Fiber Optic Based Sensor System for Determination of Protein Content in Milk

Dharitri Devi[1], Jhimli Kumari Das[2]

[1] *dharitridevi@email.com,* [2]*jhimli.das@dbuniversity.ac.in*
Don Bosco College of Engineering and Technology, Assam Don Bosco University
Airport Road, Azara, Guwahati - 781017, Assam. INDIA.

Abstract: ***Milk is the primary source of nutrition for young mammals before they are able to digest other types of food. Milk contains 3.3% total protein. As milk protein contain all 9 essential amino acids required by humans,so we select milk for protein studies. In this paper a novel method and instrumental system is used to determine the total protein concentration in a milk sample. It uses a fiber optic protein sensor based on the principles of fiber optic evanescent wave absorption. The evanescent waves at the fiber optic core-cladding interface are used to monitor the protein-induced changes in the sensor element. This system measures protein concentration in milk sample without destroying the sample. This new approach has the potential to offer significant sensitivity enhancements over more conventional protein sensing technology which may be time consuming or less economical.***

Keywords:evanescent wave, protein sensor, fiber optic sensor**.**

1. Introduction

Proteins are polymers of amino acids. Twenty different types of amino acids found in proteins. Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. So each protein have different molecular structures, nutritional values and physiochemical properties. Proteins are important constituents of foods for different reasons. Proteins are major source of energy,as it contains all essential amino-acids required for human health.

Milk contains 3.3% of total available protein. Milk proteins contain all 9 essential amino acids required by humans. There are 2 major categories of milk protein that are broadly classified according to their physical properties and chemical composition.Casein and Serum protein. The casein protein contains phosphorus and will coagulate or precipitate at pH 4.6. The serum (whey) proteins do not contain phosphorus, and it remain in solution in milk at pH 4.6.

During last few years several works has been carried out to measure protein content in different food specimen.However, each of these methods has certain limitations in its sensitivity, accuracy, and reproducibility. We consider the method which is more sensitive, accurate, portable, rapid and economically viable.

The aim of this work is to develop a portable, low cost fiber optic based sensor system to determine protein concentration of different milk sample. The main challenge for this system is to identify protein content in different milk sample by using optical fiber. The system was developed by considering 2 different milk samples such as cow milk and milk powder. The fiber optic sensor developed in this study is based on the variation in the evanescent wave phenomenon at the core-cladding interface. In the sensor design, an optical fiber is used as the transduction element. In this sensor system some chemical reagents are used to generate a spectrophotometrically detectable signal within the sensing region of the optical fiber.

2. Materials and Method

Sensor system developed in this study was based on spectrophotometric analysis. For this purpose the following steps are adopted.

2.1 Estimation of protein

The Bovine Serum albumin (BSA) solution (2.5 mg/ml) was prepared by dissolving 0.0025 g BSA in 1.0 ml deionized water. BSA solution was used as a standard for protein estimation because of its low cost, high purity and ready availability.Folin Ciocalteau Reagent (FCR) or Folin's phenol reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric in vitro assay of phenolic and polyphenolic antioxidants. This reagent will react with phenols and nonphenolic reducing substances to form chromogens that can be detected spectrophotometrically. Folin reagent solution (1N) is prepared by mixing commercial reagent (2N) with an equal volume of distilled water on the day of use (1 ml of commercial reagent + 2 ml distilled water).

Analytical reagents:

(a) 2% sodium carbonate mixed with 0.1 N NaOH solutions (0.4 gm in 100 ml distilled water.)

(b) 0.5% copper sulphate solution mixed with 1% sodium potassium tartarate solution. An analytical reagent is prepared by mixing 1 ml of (b) with 50 ml of (a).

Milk sample:

Different milk sample such as cow milk and milk powder mixed with water is used as an unknown sample for protein estimation. Milk samples are freshly prepared on the day of use. For this system five types of milk sample was cosidered and the total volume in each milk sample was 80 ml. First milk samples contains 80 ml of cow milk (pure cow milk), second milk sample contains 53 ml of cow milk and 27 ml of water, third sample contains 50 ml of cow milk and 50 ml of water fourth sample contains 27 ml of cow milk and 53 ml of water and 25% water, fifth milk sample contains 1 table spoon of milk powder mixed with 80 ml of water are used

for protein estimation. For each milk sample 0.2 ml of solution is pipette out to different test tubes and then distilled water is ddded to each sample.

2.2 Chemical validation of data by Folin Lowry method

Folin Lowry method is one of the most commonly used chemical methods for determination of protein concentration in different unknown sample. The principle behind the Folin Lowry method of determining protein concentrations of different unknown sample lies in the reactivity of the peptide nitrogen with the copper ions under alkaline conditions and the subsequent reduction of the Folin-Ciocalteay phosphomolybdic phosphotungstic acid to heteropolymolybdenum blue by the copper-catalysed oxidation of aromatic acids.

Folin Lowry method is described by the following block diagram:

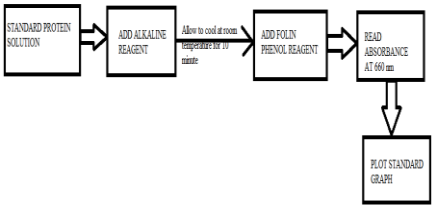

Figure1: Block Diagram of Folin Lowry method

2.2.1 Procedure of Folin Lowry Method

For Folin Lowry method different volume of standard protein (BSA) solution is taken in different test tube and then distilled water is then added to each sample so that the volume of each test tube becomes 1 ml. For milk sample 0.2 ml of different milk solution is pipette out to different test tubes and then distilled water is added to each sample. Next 5 ml of alkaline reagent was added to the sample, mixed thoroughly and allowed to stand at room temperature for 10 minutes or more. After that 0.5 ml of Folin reagent was added rapidly with immediate mixing. Rapid mixing was possible by the use of vortex mixture. Reading of the samples was taken against appropriate blank at 660 nm by using spectrophotometer. A UV-VIS Spectrophotometer, SL 159works on the principle of Beer-Lambert Law is used to measure the absorbance of the protein sample.

From the measured absorbance and the protein concentration of BSA a standard curve is prepared by plotting concentration of the protein sample on Y axis and the measured absorbance on X axis and from the curve protein concentration of the unknown was determined by interpolating the curve.

2.3 Optical Sensor System

The main aim of the work reported in this paper is to develop an optoelectronic based sensor system that consists of a Laser Source, a multimode optical fiber, a container containing milk sample and a photo detector which is used to measure the intensity of the output light, a 8051 microcontroller and a display unit which display the concentration of protein. For this fiber optic based protein sensor 632.8nm laser source is used. An objective lens is used to launch the light from the source into one end of the optical fiber.

A plastic clade silica core fiber of length 1m is used to fabricate this sensor system. Outer protective sheath of 0.04 m length optical fiber was removed in the mid region of the selected optical fiber. For this system optical fiber is used as a sensing element.

A container of length 8cm, breadth 1.2 cm is used for the test sample. In the container two holes are made to pass optical fiber through it and milk sample is placed in the container. To make this system a portion of cladding is removed and this uncladed portion is placed in the container.

A photo detector is connected at the other end of the optical fiber. Photo detector output is then applied to a microcontroller and the microcontroller output is applied to a display unit which displays the protein concentration.8051 microcontroller is used for this sensor system. Fig. 2 shows the schematic of the experimental system employed in this study.

For this system five types of milk sample was considered and the total volume in each milk sample was 80 ml. First milk samples contains 80 ml of cow milk (pure cow milk), second milk sample contains 53 ml of cow milk and 27 ml of water, third sample contains 50 ml of cow milk and 50 ml of water fourth

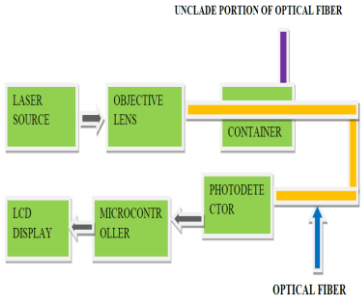

Figure 2:Block Diagram of Optical Sensor System

2.4 Measurement of transmittance and absorbance

sample contains 27 ml of cow milk and 53 ml of water and 25% water and fifth milk sample contains 1 table spoon of milk powder mixed with 80 ml of water are used for protein estimation. For each milk sample we pipette out 0.2 ml of solution to different test tubes and then add distilled water to each sample. During experiments, each sample was separately added to the sensor and the variation in the transmittance of light was recorded at the output terminal.

The Block Diagram that we have developed to measure the intensity of light is shown in the fig. 3.

Figure 3: Setup used to measure the intensity of light

From intensity of light transmittance and absorbance of each sample is determined. Using the following formula, we can measure the transmittance and absorbance of light.

Transmittance $T = I/I_0$

Where, I is the light intensity passing through the sample solution.

I_0 is the intensity of light passing through distilled water.

Absorbance A=-log (T)

Then a standard curve was prepared by considering different concentration of BSA on X axis and absorbance on Y axis. By plotting concentration and absorbance of each BSA sample, a straight line is obtained. Then we plot absorbance of each milk sample on the straight line and from the absorbance we measure its corresponding concentration from the graph. Protein concentration of each milk sample was determined by using the following formula:

Protein Concentration (microgram/ml) = (Absorbance from the graph/volume of milk in the container).

Absorbance for each protein sample was determined by using Beer Lamberts law. Beer Lamberts law is given by:

$$A= €bc$$

Where € is the absorption coefficient

b is the path length of the chamber

c is the concentration of the sample in the solution

Absorption coefficient is determined by using the following equation

$$€= (A/bc)$$

3. Result and Discussion

Milk contains 3.3% total protein. As milk contain all 9 essential amino acids required by humans, so we select milk for protein studies. Constant attention has been made on applying milk sample. 0.2 milliliter of milk sample is required for testing. The performance of the sensor system for the measurement of protein is designed, developed and tested. The milk sample is applied correctly in a reaction zone i.e. uncladed region of the optical fiber. The transmittance measurements for both the sensor system and Folin Lowry method were tested and the results were compared.

Result obtained for Absorbance vs. Concentration plot for both the spectrophotometer and the sensor system is shown below:

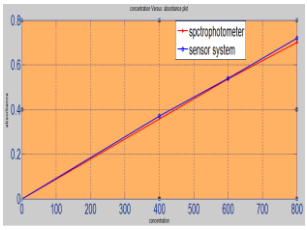

Figure. 4: absorbance vs. concentration curve for both the spectrophotometer and sensor system

From the graph it is clear that for both the designed protein sensor and Lowry method as we increase the amount of milk its absorbance also increases i.e. absorbance is directly proportional to the milk concentration. Also protein concentration is directly proportional to concentration of milk.

Protein concentration value obtained for both the spectrophotometer and the sensor system is shown below:

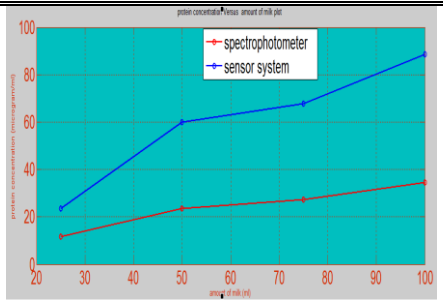

Figure 5:protein concentration vs amount of milk plot for both the sensor system and spectrophotometer

Protein concentration in milk increases as the milk become more pure. For both the sensor system and spectrophometric method protein concentration in milk was determined by interpolating the concentration vs. Absorbance curve.

Absorption coefficient for each sample was determined by using following formula:

$$€= (A/bc)$$

Where € is the absorption coefficient

b is the path length of the chamber

c is the concentration of the sample in the solution

For this sensor system b=1.1 cm

TABLE 1: Absorption coefficient for different milk sample

Milk Sample	***Absorption Coeffecient for optical Sensor System***
100% milk	1.091
75% milk	0.864
50% milk	0.773
25% milk	0.364
1Table spoon of milk powder	0.545

4. Conclusion

A new sensor technique is developed for the rapid and sensitive detection of total protein. The fiber optic protein sensor enables single-step detection of total protein directly without destroying the sample. We can easily determine the protein concentration in milk solution by using Fiber Optic based sensor system. The work reported in this paper is useful as proteins are a major source of energy, as well as it essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health. So it becomes necessary to determine the protein content of each food specimen.The optical measurement method described in this paper offers accurate is very accurate, inexpensive as the cost of each component is very less. Using this project we can optically determine the protein concentration in different milk sample. The same set up can be used to determine protein concentration in different food specimen. So it is different from other optical sensor. This project is the combination of

both optics and microcontroller. Result of the present study show the optical sensor, using optical fiber, microcontroller and a display unit. In this project milk samples such as cow milk and milk powder are diluted to various concentrations.The experimental results shows that concentration of milk increases intensity of light also increases and so the protein concentration also increases .Thus the protein concentration can be determined by considering intensity of light and milk concentration in the chamber, and after the sensor sense the protein concentration, a LCD will display the protein concentration which is connected with a microcontroller.

References

[22] K.E. Rani, Ganesh. Shanbhag and Dr.T.Srinivas., "Novel Optical Biosensor", ICOP 2009,International conference on Optics and Photonics A.

[23] P.V. Preejith, C.S. Lim, A.Kishen, M.S. John & A. Asundi, "Total protein measurement using a fiber-optic evanescent wave-based Biosensor", Biotechnology Letters 25: 105–110, 2003.

[24] Erik P.Schartner, Yinlam Ruan, Peter Hoffman,Tanya M.Monro, "An Optical Protein sensor", COIN ACOFT 2007.

[25] A Ksendzov, Y Lin, "Integrated Optics Ring Reasonator Sensor for Protein detection", OPTICS LETTER Vol. 30, No.24.

[26] Ya Hasin Chang,Tsung China Chang,E Fog Kao and Chien Chou , "Detection of Protein A by Straphylococcus Aureaus with a Fiber Optic Based Biosensor", Biosci,Biotech,Biochem, 60 (10),1571-1574,1996.

[27] Gerard L cote,Martin D fox and Robert B.Northrop, "Optical Polarimetric Sensor for Blood Glucose Measurment", 1990 IEEE .

[28] Beate Strehlitz, Nadia Nikolus and Regina Soltenburg, "Protein Detection with Aptamer Biosensor", Sensor 2008, 4296-4307.

[29] Adonis A. Yanos, Marianne N. Bautista, Mark Rickard N. Angelia and Ernesto J. del Rosario, "Digital Photometric Determination of Protein Using Biuret, Bradford and Bicinchoninic Acid Reagent",Philippine Science Letters, Vol 6, No.2, 2013.

[30] Sudad S. Ahmed, Kais A. Al-Naimee, , Afnan H. Hayat, "Optical fiber Hemoglobin concentration sensorbased laser polarization", International Journal of Engineering Research and Applications (IJERA) ISSN: 2248-9622, Vol. 2, Issue 5, September- October 2012, pp.1440-1444.

[31] Neide K.K. Kamizake, Mauricio M. Goncalves, C!assia T.B.V. Zaia,A. M. Zaia, "Determination of total proteins in cow milk powder samples: a comparative study between the Kjeldahl method and spectrophotometric methods", Journal of Food Composition and Analysis 16 (2003) 507–516.

[32] Franz-Xaver Schmid, "Biological Macromolecules:UV-visible Spectrophotometry".

[33] Fidanboylu, K, Efendioglu, H. S. "Fiber Optic Sensors and Their Applications", 5th International Advanced Technologies Symposium (IATS'09), May 13-15, 2009, Karabuk, Turkey.

[34] L. Thylén, and M. Gilbertsson, T. Rosenthal, and S. Wrenn, "An On-line Protein Sensor – from Research to Product.

[35] Angela Leung a, P. Mohana Shankar b, Raj Mutharasan, "A review of fiber-optic biosensors", Sensors and Actuators B 125 (2007) 688–703.

[36] Graham H. Cross , Andrew A. Reeves a, Stuart Brand , Jonathan F. Popplewell , Louise L. Peel, Marcus J. Swann b, Neville J. Freeman, "A new quantitative optical biosensor for protein characterization", Biosensors and Bioelectronics 19 (2003) 383_/390.

[37] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, "Protein measurement with the folin phenol reagent", J. Biol. Chem.**193**: 265–275.

[38] Sapan CV, Lundblad RL, Price NC , "Colorimetric protein assay techniques",Biotechnol. Appl. Biochem. 29: 99–108.

[39] P Suresh Kumar, C P G Vallabhan1, V P N Nampoori, V N Sivasankara Pillai2 and P Radhakrishnan, "A fibre optic evanescent wave sensor used for the detection of trace nitrites in water", J. Opt. A: Pure Appl. Opt. 4 (2002) 247–250.

[40] Guptha B D and Khijwania S K, "Experimental studies on the response of the fibre optic evanescent field absorption sensor", Fibre Integr. Opt. 17 63–73.

[41] P Neelamegam, A Rajendran, C Maruthanayagam and M Mohanraja, "Study the Protein variation by Monocrotophos in Cyprinus carpio using PIC16F877 microcontroller", Journal of Science and Indystrial research,Vol.65,August 2006,pp.655-658.

Authors Profile

MissDharitri Devi has received her M. Tech degree in Optoelectronics and Optical Communication from Assam Don Bosco University (India) in 2015. Completed B.Tech in Electronics and Communication Engineering from School of Technology (Assam Don Bosco University), Guwahati, India in 2012. Her area of interest includes Optical Electronics, Optical Communication and Biosensor.

Miss. Jhimli Kumari Das is currently working as an Assistant Professor in the Department of Electronics and Communication Engineering, Assam Don Bosco University. She has obtained her M. Tech. Degree in Bioelectronics from Tezpur (Central) University, Assam, India.She is now persuing her PhD from Assam Don Bosco University, Assam, India. Her research interests include Optoelectronics and Biosensor. Some of her papers have already been published in national and international conferences.