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Quartz Crystal Microbalance Based Hydrophobic Nanosensor for Lysozyme Detection

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***Abstract*—**A quartz crystal microbalance (QCM) nanosensor was developed to detect lysozyme enzyme by functionalizing its gold surface with the attachment of poly(methacroyl-L-phenylalanine) (PMAPA) nanoparticles. PMAPA was chosen as a hydrophobic matrix. The hydrophobic nanoparticles were synthesized by micro-emulsion polymerization method. Hydrophobic QCM nanosensor was tested for real time detection of lysozyme enzyme from aqueous solution. The kinetic and affinity studies were determined by using lysozyme solutions with different concentrations. The responses related with mass (Δm) and frequency (Δf) shifts were used to evaluate adsorption properties.

***Keywords*—**HIC, lysozyme, nanosensor, QCM.

I. Introduction

QUARTZ Crystal Microbalance (QCM), high-resolution mass sensing technique, measures changes in mass on oscillating quartz crystal surface by measuring changes in oscillation frequency of crystal in real time. QCM sensors are new generation optic sensors [1] that can be used for studying of antibody/antigen, protein/DNA, analytic polymeric material interactions. Protein adsorption techniques via hydrophobic interaction between protein and solid support, called hydrophobic interaction chromatography (HIC), can be favorable in many cases. HIC takes advantage of the hydrophobicity of proteins by promoting its separation on the basis of hydrophobic interactions between immobilized hydrophobic ligands and nonpolar regions on the surface of the proteins [2]. QCM sensor systems have very important advantages enabling real time analysis and determination unnecessary of labelling analyte molecule, high surface sensitivity and instantaneous determination as well as repeatable use potential, quick response time and analysis of many analytes simultaneously. These sensor systems have widely been employed in many fields. Lysozyme is found in a variety of vertebrate cells and secretions, such as spleen, milk, tears and egg white. Its common applications are as a cell disrupting agent for extraction of bacterial intracellular products, as an antibacterial agent in ophthalmologic preparations, as a food additive in milk products and as a drug for treatment of ulcers and infections [2]. Lysozyme has also been used in cancer chemotherapy. In the scope of the project, it is aimed to develop hydrophobic interaction [3], [4] based QCM sensor systems for lysozyme enzyme detection.

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II. Experimental

A. Polymerization Reactions

Polymethacryoyl phenylalanine (PMAPA) nanoparticles were synthesized by using MAPA as a monomer.

1. First Step

In the first step MAPA monomer was synthesized from methacryloyl chloride and L-phenylalanine aminoacid. The following procedure was applied for the synthesis of N-methacryoyl-(L)-phenylalanine (MAPA). L-phenylalanine methyl ester (5.0 g) and $NaNO_2$ (0.2 g) were dissolved in 30 mL K_2CO_3 aqueous solution (5%,w/v). This solution was cooled down to 0^oC. Methacryoyl chloride (4.0 mL) was slowly poured into this solution under nitrogen atmosphere and this solution was stirred at room temperature for 2 h. At the end of this period, pH of the solution was adjusted to 7.0 and subsequently the solution was extracted with ethyl acetate. Fig. 1 shows chemical reaction of methacryloyl chloride and L-phenylalanine aminoacid to form MAPA monomer. PMAPA nanoparticles were prepared according to the recipe which is done by [5].

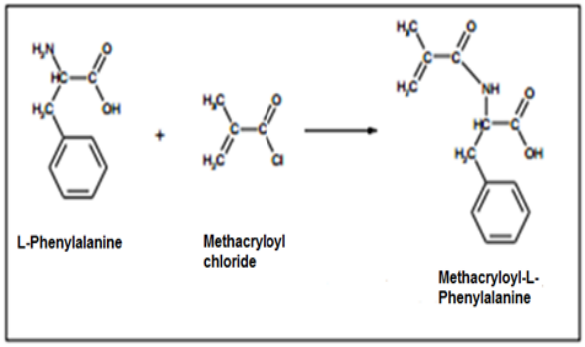

Fig. 1 Chemical reaction of L-phenylalanine with methacryloyl chloride

2. Second Step

In the second step MAPA was used as monomer for PMAPA synthesis. The nanoparticles were attached by dropping of nanoparticle solution (7μL) to the gold surface of QCM chip and then, dried at 37^oC for 6 h. After drying, nanosensor chip was ready for binding experiments. Fig. 2 shows schematic representation of polymerization reactor used during PMAPA synthesis.

Schematic representation of nanosensor chip making was shown in Fig. 3. As shown in Fig. 3 polymer solution was poured onto the surface of gold chip.

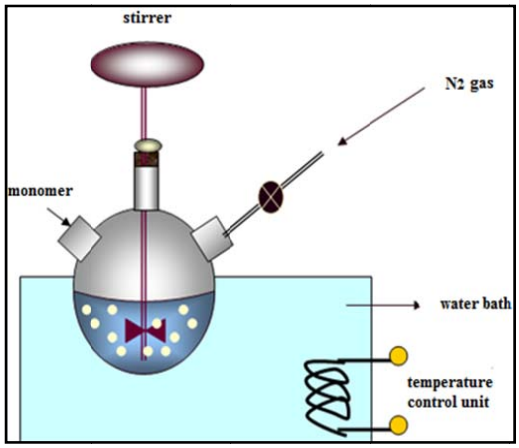

Fig. 2 Schematic representation of polymerization reactor

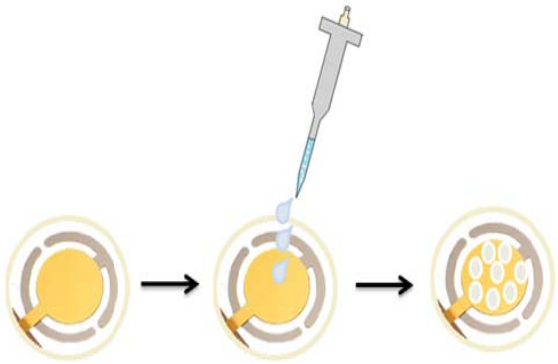

Fig. 3 Nanosensor chip making system

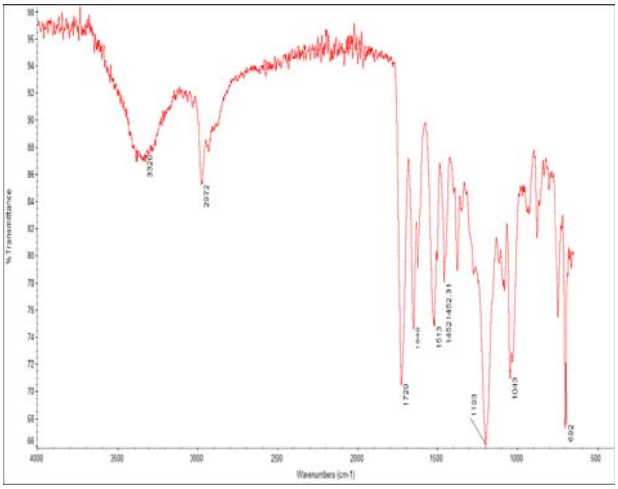

Fig. 4 ATR-FTIR spectrum of hydrophobic QCM nanosensor

III. Results

A. Characterization

PMAPA nanoparticles were prepared by miniemulsion polymerization method of hydrophobic MAPA monomer. Hydrophobic QCM nanosensor was characterized by attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy. In order to determine particle size and size distribution of the nanoparticles, zeta-sizer and atomic force microscopy (AFM) were used also. Hydrophilicity of nanoparticle attached QCM nanosensor was measured by contact angle measurement. Fig. 4 shows ATR-FTIR spectrum of hydrophobic QCM nanosensor. Fig. 5 shows AFM image of hydrophobic QCM nanosensor, Fig. 6 shows roughness of hydrophobic QCM nanosensor by taking crossection image of AFM and Fig. 7 shows contact angle measurement of nanosensor chip. Fig. 8 shows zeta size analysis of hydrophobic PMAPA nanoparticles.

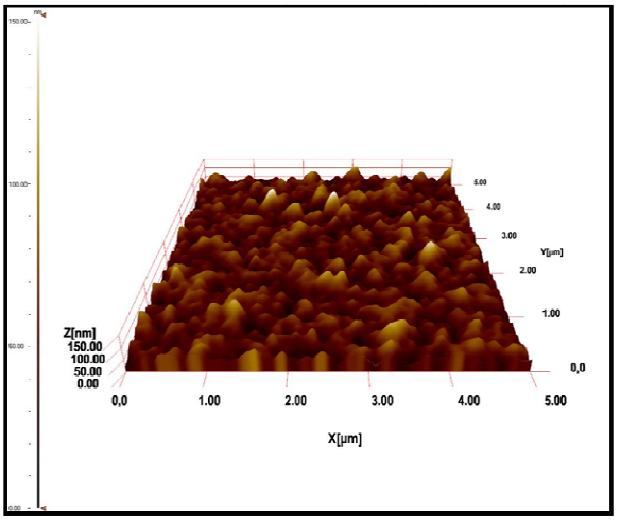

Fig. 5 AFM image of hydrophobic QCM nanosensor

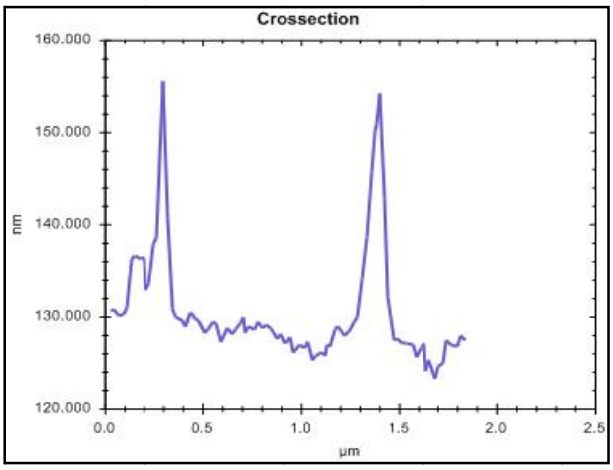

Fig. 6 Roughness of hydrophobic QCM nanosensor

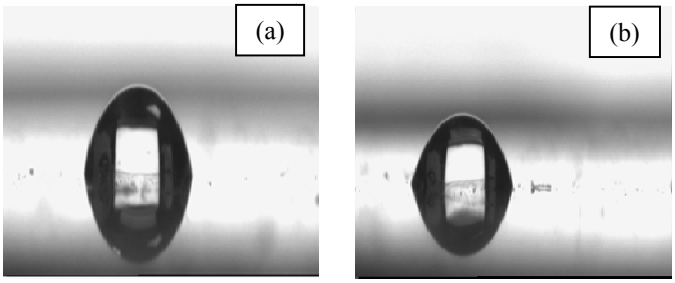

Fig. 7 The contact angle measurements of QCM nanosensor (a) nanoparticle attached and (b) empty surface

As seen in Table I, the contact angle of the unmodified QCM nanosensor increased from 76.3° to 87.2° when the hydrophobic polymer PMAPA was attached onto the modified gold surface. Increasing of the contact angle shows that the increased hydrophobic property of the surface of nanosensor chip.

TABLE I
CONTACT ANGLE MEASUREMENT FOR NANOSENSOR

Contact Angle, °	Unmodified Gold Surface	Nanosensor Chip prepared by PMAPA Nanoparticle
	76.3±0.02	87.2±0.08

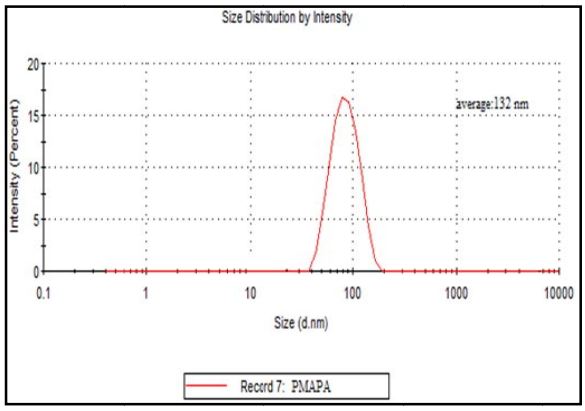

Fig. 8 Zeta size analysis of hydrophobic nanoparticles

B. Summary of Studies

PMAPA formation can be deduced from ATR-FTIR spectrum in Fig. 4. Band belongs to MAPA monomer's (C=C) group at 1633 cm^{-1} disappeared when polymerization completed.

As seen in Fig. 5, AFM image of nanoparticles shows that nanofilm deepness formed by MAPA nanoparticles is approximately 150 nm. In Fig. 6, crossection values indicate that surface of nanosensor roughness almost homogeneous. The results obtained from zeta-sizer show that nanoparticles have average particle size as 132 nm in Fig. 8.

C. Kinetic Analysis

Kinetic analyses were evaluated by using different concentration of lysozyme solution (500-10000 pg/mL). Δf and Δm sensograms of the interaction between the lysozyme and PMAPA nanoparticle bonded QCM nanosensor was shown in Figs. 9 and 10 respectively. Δm and Δf values obtained from sensorgrams indicate that there is strong interaction between hydrophobic QCM nanosensor and lysozyme solution (10000 pg/mL).

IV. CONCLUSION

Kinetic analyses indicate that prepared QCM nanosensor chip functioning correlated values. It is concluded that, QCM nanosensor via nanoparticle film may be used to detect lysozyme enzyme with low concentration values than the former studies [6], [7]. Fig. 11 shows the instrument and chip system used in QCM analysis.

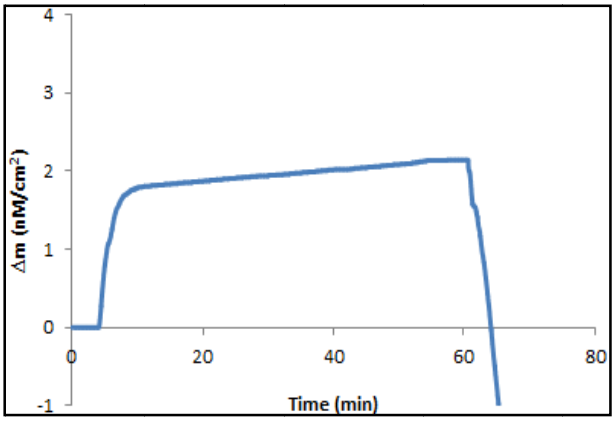

Fig. 9 Δm sensorgram of the interaction between hydrophobic QCM nanosensor and lysozyme solution (10000 pg/mL)

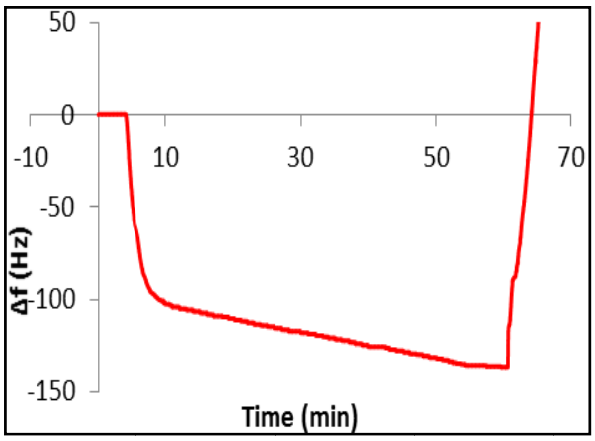

Fig. 10 Δf sensorgram of the interaction between hydrophobic QCM nanosensor and lysozyme solution (10000 pg/mL)

V. FUTURE PLANNING

In the present study, we prepared quartz crystal microbalance (QCM) nanosensor for real time lysozyme detection. Kinetic analyses will be evaluated by comparing adsorption capacity of nanosensor chip with competitive protein bovine serum albumin (BSA). Association (K_A) and dissociation constants (K_D) will be determined to estimate affinity strength. Finally detection limit (LOD) calculation will be performed.

Fig. 11 Image of instrument used in OCM analysis of hydrophobic nanoparticles

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