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Construction of a bilirubin biosensor based on an albumin-immobilized quartz crystal microbalance

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ABSTRACT

Bilirubin is a bile pigment that is produced by hemoglobin catabolism in old erythrocytes in mammals. Bilirubin accumulates in the brain tissue, where it is toxic. Bilirubin is associated with several diseases such as Gilbert's syndrome, Dubin–Johnson syndrome and other systemic pathologies. For this reason, it is very important to measure the bilirubin levels in the human body. To treat hyperbilirubinemia patients, who have high bilirubin levels, extracorporeal bilirubin removal columns are applied. The measurement of bilirubin is important for diagnosis and therapy, and it is usually measured by nonchemical photometric devices, skin test devices and laboratory analyzers. In this study, a new bilirubin biosensor using quartz crystal microbalances immobilized with albumin is proposed. To measure the effectiveness of the biosensor, a series of experiments was realized with various concentrations of bilirubin, including 1 mg/ml (1.71 mmol/L), 2 mg/ml (3.42 mmol/L), 5 mg/ml (8.55 mmol/L) and 10 mg/ml(17.1 mmol/L). Comparing gas analyzers, laboratory analyzers, skin test devices and nonchemical photometric devices, skin test devices could be used up to 200 μmol/L and nonchemical photometric devices could be evaluated as reliable up to 250 μmol/L. The low limit range of the bilirubin detection is between 1.7 μmol/L and 2.5 μmol/L for costly commercial bilirubin measurement devices. Nevertheless, this study presents measurements with a high sensitivity and includes the advantage of reusability by using cheaper materials. In the light of this study, more sensitive biosensor could be developed to detection bilirubin level in the human blood instead of current commercial products. In addition, atomic force microscopy (AFM) was used to prove albumin immobilization and the bilirubin-albumin interaction, and a good correlation was obtained from AFM images. As a result, a low cost and more sensitive bilirubin measurement device is implemented. In conclusion, an effective and reusable bilirubin biosensor could be developed with albumin immobilization. Q2

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1. Introduction

Bilirubin is a yellow pigment in the blood and it is made in body when normal heme catabolism is broken down. Bilirubin is also the end product of heme metabolism and excreted in bile and urine [22].

Heme is found in red blood cells, which is a primary hemoglobin component. In the blood, if the bilirubin level is high it can cause jaundice, brain damage thalassemia, spherocytosis, hemolytic uremic syndrome, Gilbert syndrome and sickle anemia. Bilirubin is a insoluble and toxic matter, and metabolizing bilirubin is only possible through glucuronidation. There is not a developed of this mechanism in newborns, so massive increases of bilirubin could lead to jaundice and kernicteric damage in the brain for many babies [1]. Quantitative determination of bilirubin is very important to prevent these risks. There are many studies which include the investigation of the bilirubin removal from the human blood [2–5,34].

There are many electrochemical detection methods for the determination of bilirubin amount. In a previous study, gold nanoparticles were grown in situ on the surface of graphene multi-walled carbon nanotubes–COOH to construct a biocompatible interface. To construct a biosensor that determines the bilirubin amount, bilirubin oxidase (BOx) was immobilized on the surface. As a result, this biosensor combined composite film–gold particle–bilirubin oxidase and showed an electrocatalytic activity with a linear range from 1.33 μM to 71.56 μM, where the low detection limit is 0.34 μM with S/N = 3 (signal to noise ratio) [1]. In another study, an amperometric biosensor was constructed for the determination of bilirubin amount. This biosensor was depending on the covalent immobilization of BOx similar to the previous study. However, BOx was immobilized onto a zirconium dioxide (ZrO_2) coated silica nanoparticle (SiO2@ZrONPs)/chitosan (CHIT) composite electrodeposited onto an Au electrode. For the characterization of the enzyme-coated electrode several method were used such as scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The best response of the biosensor was observed within 2 s at pH = 8.5 (0.1 M Tris–HCl) and 35 °C, when operated at 20 mV s^{-1}. An excellent detection sensitivity with a low limit 0.1 mM was exhibited by biosensor with a low response time and a larger linear range (0.02–250 mM). The constructed biosensor measurements were correlated well with the measurements of a standard colorimetric method, with r = 0.99 [6].

In addition to electrochemical bilirubin biosensor studies, BOx covalently immobilized Au electrodes could be used as an oxygen biosensor. In one study, a sensitive amperometric biosensor was developed that could work in the kinetic barrier solutions for mass transfer such as complex physiological fluids and also other buffer solutions. This developed has a lower performance comparing to the classic biosensor performance [7].

Piezoelectric sensors, called ''piezoelectric quartz crystal microbalances'', work on the basis of the production of an electrical potential under mechanical stress. It has bilateral working principal that the application of an electrical field to the crystals causes mechanical oscillation that can generate an electrical potential when heated. This principal was observed in quartz by Pierre and Jacques Curie and it is named as the ''piezoelectric effect''. The quartz crystal is homogeneously coated by thin gold layers to make both sides electrodes. Because of an alternating current from one side to another one the crystal oscillates at a certain frequency. This frequency is reduced by impact with the increased mass of the crystal micro balance by interacting with the surface of the analyte. The decrease in the frequency is directly proportional to the concentration of analyte [8]. Because of this effect, a QCM has a wide use in fields with biosensor applications [27,28,35]. In general applications, the surface of the crystal was immobilized with an antibody that reacts with the target molecule. The frequency of oscillation was then measured, and the observed decreases in the oscillation frequency were due to the binding of the target molecule to the antibody on the surface and the subsequent increase in molecular mass on the surface of the crystal. In a previous study, a QCM was used as an early detector of myocardial infarction that quantifies the affinity of mutants of troponin T to a-tropomyosin [9]. For purification of selective protein affinity a QCM was used to screen Proinsulin C and engineered anti-C peptide antibodies [10]. We found that QCM is another option to be used in the investigating the influence of electric ions and solvent on protein–protein interactions [11]. QCM, as a transducer element, which is very suitable for chemical sensors because of its properties of portable, rapid and sensitive [12]. Once a mass attaches to the recognition element on the device surface, a decrease in the resonance frequency realizes. In the literature, QCM has a wide application field [13]. There are some applications related with bilirubin and QCM; a QCM-based approach is rapidly going to be developed with different antibodies, processing and experimental setups [30–32]. In this study, cysteamine, gluteraldehyde and albumin were sequentially immobilized on a QCM and the frequency shift of the QCM with the interaction of bilirubin was repeatedly measured. The surface of the QCM was analyzed with SEM and atomic force microscopy (AFM). All measurements and graphics were evaluated according to previous studies and experimental conditions.

2. Material and methods

2.1. Chemicals

The acetone solution, methanol, NaOH, Bovine albumin (A7906), cysteamine (M9768), glutaraldehyde (G5882) and bilirubin were obtained from Sigma–Aldrich (Interlab, Istanbul, Turkey) and used in the experimental setup. All chemicals have properties of analytical purity.

2.2. Apparatus

Twelve 10 MHz QCM crystals, were taken from the TIC Company, that were used in experimental study. To provide a clean and hydrophilic surface the electrode surface was treated with pure acetone and methanol and also 0.5 M NaOH respectively (30 min for each step) prior to experimental

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