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Enzyme-based glucose biosensor using a dye couple system

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Abstract

We have developed a new type of enzyme-based glucose biosensor based on a dye couple system. A concentration dependence of glucose on a test strip containing physically adsorbed glucose oxidase, peroxidase and dye couple have been optically measured. The application of this method permits the exploitation of the biosensor during a period of more than 300 days. © 2001 Published by Elsevier Science Ltd.

Keywords: Glucose biosensor; Dye couple; Sensing strip; Glucose oxidase; Peroxidase

1. Introduction

Within the last decade, clinical practitioners have gradually moved from the diagnosis of established diseases towards symptomatic prognosis as preventive measures. Continuous tests for electrolytes, total protein, urea, glucose, creatinine, cholesterol, triglycerides, and others in blood or urine are subject of intense research and development to produce devices capable of automatically recording results by using computerized data logging and output systems.

Saccharides are nature's conveyors of energy and, therefore, essential for cell survival [1]. The breakdown of glucose transport has been correlated with certain diseases: renal glycosuria [2,3], cystic fibrosis [4], diabetes [5,6], and also human cancer [7].

The study of glucose and other monosaccharide gradients in vivo is, therefore, of seminal importance. It is important for the diabetic patient to maintain normal or near normal blood glucose levels throughout the day. The blood glucose level in a typical person after an overnight fast is 80 mg/100 mg (4.4 mM). The blood glucose level during the day normally ranges from about 80 mg/100 mg before meals to about 120 mg/100 mg after meals [8]. These levels can be obtained through appropriate diets, insulin injections and exercise patterns. However, in order to avoid over or under compensation, it is desirable for the diabetic patient to know his blood glucose level in order to take appropriate compensatory action.

The quantification of chemical and biochemical components in coloured aqueous fluids, in particular coloured biological fluids such as whole blood and urine and biological fluid derivatives such as serum and plasma, is of ever-increasing importance.

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Important applications exist in medical diagnosis and treatment and in the quantification of exposure to therapeutic drugs, intoxicants, hazardous chemicals and the like.

Currently there are available sophisticated biochemical systems which can be incorporated into dry, dip-and-read reagent strip devices, used in solution or suspension techniques, or in conjunction with spectrophotometric and other read-out systems. The detection procedures have already been considerably simplified by the provision of test strips. These strips comprise a plastic strip, having at one end a carrier portion impregnated with an enzymatic testing composition which includes the enzymes glucose oxidase (GOD) and peroxidase (POD) and an indicator compound as the principal active ingredient. Various indicators can be used including benzidine-type chromogens, e.g. benzidine, *o*-tolidine and tetramethyl-benzidine and substituted aniline chromophores. The indicator may be an oxidizable dye.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \underset{\text{(colorless)}}{\text{reduced dye}} \xrightarrow{\text{POD}} 2H_2O_2 + \underset{\text{(colored)}}{\text{oxidised dye}}$$

Unfortunately the diagnostic agents of this type leave a lot to be desired with regard to their actual use, as well as with regard to their reliability. Here we report on the improved rapid and simple methodology for the determination of analytes such as glucose, which results in the production of hydrogen peroxide as an enzyme product. Also contained in the matrix will be a second enzyme, a peroxidase, and a dye couple system which produces a light-absorbing product in conjunction with the peroxidase.

2. Experimental

2.1. Preparation of glucose sensing strip

2.1.1. Dye immobilization

- 3-Methyl-2-benzothiazolinone hydrazone hydrochloride/35 mg
- Diethanolamine/78 mg
- Acetonitrile/8 mg
- Water/8 ml

Stir until all reagents are dissolved and pour onto a glass plate. Dip a piece of Posidyne membrane (Pall Co. Supor 450, P/N 60173, Hydrophilic polyether sulfone, pore size/0.45 μm) for 20 s and then dry at 75°C for 3 min.

2.1.2. Enzyme immobilization

- Ethylenediamine tetracetic acid disodium salt dihydrate (EDTA)/35 mg
- Polypeptide (low viscosity)/2 g
- Sodium citrate/2.15 g
- Citric acid/3.25 g
- Glucose oxidase (162,000 units/g)/0.09 g
- Peroxidase (horseradish, 1310 units/g)/0.006 g
- Water/45 ml

Stir until all solids are dissolved and add 0.15 g of poly(acrylic acid sodium salt) ($M_w \sim 1200$) and pour onto a glass plate. Dip a piece of membrane previously impregnated with dye couple and dry at 75°C for 3 min.

2.1.3. Glucose sample

2.5, 5, 10, 15 and 20 mmol glucose were prepared with 0.2 M phosphate buffer adjusted to pH 7.

2.2. Optical system

The diffuse reflecting optical apparatus can be made to automatically read reflectance, calculate the reflectance change and output the glucose concentration in the sample solution. The optical apparatus consists of a light source, photodiode, prism and signal processing part. The light source is a red LED (655 nm peak). The light beam passing through the glass prism projects a glucose strip. A substantial portion of this light is diffusively reflected from the test strip and is detected by photodiode as a light detector that produces an output current proportional to the light it receives (Fig. 1).

3. Results and discussion

Thses test strips are based on a GOD–POD-dye coupled system. When a drop of blood is applied,

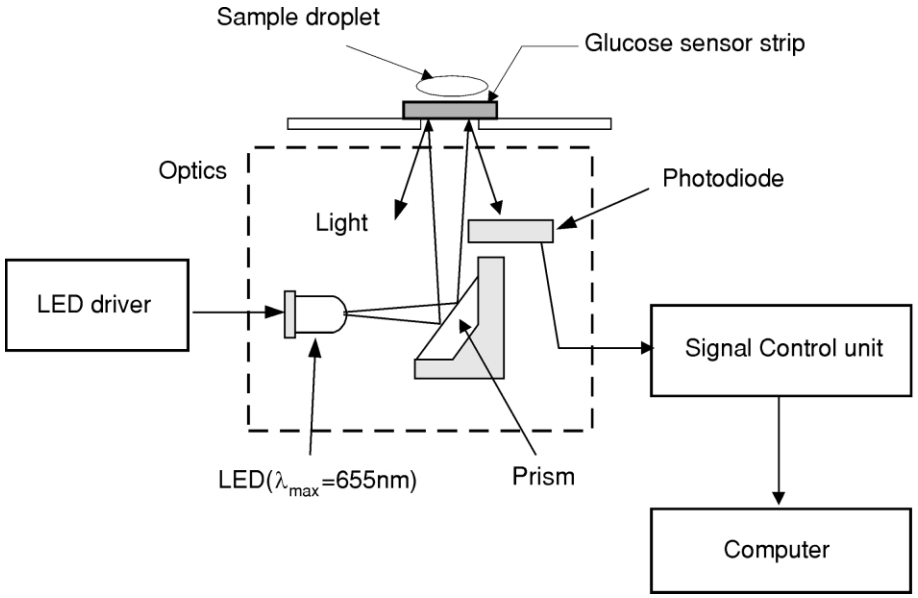


Fig. 1. Schematics design of the diffuse reflection.

glucose oxidase catalyzes the oxidation of glucose in the blood by oxygen in the atmosphere and oxygen in the blood. Glucose is oxidized to gluconic acid with the concomitant formation of hydrogen peroxide. The peroxidase then triggers the reaction of the hydrogen peroxide with 3-methyl-2-benzothazolinone hydrazone (MBT) and diethanol amine (DEA). The reaction sequence is:

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{MBT} + \text{DEA} \xrightarrow{\text{POD}} \text{MBT}-\text{DEA}$$

(colored)

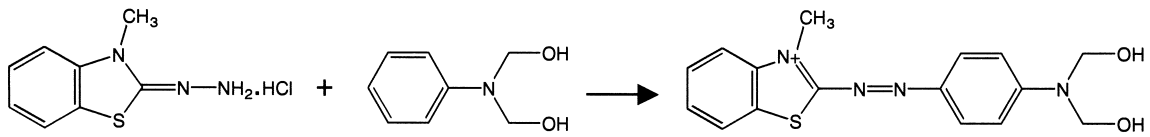
As the glucose sample is applied to the glucose sensing strip, the enzymatic reaction proceeds and the absorption peak at 515, 573 and 636 nm is apparent and the color is blue. The absorbances increase significantly, depending on the increment of the glucose concentration. The glucose test strip is based on the reaction of MBT and DEA and formation of the benzothiazolium dye. The reaction sequence is shown below.

The MBT–DEA dye couple, although previously described MBT is a suitable reagent for color development for peroxidase labels in enzyme immunoassays, has never been used in a glucose measuring reagent. This dye couple system shows improved enzymatic stability as compared to traditional dyes used for glucose measurement, such as benzidine derivatives.

Comparison of the absorption spectra in buffer solution and the reflection spectra in the test strip of benzothazolium dye are illustrated in Fig. 2.

The dye couple exhibits an absorption peak at around 500–700 nm. However, a very broad reflection spectrum from 450 to 700 nm was observed for the test strip.

Generally, the biochemical information delivered by the optical sensors can be divided into two



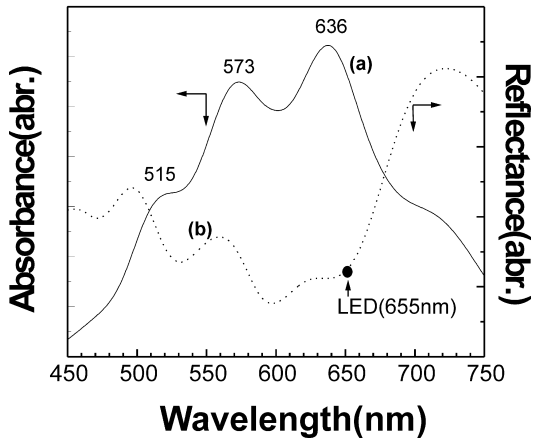


Fig. 2. Absorption spectra of a dye couple in glucose solution (solid) (a) and reflection spectra in test strip (dashed) (b).

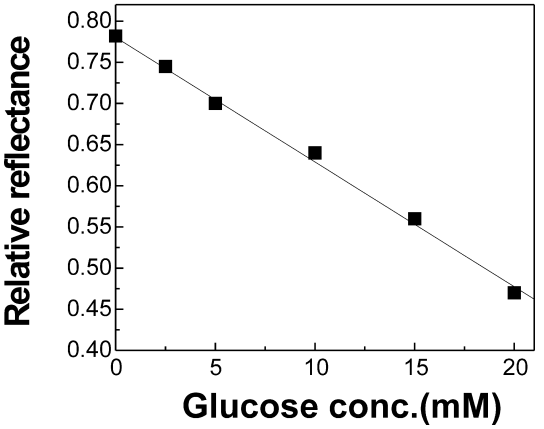


Fig. 3. Effect of glucose concentration on the reflectance of a dye couple in a test strip.

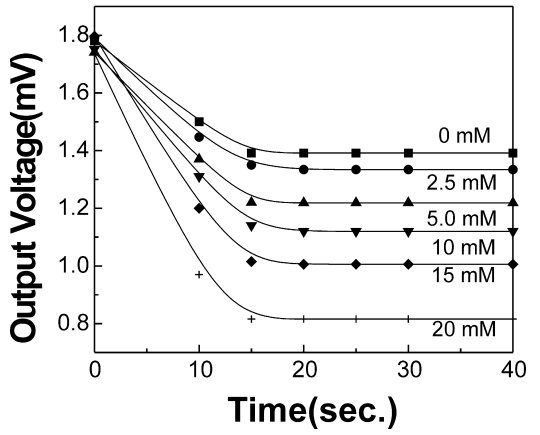


Fig. 4. Glucose sensor response time as a function of the glucose concentration.

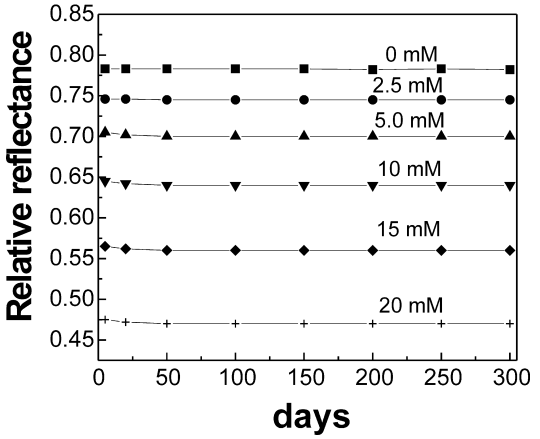


Fig. 5. Long-term stability of the glucose biosensor on various glucose concentration.

categories; in the first category, information is obtained from the direct spectroscopic study of the biological medium, for example, the determination of the oxygen saturation of haemoglobin, myoglobin and cytochrome by direct spectrometry of the blood or tissues.

In the second category, the spectroscopic study of a sensitive reagent, in contact with the biological medium, yields specific biochemical information.

Spectrophotometic analysis is a well-established method as a sensor in the analytical laboratory and based on the radiation/matter interaction such as absorption, reflection and diffraction etc. The spectral signals that are measured in spectrophotometric methods arise from a variety of different physical and chemical phenomena. Despite the recent development of many new spectroscopic, electrochemical and chromatographic analysis techniques, the spectrophotometric method based on the absorption/reflection of UV-visible radiation probably is used more often, and for a larger variety of substances, than any other method. Colour-forming chemistries are most conveniently measured by reflectance spectrometry.

R_t is the reflectivity taken at a particular endpoint time, t seconds, and is the absorbed fraction of the incident light beam described by Eq. (1), where R_{test} is the end point reflectance.

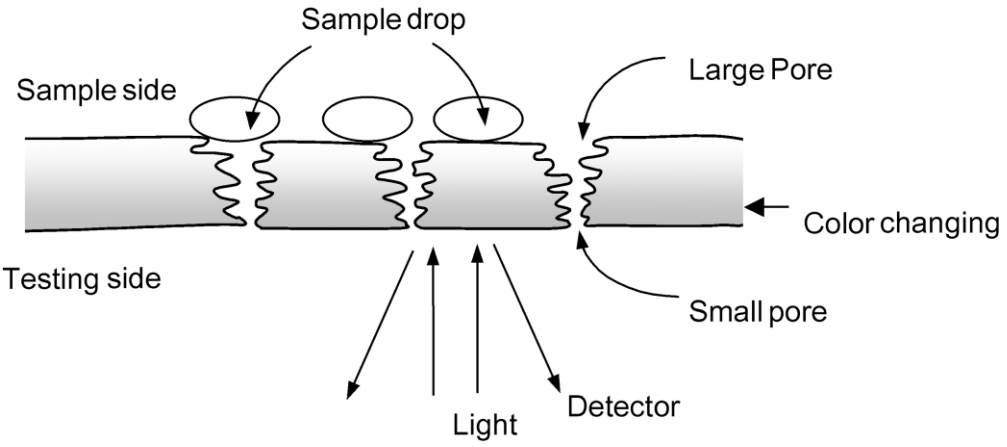


Fig. 6. Configuration of an anisotropic membrane.

Upon formation of the colored reaction product, the amount of diffused light reflected from the glucose sensing strip decreases according to the equation

$$R_t = (R_{\text{test}} - R_{\text{dark}})/(R_0 - R_{\text{dark}}) \quad (1)$$

where R_{test} is the reflected light from the sample, R_0 the reflected light from the unreacted test strip, R_{dark} the back ground reflectance. A suitably stable end point is reached in 15 s, and final reflectance, R_{15}, is taken. The matrix contains an oxidase enzyme which produces hydrogen peroxide. Also contained in the matrix will be a second peroxidase, and a dye couple system which produces a vis-absorbing product in conjunction with the peroxidase. The colored product changes the reflectance signal of the matrix system. Fig. 3 shows that a linear relationship is observed between reflectivity at 655 nm and glucose concentration in the range of 0, 2.5, 5, 10, 15 and 20 mM. As the glucose concentration increases, the relative reflectance decreases. The 655 nm reflectance reading for the automatic optical system is typically taken at 15 s (R_{15}). By this time the glucose will have completely saturated the sensing strip (Fig. 4). This enzymatic stability also makes the MBT–DEA dye couple desirable in order to ensure longer shelf life of the glucose test strips. Fig. 5 shows the functional stability of the glucose test strip. As one can see, the strip by the new method is very stable. It can be stored up to 300 days under normal conditions without loss of activity.

Fig. 6 is a perspective view of a glucose sensing strip. The key element of such a strip is a porous membrane that incorporates a testing reagent. The porous membrane undergoes a colour change in response to glucose that is applied to the strip. The membrane has relatively large pores in the sample side and a relatively smaller size in testing side. The membrane accepts the sample on the sample side and passes it towards the testing side.

4. Conclusion

This study demonstrates the possibility of glucose determination using a dye couple system. The strip utilizes an enzyme system wherein the glucose is a substrate for GOD. Glucose is oxidized to gluconic acid with the formation of hydrogen peroxide. The MBT–DEA dye couple present undergoes colour changes in the presence of hydrogen peroxide and peroxidase. The linear relationship was obtained between reflectivity at 655 nm and glucose concentration. As the glucose concentration increases, the relative reflectance decreases. The study can be performed utilizing simple instrumentation.

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