Doğal Melanin Nanopartikülleri Kullanarak Kuvars Ayar Çatalı Tabanlı Kütle Duyarlı Sensörlerin Modifikasyonu İçin Farklı Stratejilerin Karşılaştırması

A Comparison of Different Strategies for The Modification of Quartz Tuning Forks Based Mass Sensitive Sensors Using Natural Melanin Nanoparticles

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***Özetçe*—**Kuvars ayar çatalı (QTF) uygun maliyeti, kararlı ve düşük çalışma rezonansına sahip olması gibi üstün özellikleri sebebiyle, her geçen gün daha çok kabul gören bir ölçüm cihazı haline gelmektedir. Ancak kuvars ayar çatalların kimyasal veya fiziksel bir algılayıcı olarak yaygın olarak kullanılabilmesi için nasıl işlevselleştirilebileceği, halen çözülmesi gereken bir sorun olarak beklemektedir. Bu çalışmada kuvars ayar çatalların yüzeyinde melanin nonoparçacıklar yardımıyla bir tanıyıcı yüzey oluşturulması ve bu sayede belirli hedefleri algılayan bir kütle hassas biyosensör olarak kullanılabilmesi için farklı yaklaşımları ele alınmıştır. Bu amaca ulaşmak için görece düşük maliyetli olmaları ve endüstride kolay ve tekrarlanabilir olma özellikleri sebebiyle daldırma kaplama ve elektrokaplama işlemleri yöntem olarak seçilmiştir. Ayrıca daldırma kaplama yöntemindeki melanin nanoparçacıkların kuvars ayar çatallarının yüzeyine tutunumunu arttırmak için kimyasal aşındırma yöntemi de bu çalışmada uygulanmıştır.

Anahtar Kelimeler— Kuvars ayar çatalı, Melanin nanoparçacığı, Kütle Hassas Biyosensör, Nanobiyosensör

***Abstract*—**Quartz tuning fork (QTF) is a measurement tool that is gaining attraction nowadays due to remarkable features like their low cost, stable resonance frequency, and considerably low working frequency. However how to functionalize a QTF as a chemical or a physical sensor is still an important problem that need to be solved for a widespread usage. This paper describes approaches to functionalize QTFs by utilizing melanin nanoparticles (MNP) in order to create a recognition layer for the creation of a target specific mass sensitive biosensor. In order to achieve this aim, electroplating and dip coating methods are chosen for their relative ease of use and cheap operating costs for the purpose of being industry-friendly and reproducible as a product for field applications. Moreover a comparative study on chemical etching of QTFs was conducted with the goal of improving MNP attachment during dip coating process.

Keywords—Quartz Tuning Fork, Melanin Nanoparticle, Mass Sensitive Biosensor, Nanobiosensor.

I. INTRODUCTION

A piezoelectric quartz tuning fork (QTF), used in clock circuits in the market and, recently, force sensors for atomic force microscopy, has proven to be usability in mass sensitive biosensor technologies with their high quality factor, sharp frequency response, high precision, long stability and low cost [1][2][3]. All these features arisen from the cut of quartz crystal with special geometry by microfabrication methods. QTF consists of two prongs whose surface is coated with metal electrode films. These forks vibrate at a frequency under vacuum by moving sideways when stimulated by an alternating current excitation voltage. The change in frequency of oscillation is dependent on the mass adsorbed/binded/agglomerated to the prongs [4-6]. Obviously, the main challenge with mass sensitive biosensors is the need to collect selective and sensitive response from the transducer causing to the development of many materials [5-16].

Nanoparticle decorated biosensors play a critical role in biomedical applications to improve specificity, sensitivity and the detection limit. For this reason, to date, incredible progress has been made in both synthesis and usage of nanoparticles in sensor/biosensor technologies [17-18]. Among these nanoparticles, metallic and metal oxide nanoparticles have been used to modify mass sensitive crystals' surfaces to increase mass transport, effective sensor surface area, and the sensitivity. However, electrical instability, high cost, the need to use simple processes for synthesis, and, most importantly, the toxicity of such nanoparticles, constitute major disadvantages in sensor applications [19-21].

In recent years, melanin nanoparticles attract major interest in biomedical fields due to their biocompatibility, antioxidant activity, free radical scavenging, metal ion chelation, strong near infrared absorption and high photothermal conversion efficiency [18; 22-26]. Melanin is a brownish-yellow pigment produced in melanosomes and widely distributed in living organisms. Today, bacteria, fungi, black tea leaves, chestnut shells, catfish and cuttlefish (*Sepia officinalis*) ink are used as natural sources of melanin. Among them, cuttlefish ink has been the most preferred natural source of melanin because melanin can be easily produced from cuttlefish ink by a simple centrifugation and washing process without the need for several extraction or purification steps [18-19].

In this work, melanin nanoparticles were used to modify QTFs' prongs due to the aforementioned features. We have used dip coating and electroplating methods under different parameters. The efficiency of surface modification technique was evaluated with the change in resonance frequency of QTF as a function of coating parameters.

II. MATERIALS & METHOD

A. Materials

Quartz tuning forks (QTFs) with 32768 Hz resonance frequency with 3.10x8.20 mm and 2.10x6.20 mm dimensions purchased from Raltron Electronics (Miami, FL, USA). Ethanol, sulfuric acid and hydrochloric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

B. Method

1) Melanin Nanoparticle Production

Melanin nanoparticles was extracted from commercially available cuttlefish (*Sepia officinalis*) ink according to a method described by Jakubiak et al. (2019). Firstly, cuttlefish ink was diluted five times and centrifuged at 10,000 rpm for 20 minutes. The supernatant was removed and the pellet was washed with deionized water (DIW). This process was repeated for 5 times to remove salt and impurities. Then, the final pellets were dried in the oven at 80° for 1-2 days. One hundred milligrams of the collected powder dispersed in 10 ml of DIW (1 wt %) were prepared as a stock MNP solution and sonicated overnight using bath-type sonicator before use.

2) Electroplating

Electroplating is utilized to produce coating from the extracted melanin. The elements of the mechanism are designated to use 99% pure copper plate as anode and QTF as cathode. The electrolytic solution contains melanin nanoparticles (from 1% (w/v) melanin solution), deionized water, sulfuric acid and hydrochloric acid. The QTFs underwent electroplating process for; 2, 2.5, 3, 3.5, 4, 4.5 and, 5 minutes as time parameters (Figure 1).

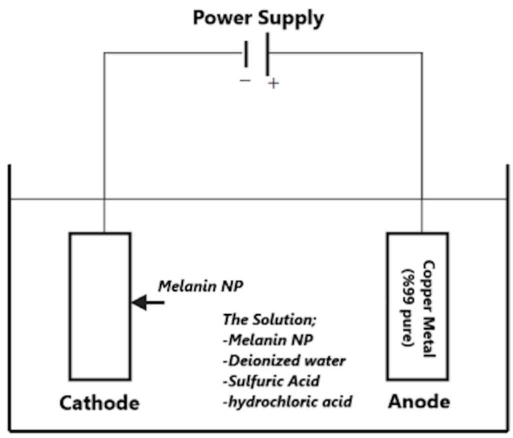

Figure 1. Electroplating diagram for melanin nanoparticle coating.

3) Dip coating

Firstly, the QTFs are washed with ethanol in ultrasonic bath for 15 minutes, then rinsed with pure water and dried with nitrogen gas, respectively. Baseline measurements of the clean QTFs are taken. Due to the unique shape of QTFs, regular droplet method for QTF could not be applied. Therefore hermetic cases of QTFs were utilized as container for the melanin droplets of 10 μL, 20 μL and, 30 μL respectively. QTFs are then placed inside the melanin solution and placed inside a furnace for drying at designated temperature conditions; 25°C, 50°C, 80°C, and, 110°C; and left for the solvent to dry out and create a deposited film over the quartz. After dip coating, all QTFs are washed individually with pure water, dried with nitrogen gas and then frequency measurements were collected.

After temperature optimization, an etching process was also tested to improve the attachment of melanin nanoparticles to QTFs with 2.10x6.20 mm and 3.10x8.20

mm dimensions during dip coating process. In order to perform chemical etching, clean QTFs were dipped into sulfuric acid for 15 minutes, 30 minutes and, 60 minutes in order to optimize etching time for the application. To understand the effect of etching process onto coating efficiency, melanin decorated QTFs with dip coating without etching process were also performed.

4) Frequency measurement

The efficiency of surface modification with melanin nanoparticles via electroplating and dip coating under different conditions were monitored with Asensis QTF F-master (Asensis, Ankara, Turkey) in terms of frequency.

III. RESULTS

The expected result to obtain stable and functional nanoparticle decoration onto QTFs' prongs could be monitor with the decrement in its oscillation frequency and the lack of adequate frequency shift as it is removed from the surface by the cleaning process. It would be challenging for the user to be very sensitive to QTFs during field application; therefore, a stable and functional coating is deemed inappropriate [4-6].

Both electroplating and dip coating have been used for different applications due to their simplicity and easiness to operate. Electroplating is an electrodeposition process for producing a dense, uniform, and adherent coating, usually of metal or alloys, upon a surface by the act of electric current [27]. The coating is usually utilized for decorative and/or protective purposes or enhancing specific properties of the surface. The surface can be conductors, such as metal, or nonconductors, such as plastics. The core part of the electroplating process is the electrolytic cell (electroplating unit). In the electrolytic cell a current is passed through a bath containing electrolyte, the anode, and the cathode [18]. The latter technique, dip coating, is a facile and economical technique widely used in many industrial fields to deposit onto any substrate, including metallic, ceramic, polymer films, and fibrous materials, etc. The process could be defined as depositing aqueous-based liquid phase coating solutions onto the surface of any substrate. Generally, target materials are dissolved in solutions which are directly applied onto the surface of the substrate, and then the sedimentary wet coating is evaporated to obtain a dry film [29].

In this study, while electroplating was optimized by conducting the experiments with different time intervals, dip coating was optimized by solvent evaporation under different temperature. The coating efficiencies were examined with the change in resonance frequencies before and after coating process. The output of controller before and after melanin decoration was given in Figure 2.

The effect of electroplating process time on the surface coating were tested after washing and drying process and given in Figure 3. All times intervals were decreased the resonance frequencies of QTFs compared to unmodified QTFs. During the study, we performed harsh cleaning process to remove unbound melanin nanoparticles from prongs. Therefore, it could be concluded that electroplating is an efficient technique for the deposition of melanin nanoparticles on QTF prongs. The change in resonance frequencies of QTFs were found around 17 Hz which is quite similar in every time intervals, thus it was seen that the change on time did not significantly affect the frequency shift.

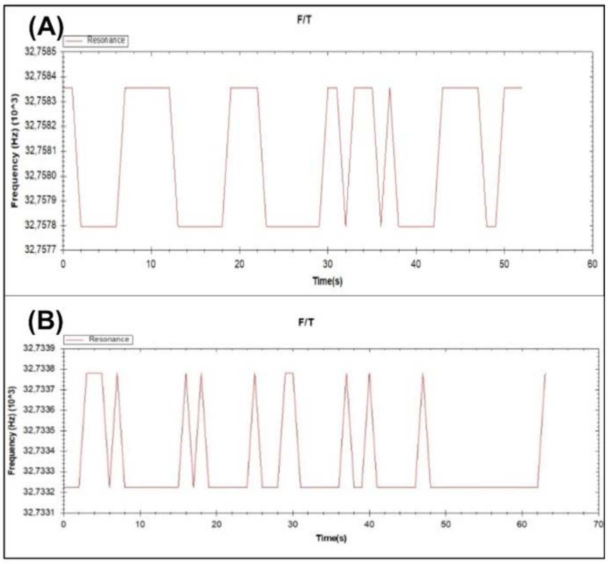

Figure 2. Resonance frequency versus time for both unmodified QTF (A) and melanin nanoparticle modified QTF (B).

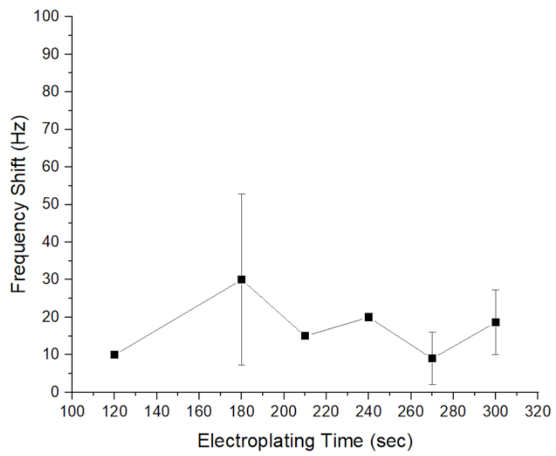

Figure 3. QTF frequency shift against electroplating time

Then, the efficiencies of second coating technique were examined and given in Figure 4. Melanin modified QTFs with 10 µL under different temperature were all decreased the frequencies around 10 Hz and no significant difference between temperatures were observed. After the volume of melanin nanoparticle solution increased to 20 µL, the shifts were found around 8 Hz at 25°C, 50°C and 80°C. Interestingly, the change in frequency at 110°C was found four times higher compared to other. The reason behind the sharp shift could be the caused by the fact that applied temperature is higher than the boiling point of solvent, water and the drying speed could affect the deposition of melanin nanoparticle onto prongs. But the reproducibility of process at 110°C with 20 µL was found quite poor. When the volume increased to 30 µL, the shifts at 50°C and 80 °C were found 6.5 Hz. However, the slight increments in resonance frequency at 25 °C were seen. Dip coating performed with 30 µL melanin nanoparticle at 110°C were decreased the resonance frequencies at approximately 16.5 Hz and the reproducible coating were obtained. Therefore, the optimum conditions were chosen as 30 µL melanin nanoparticles and 110°C for dip coating which is similar resonance frequency shift obtained with electroplating.

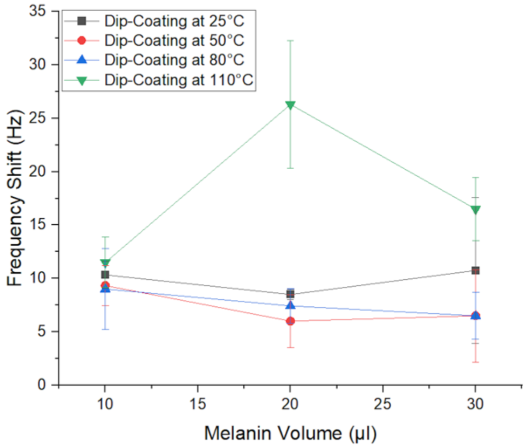

Figure 4. The temperature effect onto dip coating process in terms of frequency shift at 25°C, 50°C, 80°C and 110°C.

In this study, we also examined the chemical etching effect onto the coating efficiency and stability of QTFs with 2.10x6.20 mm and 3.10x8.20 mm dimensions. As etching removes material from the QTF surface, it is expected to showcase an increase in resonance frequency of the crystal. The average frequency shift after etching process against etching time is given in Figure 5. As expected the data shows a trend of resonance frequency increasing in relation to increment in etching time for QTFs with 3.10x8.20 mm dimensions except 60 min. There were no significant difference between 30 min and 60 min etching time. Surprisingly, QTFs with 2.10x6.20 mm were etched similar amount for each time intervals. It could be concluded that the optimum condition for chemical etching of QTF was found 30 min for both dimensions.

Then, as a comparative study the optimized temperature condition of 110°C was utilized for QTF specimen that were subjected to chemical etching prior to dip coating process (Table 1). It was expected to create a surface more capable of attachment as a result of etching process however the resulting frequency shifts seem to be less effective for melanin deposition than its non-etched prongs. These results indicated that only dip coating technique is efficient for deposition of QTFs.

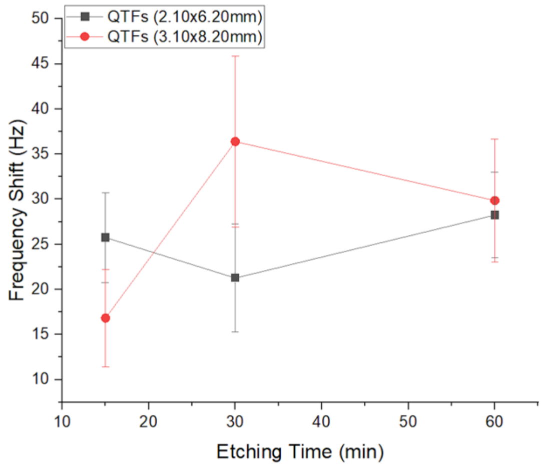

Figure 5. Frequency shift created by etching for QTFs with 2.10x6.20 mm and 3.10x8.20 mm dimensions

Sample with dimensions	**Etching process**	**Frequency shift after dip coating process**
QTF (2.10x6.20 mm)	-	36.4 ±9.5
QTF (2.10x6.20 mm)	30 min	12 ± 2.4
QTF (3.10x8.20 mm)	-	21.3 ± 6.0
QTF (3.10x8.20 mm)	30 min	37.4 ± 14.8

Table 1. The The effect of chemical etching onto frequency shift QTFs with 2.10x6.20 mm and 3.10x8.20 mm dimensions under 110 °C using 30 µL melanin nanoparticle solution

IV. CONCLUSION

In this study, we tried various methods for the modification of QTF-based biosensors. The deposition for melanin nanoparticles were found quite similar for every electroplating time interval and the obtained melanin nanoparticle layer on prongs were found stable. Additionally, we examined that the dip coating method applied at 110°C using 30 µL of melanin nanoparticle solution gave highest decrement in resonance frequencies within dip coating conditions. As a result of the comparison of the data we received, both electroplating and dip coating caused similar frequency shift, thus similar melanin nanoparticle deposition. In further studies this paper showcases a work that does not exist in literature yet which is the creation of a QTF based nanobiosensor modified with melanin nanoparticles. Due to its cost effectiveness, QTF is a perfect option for use in single-use diagnostic tests, and furthermore due to its fast reaction time uses of QTF are significant for early diagnosis which is of high importance in clinical applications.

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