ROHINI
COLLEGE OF ENGINEERING AND TECHNOLOGY
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DEPARTMENT OF BIOMEDICAL ENGINEERING

BM3491 Biomedical Instrumentation

UNIT- V BIOCHEMICAL MEASUREMENTS

5.3 Blood Glucose Sensors

Accurate measurement of blood glucose is essential in the diagnosis and long-term management of diabetes. This section reviews the use of biosensors for continuous measurement of glucose levels in blood and other body fluids.

Glucose is the main circulating carbohydrate in the body. In normal, fasting individuals, the concentration of glucose in blood is very tightly regulated—usually between 80 and 90 mg/100 ml, during the first hour or so following a meal. The hormone insulin, which is normally produced by beta cells in the pancreas, promotes glucose transport into skeletal muscle and adipose tissue. In those suffering from diabetes mellitus, insulin-regulated uptake is compromised, and blood glucose can reach concentrations ranging from 300 to 700 mg/100 ml (hyperglycemia).

Accurate determination of glucose levels in body fluids, such as blood, urine, and cerebrospinal fluid, is a major aid in diagnosing diabetes and improving the treatment of this disease. Blood glucose levels rise and fall several times a day, so it is difficult to maintain normoglycemia by means of an "open-loop" insulin delivery approach. One solution to this problem would be to "close the loop" by using a self-adapting insulin infusion device with a glucose-controlled biosensor that could continuously sense the need for insulin and dispense it at the correct rate and time. Unfortunately, present-day glucose sensors cannot meet this stringent requirement (Peura and Mendelson, 1984).

Glucose Oxidase Method The glucose oxidase method used in a large number of commercially available simple test strip meters allows quick and easy blood glucose measurements. A test strip product, One Touch UltraMini (www.LifeScan.com), depends on the glucose oxidase–peroxidase chromogenic reaction. After a drop of blood is combined with reagents on the test strip, the reaction shown in (10.18) occurs.

$$\text{Glucose} + 2H_2O + O_2 \xrightarrow{\text{glucose oxidase}} \text{Gluconic Acid} + 2H_2O_2 \qquad (10.18)$$

Adding the enzymes peroxidase and o-dianiside, a chromogenic oxygen, results in the formation of a colored compound that can be evaluated visually.

$$\text{o-dianisine} + H_2O_2 \xrightarrow{\text{peroxidase}} \text{oxidized o-dianisine} + H_2O \qquad (10.19)$$

Glucose oxidase chemistry in conjunction with reflectance photometry produces a system for monitoring blood glucose levels (Burtis and Ashwood, 1994). In the One Touch system (Figure 10.23), a test strip is inserted into the meter, a drop of blood is applied to end of the test strip, and a digital screen displays the results 5 s later.

Electroenzymatic Approach Electroenzymatic sensors based on polarographic principles utilize the phenomenon of glucose oxidation with a glucose oxidase enzyme (Clark and Lyons, 1962). The chemical reaction of glucose with oxygen is catalyzed in the presence of glucose oxidase. This causes a decrease in the partial pressure of oxygen (P_{O_2}), an increase in pH, and the production of hydrogen peroxide by the oxidation of glucose to gluconic acid according to equation (10.18).

Investigators measure changes in all of these chemical components in order to determine the concentration of glucose. The basic glucose enzyme electrode utilizes a glucose oxidase enzyme immobilized on a membrane or a gel matrix, and an oxygen-sensitive polarographic electrode. Changes in oxygen concentration at the electrode, which are due to the catalytic reaction of glucose and oxygen, can be measured either amperometrically or potentiometrically.

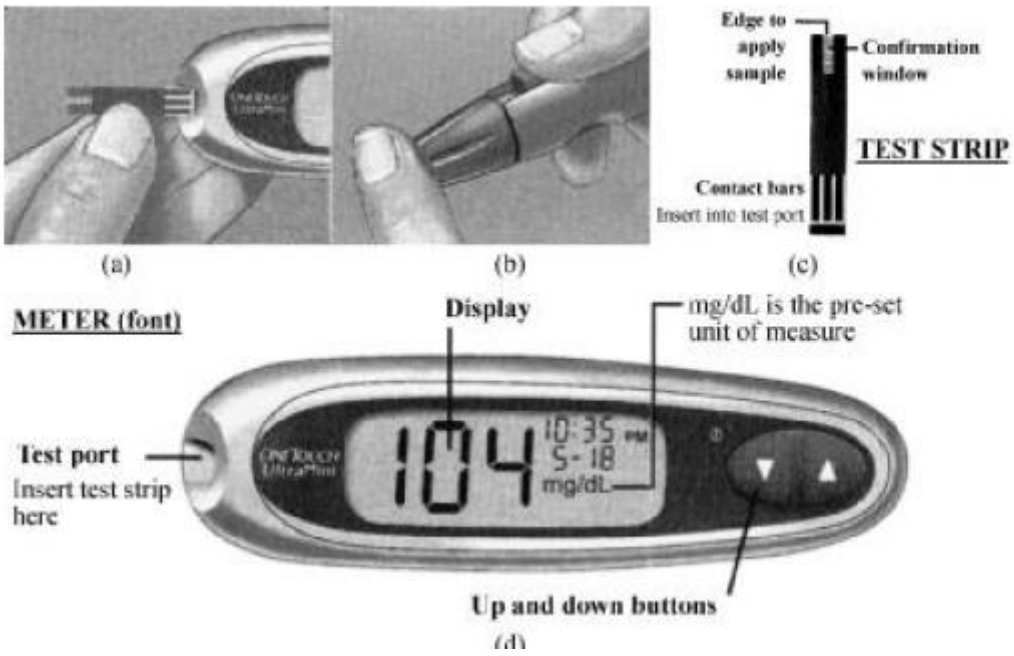
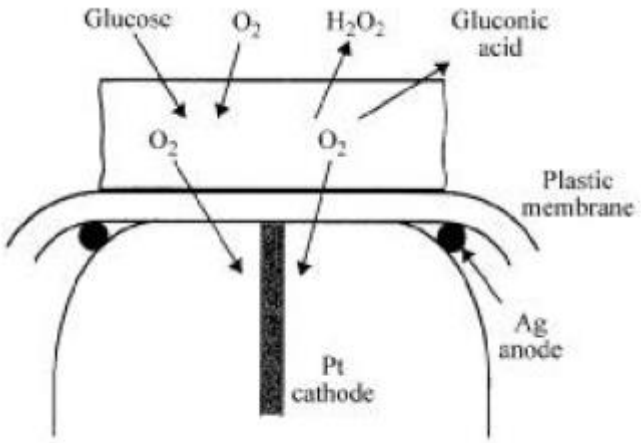


Figure 10.23 (a) A test strip is inserted into the meter. (b) A lance is released to lance the skin less than 1 mm. (c) The 1 μL blood sample is applied to the end of the test strip and drawn into it by capillary action. (d) Then 5 s later, the meter displays the blood glucose in mg/dL.

Because a single-electrode technique is sensitive both to glucose and to the amount of oxygen present in the solution, a modification to remove the oxygen response by using two polarographic oxygen electrodes has been suggested (Updike and Hicks, 1967). Figure 10.24 illustrates both the principle of the enzyme electrode and the dual-cathode enzyme electrode. An active enzyme is placed over the glucose electrode, which senses glucose and oxygen. The other electrode senses only oxygen. The amount of glucose is determined as a function of the difference between the readings of these two electrodes. More recently, development of hydrophobic membranes that are more permeable to oxygen than to glucose has been described (Gilligan *et al.*, 2004). Placing these membranes over a glucose enzyme electrode solves the problem associated with oxygen limitation and increases the linear response of the sensor to glucose.

The major problem with enzymatic glucose sensors is the instability of the immobilized enzyme and the fouling of the membrane surface under physiological conditions. Most glucose sensors operate effectively only for short periods of time. In order to improve the present sensor technologies, more highly selective membranes must be developed. The features that must be taken into account in designing and fabricating these membranes include the diffusion rate of both oxygen and glucose from the external medium to the surface of the membrane, diffusion and concentration gradients within the membrane, immobilization of the enzyme, and the stability of the enzymatic reaction (Jaffari and Turner, 1995).



(a)

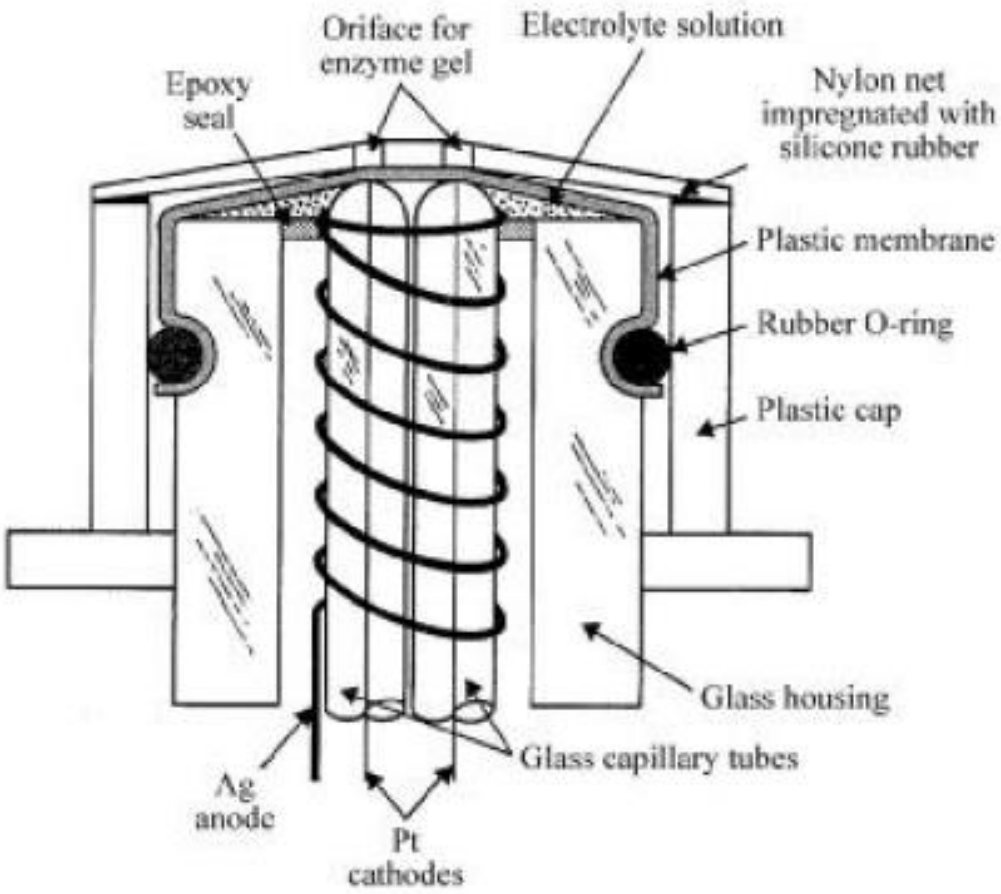


Figure 10.24 (a) In the enzyme electrode, when glucose is present it combines with O_2, so less O_2 arrives at the cathode. (b) In the dual-cathode enzyme electrode, one electrode senses only O_2 and the difference signal measures glucose independent of O_2 fluctuations. (From S. J. Updike and G. P. Hicks, "The enzyme electrode, a miniature chemical transducer using immobilized enzyme activity," *Nature*, 1967, 214, 986–988. Used by permission.)

Optical Approach A number of innovative glucose sensors, based on different optical techniques, has been developed in recent years. A new fluorescence-based affinity sensor has been designed for monitoring various metabolites, especially glucose in the blood plasma (Schultz *et al.*, 1982). The method is similar in principle to that used in radioimmunoassays. It is based on the immobilized competitive binding of a particular metabolite and fluorescein-labeled indicator with receptor sites specific for the measured metabolite and the labeled ligand (the molecule that binds).

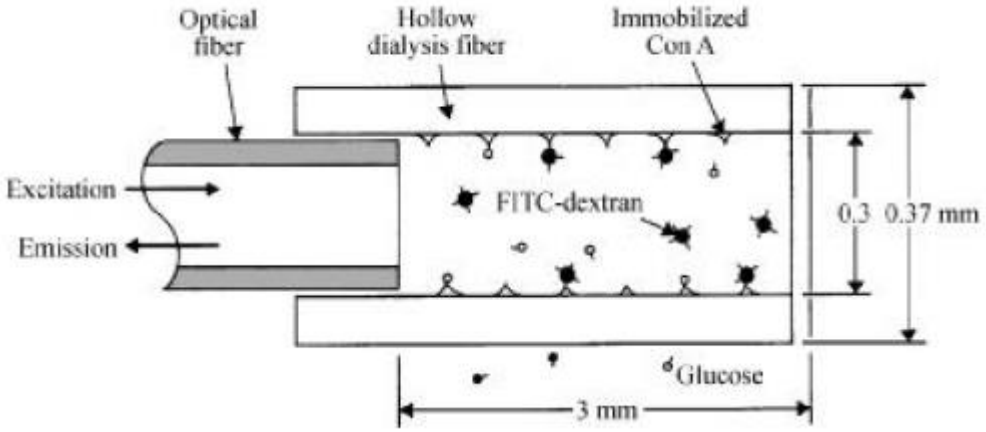


Figure 10.25 The affinity sensor measures glucose concentration by detecting changes in fluorescent light intensity caused by competitive binding of a fluorescein-labeled indicator. (From J. S. Schultz, S. Manouri, *et al.*, "Affinity sensor: A new technique for developing implantable sensors for glucose and other metabolites,"*Diabetes Care*, 1982 5, 245–253. Used by permission.)

Figure 10.25 shows an affinity sensor in which the immobilized reagent is coated on the inner wall of a glucose-permeable hollow fiber fastened to the end of an optical fiber. The fiber-optic catheter is used to detect changes in fluorescent light intensity, which is related to the concentration of glucose. These researches have demonstrated the simplicity of the sensor and the feasibility of its miniaturization, which could lead to an implantable glucose sensor. Figure 10.26 is a schematic diagram of the optical system for the affinity sensor. The advantage of this approach is that it has the potential for miniaturization and for implantation through a needle. In addition, as with other fiber-optic approaches, no electric connections to the body are necessary.

The major problems with this approach are the lack of long-term stability of the reagent, the slow response time of the sensor, and the dependence of the measured light intensity on the amount of reagent, which is usually very small and may change over time.

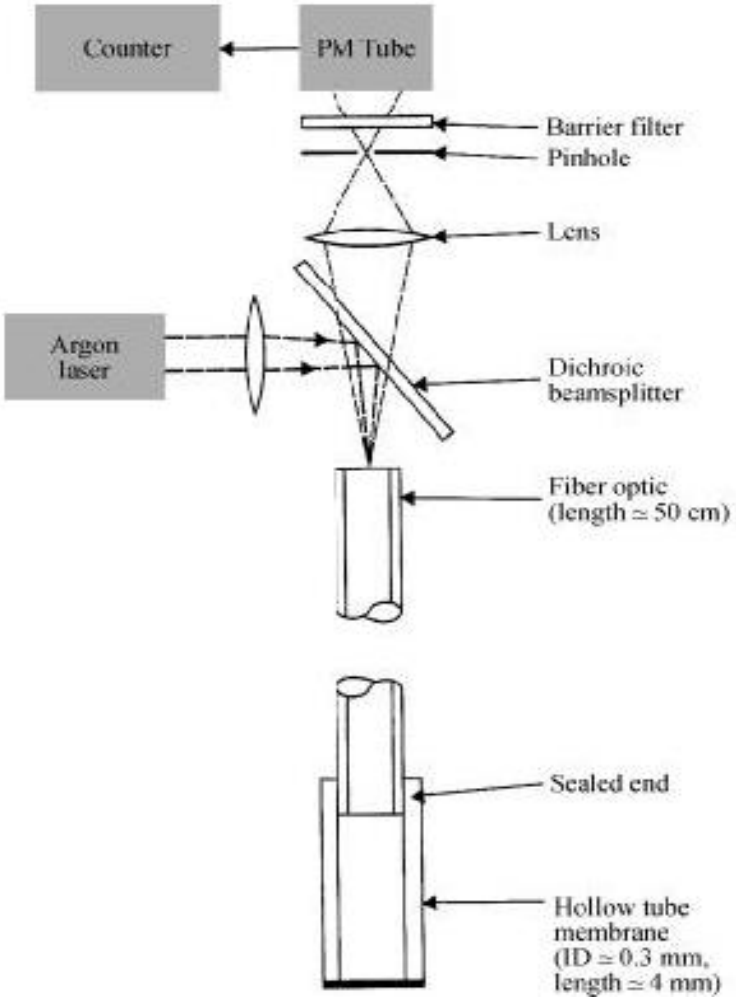


Figure 10.26 The optical system for a glucose affinity sensor uses an argon leaser and a fiber-optic catheter. (From J. S. Schulz, S. Manouri, *et al.*, "Affinity sensor: A new technique for developing implantable sensors for glucose and other metabolites," *Diabetes Care*, 1982, 5, 245–253. Used by permission.)

Attenuated Total Reflection (ATR) and Infrared Absorption Spectroscopy

The application of multiple infrared ATR spectroscopy to biological media is another potentially attractive noninvasive technique. By this means, the infrared spectra of blood can be recorded from tissue independently of the sample thickness, whereas other optical-transmission techniques are strongly dependent on the optical-transmission properties of the medium. Furthermore, employing a laser light source makes possible considerable improvement of the measuring sensitivity. This is of particular interest when one is measuring the transmission of light in aqueous solutions, because it counteracts the intrinsic attenuation of water, which is high in most wavelength ranges.

Absorption spectroscopy in the infrared (IR) region is an important technique for the identification of unknown biological substances in aqueous solutions. Because of vibrational and rotational oscillations of the molecule, each molecule has specific resonance absorption peaks, which are known as *fingerprints*. These spectra are not uniquely identified; rather, the IR absorption peaks of biological molecules often overlap. An example of such a spectrum is shown in Figure 10.27, which is the characteristic IR spectrum of anhydrous D-glucose in the wavelength region 2.5 to 10 μm. The strongest absorption peak, around 9.7 μm, is due to the carbon–oxygen–carbon bond in the molecule's pyran ring.

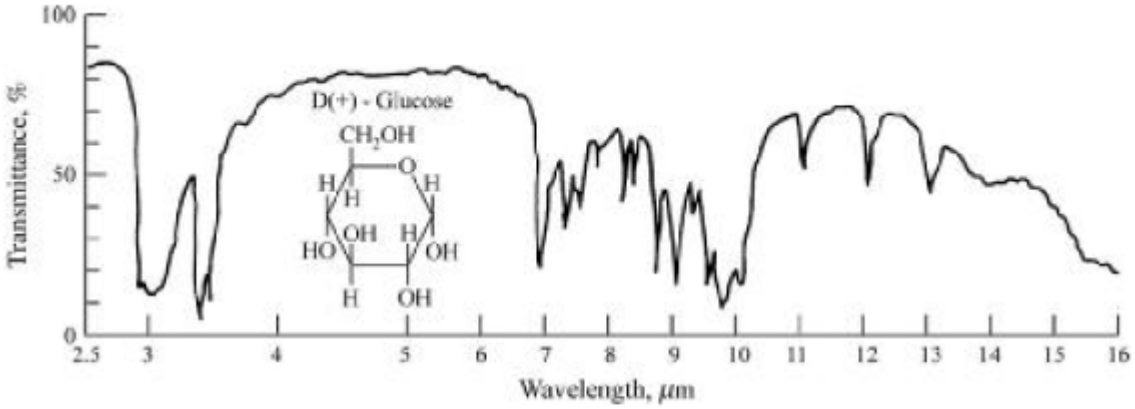


Figure 10.27 The infrared absorption spectrum of anhydrous D-glucose has a strong absorption peak at 9.7 mm. (From Y. M. Mendelson, A. C. Clermont, R. A. Peura, and B. C. Lin, "Blood glucose measurement by multiple attenuated total reflection and infrared absorption spectroscopy," *IEEE Trans. Biomed Eng.*, 1990, 37, 458–465. Used by permission.)

The absorption-peak magnitude is directly related to the glucose concentration in the sample, and its spectral position is within the wavelength range emitted by a CO_2 laser. Thus a CO_2 laser can be used as a source of energy to excite this bond, and the IR absorption intensity at this peak provides, via Beer's law, a quantitative measure of the glucose concentration in a sample.

Two major practical challenges must be overcome in order to measure the concentration of glucose in an aqueous solution, such as blood, by means of conventional IR absorption spectroscopy. (1) Pure water has an intrinsic high background absorption in the IR region, and (2) the normal concentration of glucose and other analytes in human blood is relatively low (for glucose, it is typically 90 to 120 mg/dl, or mg%).

Significant improvements in measuring physiological concentrations of glucose and other blood analytes by conventional IR spectrometers have resulted from the use of high-power sources of light energy at specific active wavelengths. In the case of glucose, the CO_2 laser serves as an appropriate IR source.

Reference : John G. Webster, "Medical Instrumentation Application and Design", 4th edition, Wiley India Pvt Ltd, New Delhi, 2015