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A quartz crystal microbalance biosensor based use of pesticide as recognition bed for detection of HSA

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A quartz crystal microbalance biosensor on the interaction of thiophanate methyl (MT) with human serum albumin (HSA) has been prepared. The human serum albumin was immobilized onto surface of Au coated MT as a biosensor for the detection of HSA protein by quartz crystal. Kinetic parameters of the HSA and MT interactions were also determined. The first-order rate constant k value was found to be 0.047 min^{-1}. The frequency–time profile suggests that the adsorption of HSA is controlled by activation kinetic. The obtained results indicate that HSA can provide a promising material for the biosensor designs and other biological applications.

Keywords: Biosensor, human serum albumin, thiophanate methyl, quartz crystal microbalance QCM.

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1. Introduction

Nowadays, biosensors are technological hot topics due to the possible applications in different interest fields such as medical diagnostic and health caring, monitoring of environmental pollutants, environmental protection, food analysis and home and defense security. Besides the signal generated by the sensing device, the biosensor is constituted by the molecular recognition element and the transducer material. The molecular recognition element can be a biological molecule, such as DNA single strand, proteins, enzymes, or a biological system, such as membrane, cell, and tissues: in this way, the sensing mechanism takes advantage of the natural sensitivity and specificity of the biomolecular interactions [1-3].

With the development of biotechnology and modern electronics, quartz crystal microbalance (QCM) sensors, due to their low cost and simple operation, have been widely used because they allow the real-time analysis of reactions without labeling requirements and provide quantitative information on the rate and equilibrium association constant. The QCM is a simple, cost effective, high resolution mass sensing instrument, which has been favorably adopted for analytical application due to its extreme sensitivity to the nanogram level of mass change loaded onto surface of the QCM resonator [4-9]. As a mass sensor, QCM has been widely used in biochemistry, environment, food, and clinical analysis because the instrument provides a label-less method for the direct study of biospecific interaction process. With immobilized antibodies on the surface of crystal, some QCM immunosensors have been employed for the detection of viruses, bacteria, and DNA [9].

Quartz crystal microbalance (QCM) devices were the first piezoelectric devices used in detection applications [10] and QCM has been extensively used in biosensors applications, in recent years. Human serum albumin (HSA) is the most abundant protein in the blood stream (accounting for about 60% of total plasma protein), which plays an

important role in the transportation and deposition of many endogenous and exogenous drugs ligands in blood [11,12].

The concentration of the HSA of a healthy person is about 20 µg/ml, whereas it can be up to 200 µg/ml in patients with renal disorders [13]. Most drugs travel in plasma and reach the target tissue by binding to HSA. Therefore, binding to HSA strongly influences the free drug concentration in plasma and has a large impact in the transfer of drugs to the tissue. So the level of the drug binding to HSA is an important factor in the drug discovery processes [14]. Thiophanate methyl (MT) is one of the widely used to control the fungal diseases of crops due to its broader range of activity in comparison with others [15,16]. Binding of pesticides to plasma proteins has toxicological importance, since the degree and time of action in the body affect the duration and intensity of their effects [17,18]. The determination of human albumin (HSA) is one of the significant indicators for early diagnosis of renal disease of diabateic patients [19]. The quartz microbalance is suitable to be used for determination of concentration levels in blood. Biosensors based on a quartz crystal microbalance can be improved for medicine applications because of low cost and potential of high integration. Thus, it is evaluated that the fabrication of a QCM biosensor is useful for practicing physicians. With this aim, we have fabricated a quartz crystal microbalance biosensor based on immobilization of HAS onto quartz crystal substrate with MT.

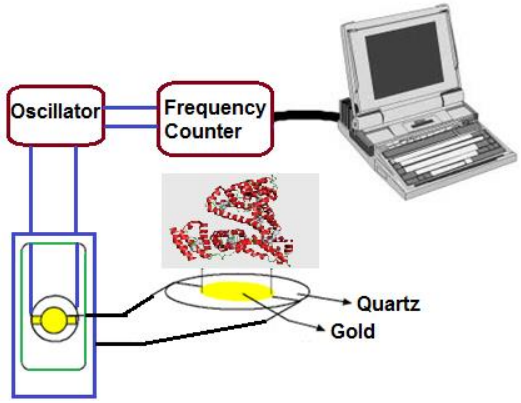
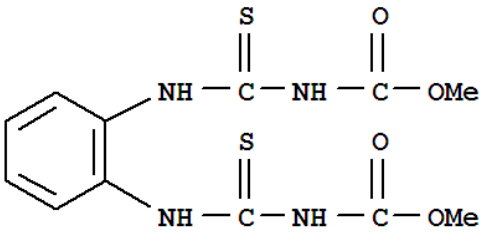


Fig.1: Experimental setup for QCM sensors.

2. Experimental details

AT-cut quartz crystals (5.0 MHz) of 13 mm diameter with Au electrodes were used for sensor measurements. Firstly, the surface of quartz crystal was cleaned by immersion in piranha solution, H_2SO_4/H_2O_2 (3:1, v/v). Subsequently, the quartz crystal was replaced in a probe made of Teflon. For QCM measurements, the stock solution of MT (4mg/ml) was prepared in DMSO and 20 µl of MT was dropped onto quartz crystal and was dried at 60 ^oC. The frequency of the quartz crystal was then monitored as a function of time. Then after, HSA solution was prepared in pH 7.40 trisHCl buffer solution as ($3.0x10^{-5}$mol/L) and 20 µl of the HSA solution was added to MT. Experimental setup for QCM sensors is shown in Fig.1. The structural properties of the MT and HSA+MT materials were investigated using a Park System XE-100E atomic force microscopy.



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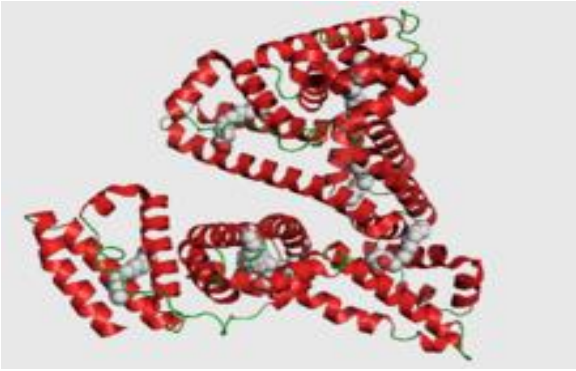

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Fig.2: The chemical structures of thiophanate methyl and human serum albumin a) MT b) HSA

3. Results and discussion:

3.1. Determination of structural changes between MT and HAS molecules by AFM

Atomic Force Microscope (AFM) is a surface characterization technique in determination of structural properties of biocompatible materials. AFM was employed for assessment of the interaction between thiophanate methyl and human serum albumin. The chemical structures of thiophanate methyl and human serum albumin are shown in Fig.2. The two (2D) and three dimensional (3D)

AFM images of the MT and MT+HSA are shown in Fig.3. As seen in Fig.3, the MT compound is formed from particles like leaf. The surface roughness of the MT film was determined to be 85.97 nm. However, upon interaction with HSA, the structural properties

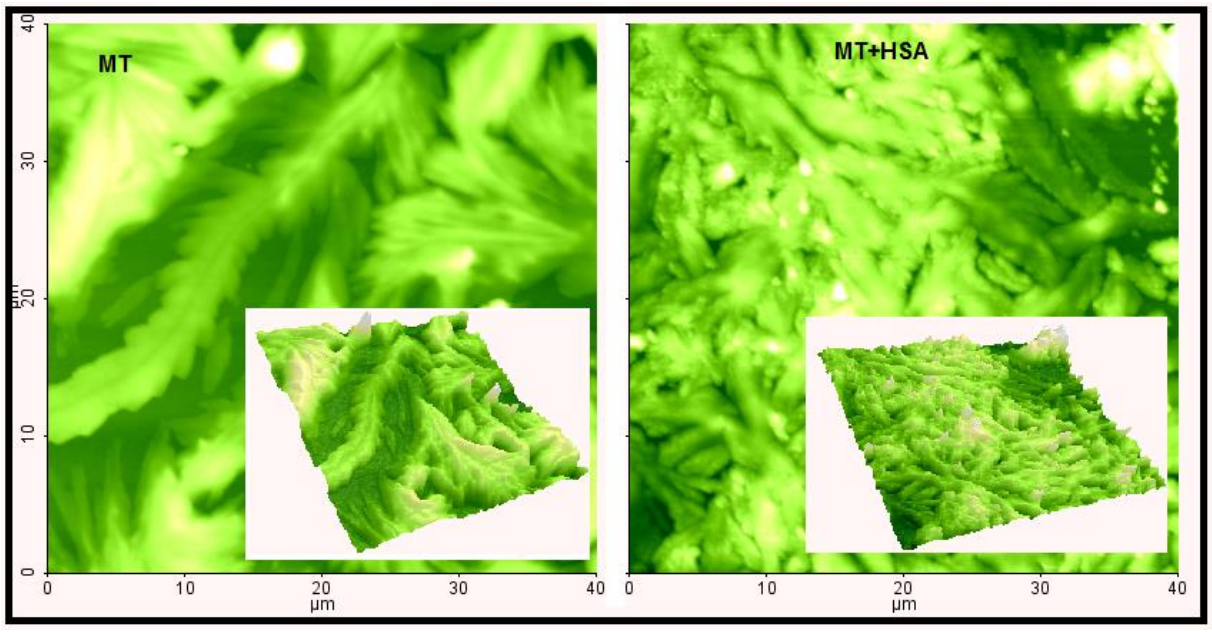

Fig.3: The. AFM images of (40x40 µm^2) of MT and MT+HSA

have been changed and the structures like branch are formed with surface roughness of 73.18 nm. These results confirm that an interaction between MT and HSA molecules has been occurred.

3.3. X- Ray Diffraction

In an attempt to investigate 'whether MT interacts with HSA, QCM measurements were performed. QCM measurement is an important method to study interactions of albumin with several substances. The frequency-time measurements of the biosensor were performed at room temperature. Fig.4 shows the plot of Δf vs. time for the MT deposited onto gold substrate. Fig.4, explicitly shows a decrease in the frequency shift with the time for MT with the value of $f = -7.39$ Hz. This suggests that MT is adsorbed on the sensor surface by physical adsorption. Addition of HAS onto MT resulted in a sharp decline in frequency during the first 65 second, followed by a gradual and linear decrease frequency. The data confirm that the interaction has occurred between HSA and MT molecules. The higher frequency change indicates a strong binding of HSA onto MT, which validates the immobilization of HSA on the sensor surface through the process of adsorption.

The frequency–time profile of HSA can be analyzed to study the kinetics of adsorption. For this, we analyzed the plot of Δf versus time via $\Delta f = At^n$, where A is constant and t is the time. The n value was determined from the plot of Δf vs time and was found to be 0.5. This suggests that the adsorption of HSA is controlled by activation kinetic. Since the QCM is a dynamic system, the possibility for assessing the kinetics of interaction exists. The reaction between MT and HSA is a pseudo first-order reaction [20] and the concentration of HSA coated on the sensor is constant [21],

$$\mathrm{MT} + \mathrm{HSA} = \mathrm{MT}-\mathrm{HAS} \qquad (1)$$

$$\frac{dC_x}{dt} = k(\mathrm{C}_o - C_x) \qquad (2)$$

Where C_o is the initial concentration of HSA and C_x is the reacted concentration of HSA, k the rate constant. Integrating Eq. (2), one can find:

$$C_x = C_o(1-e^{-kt}) \qquad (3)$$

and thus, the first-order rate constant k is can be analyzed by the following relation [22]:

$$\Delta f = \Delta f_{\max}.(1-\mathrm{e}^{-kt}) \qquad (4)$$

Where ΔF_{max} is the frequency change between the initial and the final steady-state frequencies, and k is the first-order rate constant expressed in min^{-1}. The k value and Δf_{max} values were found to be 0.047 min^{-1} and -76.14 Hz, respectively.

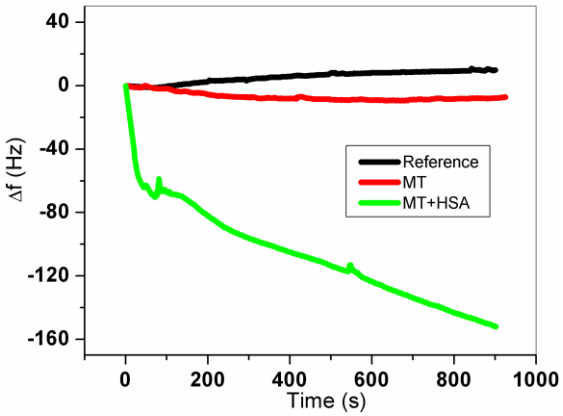

Fig.4: Plots of Δf vs. time of the sensor.

The decrease in frequency is due to the change in mass arising from the adsorption of the HSA and thus, the Δf can be expressed by the following relation [10,23],

$$\Delta f = -2\frac{f_o^2}{\sqrt{\rho_q \mu_q}}\frac{\Delta m}{A} \qquad (5)$$

Where Δm is the mass change, f_o is the resonance frequency, ρ_q and μ_q are the density and the shear modulus of the QCM sensor, A is the electrode surface area. The plots of Δ_m vs time for MT and MT+HSA materials are shown in Fig.5. As seen in Fig.5, the Δ_m value increases suddenly with time for the first 75 s and then, Δ_m is linearly increases with time. The immobilization of HSA on the QCM is resulted in a decrease in frequency and an increase in motional resistance is observed (Fig.6). The change in resistance reaches to about 41 Ω. This has further substantiated the binding of HSA molecule with MT.

For the interaction of MT and HSA, the association constant k_a and dissociation constant k_d can be analyzed by the following relation [24]:

$$\frac{d(\Delta f)}{dt} = k_a C(\Delta f_m - \Delta f) - k_d(\Delta f) \qquad (6)$$

Where C is the concentration of HSA. To determine k_a and k_d values, the curve of $d(\Delta f)/dt$ vs. Δf was plotted and the values of k_a and k_d values were determined from slope $(-(k_aC+k_d)$ and intercept $(k_a\Delta f_m C)$ of Fig.4 and were found to be $8.45\pm0.2\times10^2$ ($M^{-1}.s^{-1}$) and $1.69\pm0.01\times10^{-3}$(s^{-1}), respectively. The change in frequency resulted from immobilization of human serum albumin is higher than that of the interaction of bovine serum albumin with Pb [25].

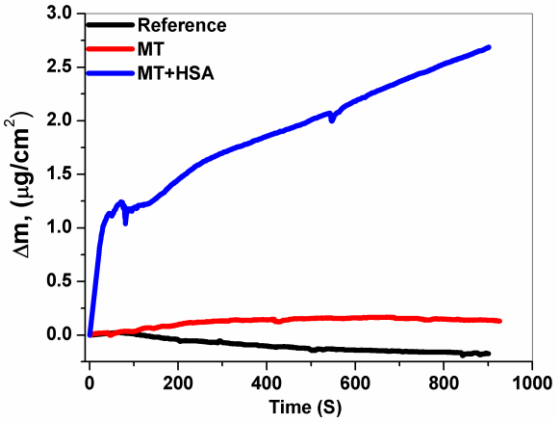

Fig.5: Plots of Δm vs. time of the sensor.

This has reaffirmed the strong interaction between HSA and MT molecules.

Finally, the effect of MT on HSA caused considerable changes in the protein secondary structure, and it may be the result of the formation of MT–HSA complex [26]. The results obtained from molecular modeling showed that the interaction between MT and HSA was dominated by hydrophobic force, and there was also hydrogen bond interaction between the pesticide and the residues of HSA, which is in good agreement with the result of binding mode. Our work based on AFM micrographs and QCM measurements are in good agreement with Jinhua Li et al for monitoring the interaction between MT and HSA [26].

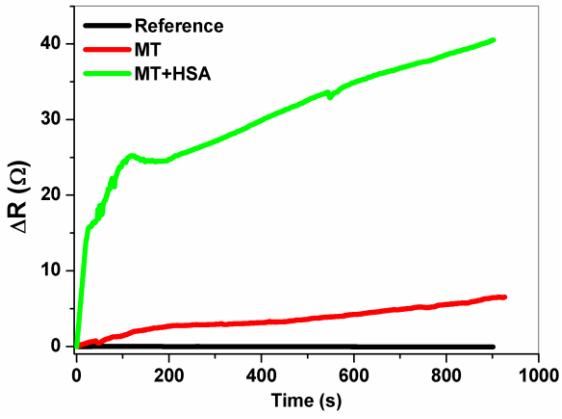

Fig.6: Plots of ΔR vs. time of the sensor.

Conclusions

The quartz crystal microbalance sensor based on human serum albumin was prepared by means of thiophanate methyl. The structural properties of HSA and MT films were investigated

by atomic force microscopy. AFM results confirmed the interaction between HSA and MT molecules. The kinetic parameters of HSA QCM biosensor were determined. The obtained results suggest that HSA-QCM biosensor is a convenient and valuable tool for real-time kinetic analysis of protein-pesticide interactions.

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