

## Micro-Sized glucose biosensor based on composite pH sensor Mikro boyutta pH temelli potansiyometrik glikoz biyosensör

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### Abstract

Today's scientific and technical advances have led to significant advances in biosensor technology, leading to widespread use of biosensors in areas such as food technology, the environment, pharmaceuticals and clinical diagnostics, biochemistry, and analytical chemistry. Improvements in the ion-selective sensor technology used in the preparation of potentiometric biosensors and the increase in the number of sensor materials have also accelerated the development of new types of potentiometric biosensors. Thus, in this study, information is given on the development of a new micro sized potentiometric glucose biosensor based on composite pH sensor. Glucose oxidase was immobilized on the composite layer as the biorecognition element. The surface of glucose biosensor based composite pH sensing matrices was first examined for electrochemical elucidation by using cyclic voltammetry and electrochemical impedance spectroscopy. The rate of electron transfer resulted in a stable response behavior of the biosensor and made it a suitable potentiometric sensor for detection of glucose. A linear response in concentration range from  $5 \times 10^{-5}$  to  $1 \times 10^{-1}$  mol/L was obtained with a detection limit of  $2 \times 10^{-5}$  mol/L for the potentiometric detection of glucose. The biosensor exhibited a fast response time (10 s), had good stability, and an extended lifetime.

**Keywords:** Solid-state composite sensor, Miniaturization, Diabet follow-up, Glucose biosensor

### Öz

Günümüzdeki bilimsel ve teknik ilerlemeler, biyosensör teknolojisinde önemli gelişmelere yol açarak biyosensörlerin gıda teknolojileri, çevre, farmasi ve klinik teşhis, biyokimya, analitik kimya gibi alanlarda yaygın olarak kullanılmasına neden olmuştur. Potansiyometrik biyosensörlerin hazırlanmasında kullanılan iyon-seçici sensör teknolojisindeki gelişmeler ve sensör materyallerin sayılarının artması, yeni tip potansiyometrik biyosensörlerin geliştirilmesini de hızlandırmıştır. Nitekim, bu çalışmada, kompozit pH sensörüne dayalı yeni bir mikro boyutlu potansiyometrik glikoz biyosensörünün geliştirilmesi ele alınmıştır. Glikoz oksidaz, biyotanıma elemanı olarak kompozit tabaka üzerinde immobilize edildi. Kompozit pH algılama esaslı glikoz biyosensör matrisinin yüzeyi ilk olarak elektrokimyasal tanımlama için dönüşümlü voltametri ve elektrokimyasal impedans spektroskopisi kullanılarak incelendi. Yüksek elektron transfer hızı, biyosensörün kararlı bir tepki davranışına sahip olduğunu ve glikozun saptanması için uygun bir potansiyometrik sensör olduğunu göstermektedir. Glikozun potansiyometrik tespiti için  $2 \times 10^{-5}$  mol/L saptama sınırı ile  $5 \times 10^{-5}$  ila  $1 \times 10^{-1}$  mol/L konsantrasyon aralığında lineer bir cevap elde edildi. Biyosensör hızlı bir tepki süresi (10 s) gösterdi, iyi bir kararlılığa ve uzun bir ömre sahipti.

**Anahtar kelimeler:** Katı-hal kompozit pH sensör, Minyatürizasyon, Diyabet takibi, Glikoz biyosensör

## 1 Introduction

In general, biosensors are defined as analytical/bioanalytical devices which are developed by combining the selectivity characteristics of chemical and biological molecules and the process capability of modern electronic techniques by making use of a great number of scientific fields such as chemistry, biochemistry, biology and engineering. Electrochemical biosensor systems which are based on specific enzyme-substrate reactions have important features such as being miniaturized, economical, portable and showing high selectivity and sensitivity towards special types. Since the immobilization of biological molecules and the design of transducer sensor membrane interface technology have a significant effect on biosensor performance (linear operating range, selectivity, response time, sensitivity, economic life, stability and sensitivity against interfering agents), they play a key role in electrochemical biosensor systems [1]. In addition, they are important for biosensor production which has a close micro environment not influenced by external environment conditions. Thus, researches in biosensor systems have focused on new immobilized enzyme systems and designs for an effective detection [2],[3]. The aim of this study is to develop a

new type glucose sensitive potentiometric biosensor by using micro size composite pH sensitive sensor.

### 1.1 Potentiometric sensor systems

In potentiometric sensory systems, the potential difference between the reference electrode and the indicator electrode, which can be considered small, is measured without giving any current to the cell. While reference electrode shows fixed potential, indicator electrode shows a potential which changes according to the activity of the ion it is sensitive to. Potential difference is within logarithmic association of the concentration (eq. 1).

$$E = E^0 + RTZ_i F \ln a_i / a_\beta \quad (1)$$

$E$  : Standard electrode potential,

$a_i$  and  $a_\beta$  : ionic activity,

$R$  : Gas constant,

$T$  : Absolute temperature,

$F$  : Faraday constant,

$Z$  : Ionic charge.

Ion selective electrode, which selectively measures ionic types, is a potentiometric sensor. Potentiometric sensor generally includes a membrane with a special composition which is solid (like glass) or polymer. Membrane composition in the membrane-solution interface determines membrane's selectivity towards ion.

Figure 1a shows the pattern of a classical ion selective electrode. Such an electrode includes internal reference electrode and internal reference solution. Figure 1b shows composite pH sensor which does not include internal reference electrode and internal reference solution and which allows for preparation in desired size and type. Figure 2 shows a comparative image of composite pH sensors versus glass pH electrode.

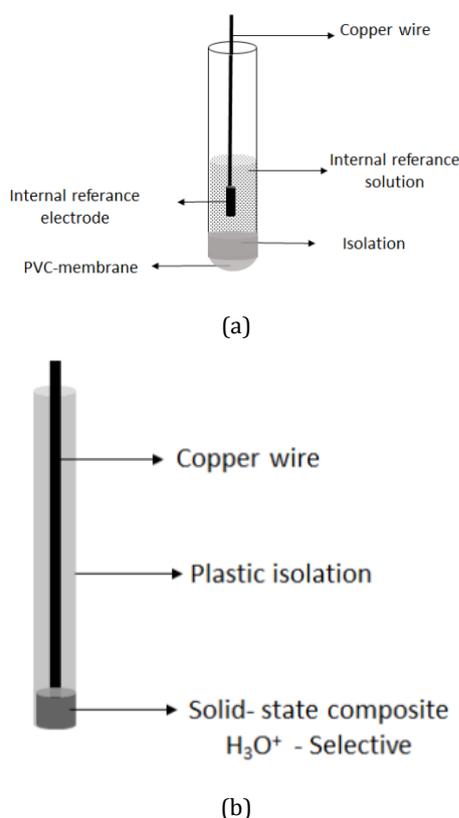


Figure 1(a): Classical ion selective electrode. (b): Composite pH sensor.

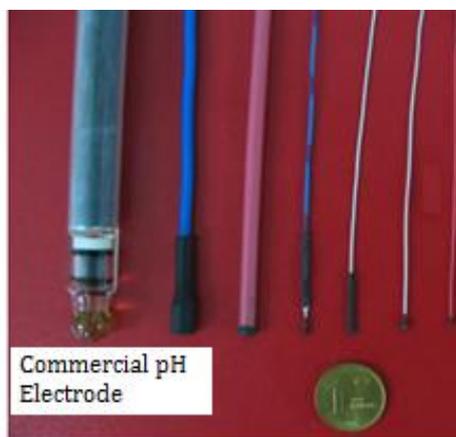


Figure 2: Composite pH sensors and commercial pH electrode.

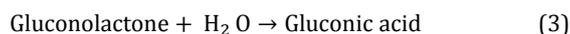
## 1.2 Potentiometric biosensors

The developments in ion-selective sensor technology used in the preparation of potentiometric biosensors and the increase in the number of sensor materials have accelerated the development of new type potentiometric biosensors [4]-[6]. Polyvinylchloride (PVC) type membrane electrodes prepared by using suitable ionophore agents have the advantage of developing simple biosensors which show short response time and give repeatable results [7],[8].

In general, enzymes are fixed on a PVC type ion selective electrode membrane surface by using glutaraldehyde cross-linker [9],[10]. However, adherence of the enzyme with cross-linker to the surface of a PVC type ion selective electrode membrane surface with a hydrophobic surface is very weak. Thus, the enzyme has begun to be adhered effectively by using functionalized PVC (aminized or carboxylated) instead of PVC [11]. Potentiometric behaviors of such functional PVC membrane ion selective electrodes have been analyzed in detail [12],[1],[2]. At the same time, it has been discussed whether functional PVC can be used in developing new biosensors [11],[12]. On the other hand, in clinical works, it is also very important to develop miniaturized biosensors to minimize enzyme and material use for simple measurement methods [13]. In this regard, the interest in using totally solid-state ion selective membrane sensors has been on the increase recently [14],[15]. These types of sensors are different from those used with classical ones. These sensors are prepared by adhering the membrane cocktail on a solid-state contact (conductive) construction which is a mixture of polymer and modified graphite. At the same time, totally solid-state membrane sensors also allow for the design of biosensors and flow-through flow cells which are economical and which can be miniaturized easily.

## 1.3 Potentiometric Glucose Biosensors

Glucose oxidase is a glucose-specific enzyme. It catalyzes glucose and releases gluconic acid in the environment as shown in the following reaction eq.2 and eq. 3:



Gluconic acid, which is the bio-catalytic product of the reaction, changes the pH of the medium. Hence, this change which occurs on the bio-layer of the sensor and which occurs because of bio-catalytic reaction is felt by pH sensor. Thus, glucose oxidase enzyme should be adhered effectively on the surface or within the pH sensor membrane. A vast number of potentiometric glucose biosensors which were based on the basis of pH sensor, have been developed. In all of these biosensors, glucose oxidase has been adhered on glass or conductive polymer electrode surface with cross-linkers. Composite pH sensor can be adhered on bio-membrane surface or within membrane physically or chemically or in the form of a thin film. Glucose-sensitive biosensor, which is prepared like this, measures hydronium ion that emerges because of bio-catalytical reaction ( $\text{H}_3\text{O}^+$ )[13]. The possible structure of glucose biosensor, which was based on composite pH sensor, given in Figure 3. As can be seen in Figure 3, glucose biosensor, which that has no inner-reference solution and inner-reference electrode thus allows for a micro-sized glucose biosensor preparation.

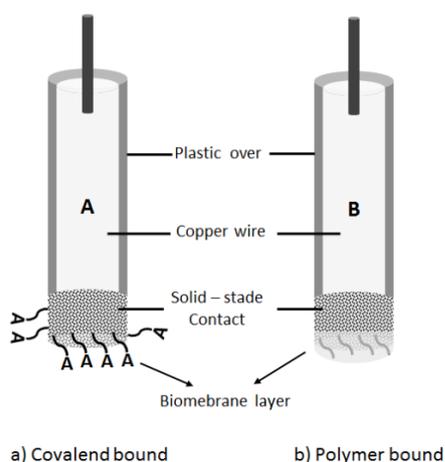


Figure 3: Micro-size potentiometric glucose biosensors  
(a): Covalent bound (b): Polymer bound.

## 2 Materials and method

### 2.1 Materials

Polyvinylchloride (PVC), *o*-nitrophenyloctylether (*o*-NPOE), methanol, tetrahydrofuran (THF) and graphite were obtained from Fluka (Milwaukee, USA). Glucose oxidase, glutaraldehyde, potassium chloride, glucose, quinhydrone and 1,4-diamino butane were obtained from Merck (Darmstadt, Germany). Epoxy resin was received from Araldite. Polymethylmethacrylate (PMMA), potassium dihydrogen phosphate and acrylic liquid were obtained from Sigma Aldrich (St. Louis, USA). Doubly distilled, deionized water was used for preparing the solutions and throughout the experiments. The supporting electrolyte solutions were 0.05 mol/L phosphate buffers (pH: 5.0-7.4) and were prepared from 0.1 mol/L solutions of phosphoric acid, potassium dihydrogen phosphate, potassium hydrogen phosphate, and potassium hydroxide, adjusted by a pH meter. Solutions of glucose were freshly prepared as required, in phosphate buffer at the desired pH and protected from light during investigation. Voltammetric studies were carried out in phosphate buffer solutions, deoxygenated by pure nitrogen. Impedimetric measurements were carried out by high-impedance input galvanostate (Princeton Applied Research, Oak Ridge, USA). A conventional three-electrode cell was used with a glucose biosensor, a saturated Ag/AgCl reference electrode (BASi), and a Pt wire as the counter electrode. Potentiometric measurements were made by a multi-channel potentiometric measurement system that was controlled by a home-made software program.

### 2.2 Preparation of glucose biosensor

A that was coated by hydrogen ion selective membranes. Firstly polyvinylchloride-aminated (PVC-NH<sub>2</sub>) polymer was synthesized. Briefly; 1.43 g PVC, 11.5 mL 1,4-diaminobutane and 3.5 mL triethylamine were mixed and refluxed under atmospheric pressure for 3.5 hours. Then the obtained polymer was washed with HCl and methanol. Subsequently, the impurities were removed by THF and the solution was filtrated and dried. The solid-state pH layers were prepared by coating one end of a copper wire with quinhydrone-PVC-NH<sub>2</sub> and modified graphite material mixed with the epoxy-hardener [14].

The pH sensitive composite sensor was dried in room temperature for 3 d. Afterwards glucose oxidase was

immobilized onto the pH layer via glutaraldehyde, a bifunctional linker.

## 3 Results and discussion

### 3.1 Voltammetric behaviour of the composite glucose biosensor

This biosensor layer was characterized with three electrode combinations in phosphate buffer solution containing 0.1 mol/L KCl at pH 7.2 including Fe(CN)<sub>6</sub><sup>3-/4-</sup> redox couple by using CV and EIS data. CV method was performed under the experimental conditions: scan rate of 50 mV/s, 0 V to 0.5 V potential range. EIS spectrums were obtained by applying 0.180 mV bias potential between 10000 and 0.05 Hz. The direct oxidation of glucose at the surface of the composite pH sensor showed no measurable wave in plain supporting electrolyte including in the potential range studied. The biosensor exhibited a remarkable increase in the anodic and cathodic peak currents when the glucose oxidase immobilized onto the composite pH sensor membrane. When the glucose biosensor is thoroughly rinsed with water and a new voltammogram is recorded in electrolyte solution, the response was retained, and two pairs of anodic and cathodic peaks were observed as reported in literature [16]-[18]. The behavior, which was observed at different concentrations of glucose and several potential scan rates, clearly demonstrates that the electrode functions electrocatalytically toward glucose.

### 3.2 Potentiometric Behavior of the Composite Glucose Biosensor

Glucose oxidase modified composite biosensor experiments on glucose measurements were carried out potentiometrically by two electrode systems. The composite pH sensitive layer based on the quinhydrone exhibited Nernst response to hydronium ions as seen in Figure 4.

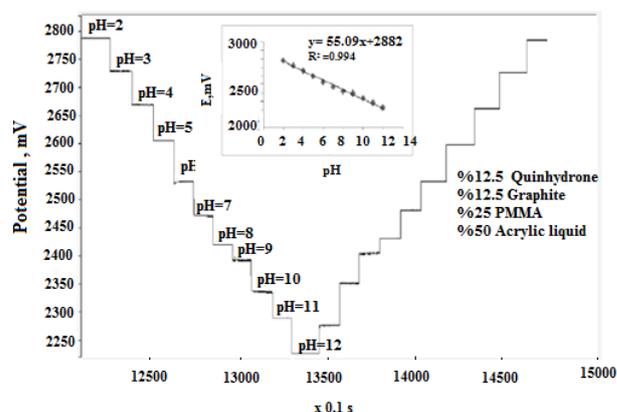


Figure 4: Potentiometric behavior of composite pH sensor.

For the selectivity coefficient measurements of the composite pH sensor,  $1 \times 10^{-4}$ - $1 \times 10^{-1}$  mol/L solutions of interfering ions were used in buffers at pH 7 and 12. It can be seen from the results that the composite pH sensor was highly selective for hydronium ions. Selectivity constants of composite pH sensor against H<sub>3</sub>O<sup>+</sup> ion next to Na<sup>+</sup>, K<sup>+</sup> and Li<sup>+</sup> ions ( $-\log k_{H^+, M^{n+}}^{\text{pot}}$ ) were calculated as 5.33, 5.63 and 5.36 at pH 7 buffer and as 11.75, 10.42 and 11.23 at pH 12 buffer, respectively. The obtained results indicated that the developed composite pH sensor acts quite selective against hydronium ion in the presence of other ions.

The repeatability of the composite pH sensor response was tested with measurements taken randomly in the pH 2 and pH 12 buffer solutions as shown in Figure 5. The composite pH sensor exhibited a good repeatability when compared with the results [19]-[22].

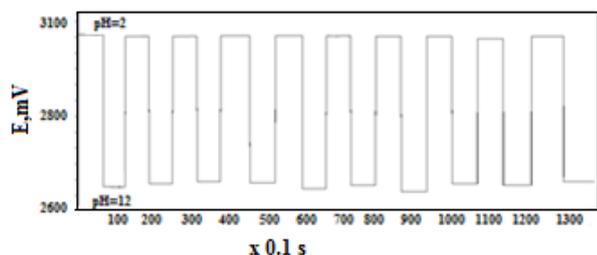


Figure 5: The potential repeatability of the composite pH sensor.

The slope of the potentiometric glucose biosensor was dependent on the solution pH and calculated to be between 20-25 mV/decade of glucose concentration indicating an under-Nernstian response of the glucose biosensor. Maximum sensitivity was observed at pH 7.2 of Tris-HCl buffer with a slope of 25 mV/decade (Figure 6).

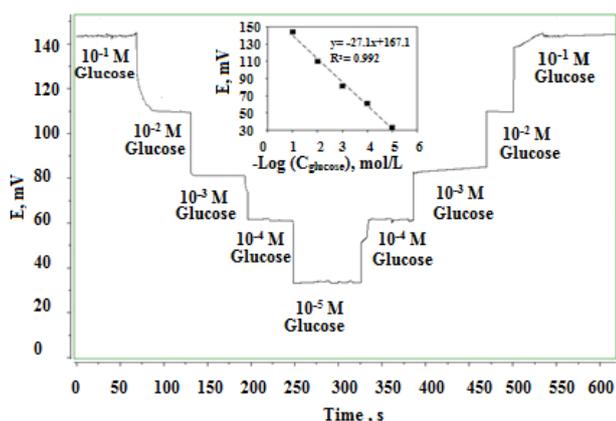


Figure 6: Potentiometric behavior of composite glucose biosensor in Tris-HCl buffer at pH 7.2.

The linear dependence of the glucose concentration has a correlation coefficient of 0.9943. The composite glucose biosensor exhibited a detection limit of  $2 \times 10^{-5}$  mol/L, based on three times measurement for the standard deviation of the blank noise. The reproducibility of the potential response of the glucose biosensor was obtained by determining the slope of the calibration graph in a period of one month (n: 5). The biosensor retained its full activity during this period and the slopes of the calibration graphs were reproducible to within 1.5 mV/decade of glucose concentration.

#### 4 Conclusion

A new type glucose sensitive potentiometric biosensor by using micro sized composite pH sensitive sensor is developed. The sensor was characterized by EIS and CV measurements, and it showed excellent potentiometric response with high selectivity. The developed glucose biosensor based on composite pH sensor has been supposed to give correct, selective and long term repetitive responses to glucose. It is thought that such a sensor can have the potential of measuring glucose continually in a measurement system such as "a glucose

watch". Thus, in the next process, the plan is to use the micro sized glucose biosensor to develop "a glucose watch" which can measure the glucose level in blood from the skin without taking blood and through reverse-iontophoresis. In addition, the fact that the glucose biosensor developed here have a longer shelf life, increased heat resistivity and a wider pH interval that reveals its potential to be used for continuous measurements.

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