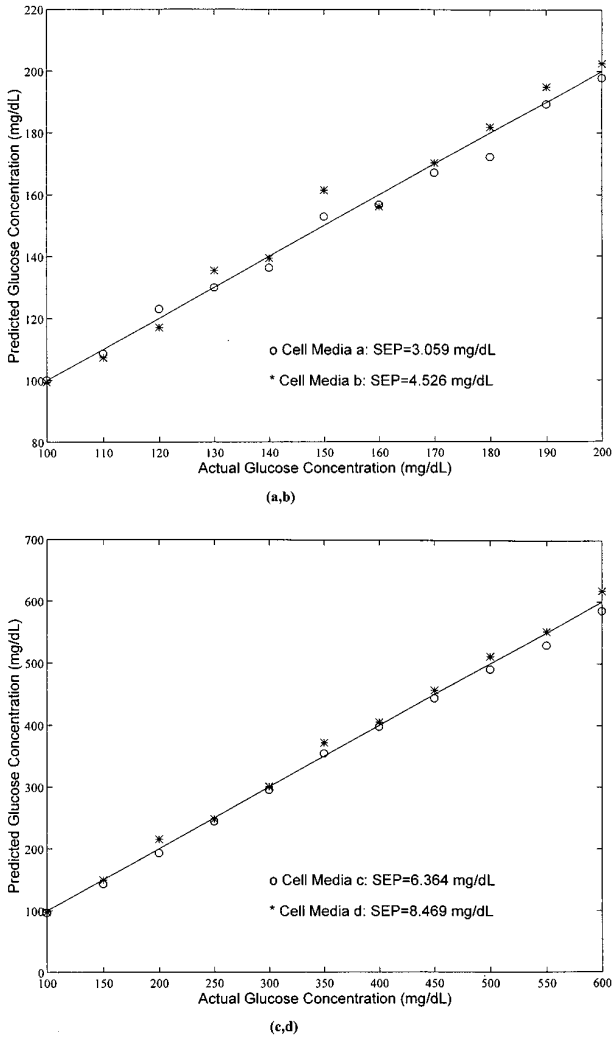

Fig. 3 Glucose-doped bovine serum-based cell culture medium showing true prediction of the glucose concentration of one data set based on a linear regression model using the other data set for both (a,b) the low concentration and (c,d) high concentration ranges. The data sets were taken with the digitally controlled benchtop polarimeter system using a 1 cm test cell. The line is shown for reference as the theoretical perfect fit.

Each cell culture data set possesses a high degree of linearity, with correlation coefficients exceeding 0.9924 and 0.9990 for the lower and upper ranges, respectively (see Table 1). The average SEPs between the cell culture-based calibration and validation data sets are 4.99 mg/dl and 5.60 mg/dl, respectively. In the cell culture medium calibration models, there is a high probability of a small fixed bias in the models since the calculated 95% confidence intervals did not bound the true zero intercept in every case. This bias is expected since there is rotation due to the other optical rotatory chemicals in the cell culture medium, which act as a dc shift in the calibration model.

4 DISCUSSION

The results of these experiments indicate that the investigated polarimetric approach is very robust, possessing strong linearity and repeatability across days. In each case, the glucose concentrations predicted from each of the data sets were determined using a calibration model generated from a separate independent data set. The average validation SEPs of this sensing approach in both glucose-doped water and cell culture media was less than 5.81 mg/dl and 5.60 mg/dl, respectively, which is comparable to the precision of most invasive lab-based glucose meters. In addition, the repeatability of the system can be seen due to the minimal SEPs in both the calibration and validation analyses (see Table 1).

The collected data suggest that minimal scattering and depolarization occur due to the other chemical components in the media, since millidegree rotational measurements corresponding to an average of 5.60 mg/dl glucose changes were possible. There were no cells present in the medium used for this investigation since, in the final system, the sensor would be placed after the filter membranes, which are used to prevent the cells and large molecules from leaving the rotating vessel chamber. For this closed-loop system, the fluid leaving the windowed interface then flows back into the bioreactor. A net baseline optical rotation was observed in this study that was due to the other optically active components, such as the amino acids, proteins, fructose, and galactose found in most cell culture media. These components were not varied in this study since, with only one wavelength, the sensor may not have the specificity to distinguish glucose variations from those of other chiral molecules. To further improve the robustness and ensure the specificity of this approach, a multiple wavelength system is being investigated to quantitatively distinguish glucose in the presence of other optically rotatory components that vary in concentration.

5 CONCLUSIONS

The aim of this research was to develop a novel polarimetric-based sensing system in an effort to noninvasively and continuously determine glucose concentrations in cell culture medium for the eventual real-time control of this nutrient for enhanced cell production. In addition to monitoring glucose, our polarimetric system has the potential for meeting both the size and power requirements needed for space flight. The variation of the glucose in the presence of other optically rotatory components found in the cell culture medium, using a single wavelength of light, has been accurately determined. The collected data indicate that the sensor is well within the range of sensitivity needed for practical monitoring of glucose in standard cell bioreactors. This sensing approach shows an accuracy comparable to that of a standard benchtop glucose measurement system but with the added advantage of the potential for continuous noninvasive monitoring.

Table 1 Summary of the various linear regression statistics for the calibration and validation analyses for both the glucose-doped water and cell culture medium experiments. Models a and b for each medium represent the lower concentration ranges while c and d are indicative of the higher concentration ranges.

Medium	Model	Correlation coefficient (*r*)	Standard error of prediction in calibration (mg/dl)	Standard error of prediction in validation (mg/dl)
Water	2a	0.9754	7.490	5.159
	2b	0.9881	5.170	7.964
	2c	0.9998	4.340	5.775
	2d	0.9996	5.770	4.338
Cell	3a	0.9924	4.120	3.059
culture	3b	0.9963	2.850	4.526
	3c	0.9990	7.540	6.364
	3d	0.9995	5.460	8.469

Acknowledgments

The authors wish to acknowledge the support of the NASA-JSC Biotechnology group under the direction of Neal Pellis for contributing the cell culture medium. This work was supported in part by grants from the National Aeronautics and Space Administration (Grant No. NAG9-821) and National Science Foundation (Grant No. BCS-9309147).

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