



PIEZOELECTRIC RESONANCE SENSOR FOR THE DETERMINATION OF AMMONIUM IN AQUEOUS ENVIRONMENT

SERAP DURKUT AND ŞÜKRAN ŞEKER *

*Ankara University Faculty of Science, and Ankara University Stem Cell Institute,
Tissue Engineering, Biomaterials and Nanobiotechnology Laboratory
Ankara, Turkey*

* *Corresponding author e-mail: sukran.seker@gmail.com*

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ABSTRACT

In this study, a novel ammonium biosensor based on quartz crystal microbalance (QCM) system for determination of ammonium ion, a reaction product of urea by urease was described. Piezoelectric quartz crystals were coated with poly(lactic-co-glycolic acid) (PLGA; 85:15) nanofiber containing fullerene C60. Then, urease (EC 3.5.1.5) was immobilized on nanofibrous electrospun PLGA membrane. The nanofibers were characterized using scanning electron microscopy. Changes in the resonance frequency of the PLGA/C60-coated quartz crystal were evaluated as an indicator of changes in urea concentration. The oscillating frequency decreased accordingly to the deposition of ammonium on the quartz crystal surface. Our results indicate that the PLGA/C60 nanofiber coated piezoelectric sensor exhibited ability to determine the ammonium ion in the solution.

KEYWORDS: Quartz Crystal Microbalance, Piezoelectric Sensor, Ammonium, Resonance Frequency, Urease, Enzyme.

1. INTRODUCTION

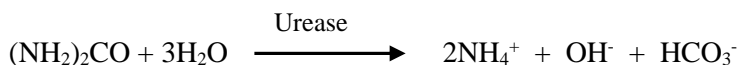
The quartz crystal microbalance (QCM) is a powerful technique that has been successfully employed to investigate the bio-molecular interaction in real-time. The active element of a QCM system is a piezoelectric quartz crystal. The working principle is based on the piezoelectric (PZ) properties of quartz crystals. This means that applying an electrical field through quartz

causes vibration of crystal near its resonant frequency. Deposition of a mass on the surface of the quartz crystals results in a decrease in resonance frequency of the crystal [1]. The change in resonance frequency is inversely proportional to the mass change, which is first described by Sauerbrey [2]. Developed on this principle, QCM device enables the conversion of a binding on the quartz surface into a change in resonance frequency of the crystal and into a measurable electrical signal.

Electrospinning is a versatile and cost-effective method used to fabricate long continuous very fine polymeric nanofibers for many applications, such as materials for enzyme immobilization, tissue engineering and wound dressing [3]. Electrospinning technique has received significant interest for the development of nanostructured coatings on the surface of PZ crystal to enhance sensor sensitivities in QCM application. Nanofiber coatings formed by electrospinning provide high porosity, large surface area, and good interconnectivity, which improve the sensing performance [4, 5].

Buckminsterfullerene (C60) is the most popular fullerene molecule with spheroid shape, having closed cage (buckyballs) structure comprising carbon atoms interconnected in 20 hexagonal and 12 pentagonal rings. The carbon atoms within C60 molecule are sp^2 hybridized. 60 π -electrons in the C60 that potentially undergo nucleophilic attack by some molecules such as gluconic acid, ammonium due to its electron-accepting properties [6, 7]. C60 has many potential applications in different fields including superconductors [8], sensors [9], polymer composites [10], solar cells [11, 12], electronic and optical devices [13]. Fullerene-coated PZ quartz crystal has been applied as a sensor for the detection of several organic compounds, including amines, thiols, carboxylic acids, aldehydes, alcohols, alkenes and alkynes [7, 14].

Determination of urea is very important in clinical diagnostics, food industry and environmental monitoring [15]. Urea is the basic nitrogenous end-product of protein metabolism in mammals [16]. Determination of urea levels in serum and urine specimens is an essential and routinely requested test by clinicians for the monitoring of dialysis efficiency, glomerular filtration rate and renal function [17]. Several analytical approaches have been developed for the determination of urea, including potentiometric, optical, thermal, amperometric, conductometric, and PZ methods [18, 19]. Urease enzyme (EC 3.5.1.5) can be used for the determination of urea, which is hydrolyzed by urease according to the reaction as given below:



The aim of this study is to evaluate the QCM system for sensing of the ammonium ion, as the reaction product of urea catalyzed by immobilized urease. Quartz crystals were coated with PLGA/C60 nanofibers to adsorb ammonium ions. The effect of urea concentration on the frequency response of the PLGA/C60-coated quartz crystal was evaluated.

2. MATERIALS AND METHODS

Chemicals

PLGA (85:15, average Mw 50.000-75.000) and Fullerene-C60 (98%) was purchased from Aldrich. Urease (EC 3.5.1.5) enzyme was obtained from Merck. Urea was obtained from Fischer. All chemicals were of either HPLC or analytical-grade reagents. Highly purified double-distilled water ($\geq 20\text{M}\Omega\text{cm}$ at 25°C) was used in all experiments.

QCM System

AT-cut quartz crystals with a fundamental frequency of 7.995 MHz were used, with 5.1 mm diameter gold electrodes. Frequencies of the crystals were measured by an electrochemical quartz crystal microbalance (EQCM, 400B, CH Instrument, USA) system.

Preparation of polymer solution for electrospinning

A saturated solution of fullerene-C60 (98%) was prepared in dichlorometane (ACS reagent 99.6%, Sigma-Aldrich). Then, tetrahydrofuran (THF) (puriss, stabilized min 99%, Riedel-de Haen) and N,N'-dimethyl formamide (DMF) (>99.0%, Fluka) were added into the solution. PLGA (85:15) was added into the solutions to the final concentration of 20% (w/v).

Coating of quartz crystal by electrospinning method

Prior to coating, the crystals were cleaned with Piranha solution (30% H_2O_2 , 70% H_2SO_4) for 1 min, washed in ethanol and ultrapure water, and then the crystals were dried in air. The freshly cleaned crystals were coated by electrospinning as detailed elsewhere [8]. The frequency shifts were calculated by subtracting the frequencies of the crystals, recorded prior (F_0) and after (F_1) coating ($\Delta F = F_0 - F_1$).

Immobilization of urease

To prevent an uncontrolled enzyme adhesion onto the quartz surfaces, the immobilized enzyme was used in the experiments. Urease was immobilized within PLGA nanofibers on a coverslip. The coverslip was coated with PLGA nanofiber by electrospinning technique. The coated coverslip was incubated with 0.2% urease solution in 0.2 M phosphate buffer (pH 6.5) for 20 hours at +4°C. After 20 hours, to remove the free enzyme molecules, the coverslip was washed with buffer solution, and stored in buffer at + 4°C.

Scanning electron microscopy (SEM)

Three-dimensional morphologic evaluation of the nanofiber coating was performed by SEM analysis. Nanofiber-coated quartz crystals were fixed in 2.5% GA in cacodylate buffer. After dehydration with a graded ethanol series (60%–95%), samples were sputter-coated with gold and visualized by SEM (JSM 5600, JEOL, Tokyo, Japan) at 15 kV.

Determination of ammonium

Determination of ammonium in the solution was performed with a dip-and-dry technique according to the following procedure: Initial frequency (F_0) of PLGA/C60 coated quartz crystal was recorded. The immobilized urease was placed in the phosphate buffer solution. Varying urea concentrations were added into the solution. Then, the coated quartz crystal was immersed into the buffer solution. After thoroughly washing with buffer to remove free NH_4^+ , it was dried at room temperature, and the resonance frequency was then recorded (F_1). Frequency shifts ($\Delta F = F_0 - F_1$) in response to adsorption of ammonium was calculated.

3. RESULTS AND DISCUSSION

Coating of the PZ Crystal

The frequency changes of quartz crystals were recorded before and after coating. The decrease in the resonance frequency of the crystal is an indicator of successful coating of quartz crystal. The frequency shift reflects the changes in the mass on the surface of the piezoelectric crystal. The average of the frequency shifts of the coated piezoelectric crystal was calculated as 236 ± 62 .

Enzyme immobilization

Efficient binding and maintenance of the biological activity of the enzyme is a crucial aspect of enzyme immobilization. Therefore, it is essential to select a proper immobilization method for the biological component [20]. In this study, urease enzyme was immobilized on PLGA electrospun nanofibers. The use of immobilized urease allowed the easy removal of the enzyme from the reaction solution after incubation. Scanning electron micrograph shows immobilized urease within PLGA nanofibers in Fig. 1. SEM images show the randomly oriented electrospun nanofibers.

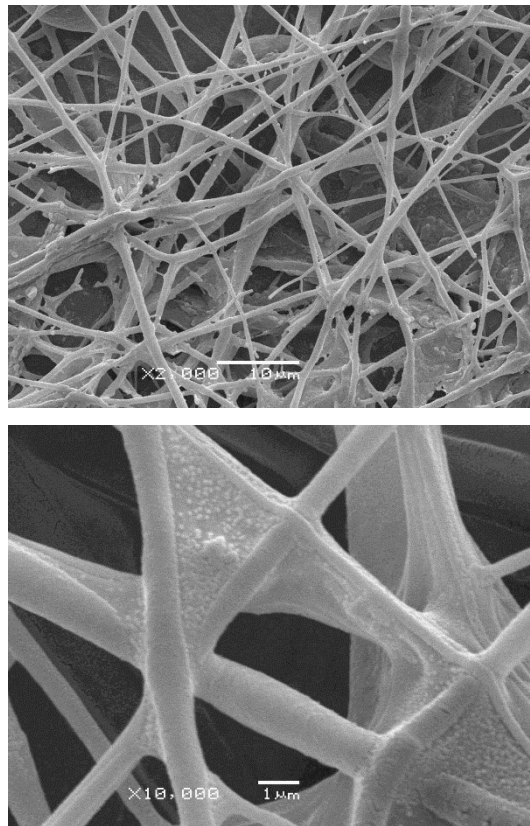


Figure 1. Representative SEM images of urease-immobilized nanofiber coatings on coverslips.

Measurement of ammonium concentration

Ammonium, an enzymatic reaction product of urea by urease, deposited on the PLGA/C60-coated PZ crystal was determined using the QCM system. The ammonium adsorption on the coated quartz crystal was monitored at different urea concentrations by following the frequency shifts of the coated PZ crystals. As shown in Fig. 2, the frequency shift of the PZ crystal increased by the increase in urea concentration.

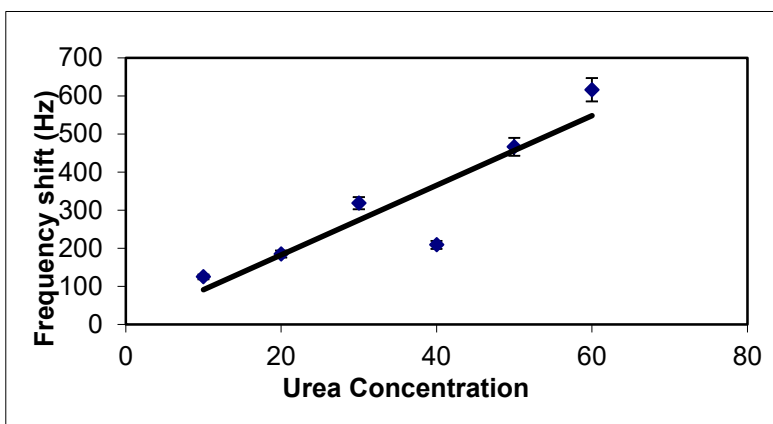


Figure 2. Frequency shift of the PLGA/C60-coated PZ crystal against various concentrations of urea.

4. CONCLUSION

This work demonstrates that the QCM system coated with PLGA/C60 nanofibrous membrane is a suitable tool for the determination of ammonium ion, the reaction product of urea catalysis by urease enzyme.

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ÖZET

Bu çalışmada, üreinin üreaz enzimi ile hidrolizi sonucu oluşan amonyum iyonunun belirlenmesine yönelik olarak geliştirilen, kuartz kristal mikrobalans (KKM) sistemine dayalı bir amonyum biyosensörü anlatılmıştır. Piezoelektrik kuartz kristaller, fulleren C60 içeren poli(laktik-ko-glikolik asit) (PLGA) nanolif membran ile elektroegirme yöntemi ile kaplanmış, ardından taramalı elektron mikroskobu ile tanımlanmıştır. Üre konsantrasyonunun, PLGA/C60

kaplı kuartz kristalin frekans cevabına olan etkisi incelenmiştir. Amonyum iyonunun kuartz kristali üzerindeki birikimine bağlı olarak, kristalin titreşim frekansının azaldığı belirlenmiştir. Elde edilen sonuçlarla, PLGA/C60 nanolif kaplı piezoelektrik sensör ile, amonyum iyonunun belirlenebildiği gösterilmiştir.

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